

Study on the Enzymatic Degradation of Aliphatic Polyester–PBS and Its Copolymers

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ABSTRACT: The catalytic degradation of prepared matrix poly(butylene succinate) (PBS) and its copolymers by immobilized lipase is carried out in the mixed organic solvent containing a small amount of water. The degradation products were studied with various characterizations techniques, including gel permeation chromatography (GPC), time of flight mass spectrum (TOF-MS), nuclear magnetic resonance (NMR), and Fourier transform-infrared spectroscopy (FT-IR). The results showed that under atmospheric pressure, 60°C, after 24 h catalytic degradation of PBS and its copolymers by immobilized lipase, the yellow oil-like degradation products can be obtained. The lipase has cata-

lytic activity on various copolymers. At the first time, the monomer of BDO was found in the degradation products and the molecular weight of product with aromatic smell is below 1000. The products consisted of cyclic oligomer, linear oligomer and monomers, and cyclic oligomer is at least dimmer. The minimum and maximum degradation yields correspond to PBS (40%) and P(BS-co-CL-co-CHDM) (54%). © 2011 Wiley Periodicals, Inc. *J Appl Polym Sci* 124: 2902–2907, 2012

Key words: immobilized lipase; degradation; dimmer; terpolymers; cyclic oligomer; linear oligomer

INTRODUCTION

Immobilized lipase has received a lot of attention as a natural and green catalyst due to its mild reaction conditions, recycling, and high activity in various organic solvents. In recent years, a series of good results have been obtained by researching on enzymatic synthesis of polyesters^{1–7} and the catalytic hydrolysis of polyesters.^{8–17} Aliphatic polyester is considered to be an important way to solve white pollution because of its good biodegradation. After been discarded, aliphatic polyester was eroded by micro-organisms in nature and transformed into CO₂ and H₂O. So polyester can be recycled after abandon, degraded by lipase, and get oligomer which can gather once again and synthesize polymer,⁷ and also for modified polyester by plasticizers, crosslinking agent.

At present, Sebastien Lenglet in France,¹⁸ Okajima in Japan,⁷ Paola Rizzarelli and Giuseppe Impaement in Italy¹⁹ have studied the degradation of copolymer

by different types of lipase, which have verified the relationship between the high lipase activity and the good hydrolysis of polyester. However, as to the use of mixed solvents for enzymatic degradation of PBS and its copolymer, and the analysis of the properties of degradation products, such as molecular weight, degradation yield, the structure of products, these studies are relatively less.

This article delivers the investigation of lipase which is used as a catalyst to degrade the new polymers with molecular weight of more than 100,000, and the molecular weight of products was hundreds; the degradation yield was calculated and analyzed on various structures of the degradation products.

EXPERIMENT

Materials and equipment

Materials

Poly(butylene succinate) (PBS), PBS and ϵ -caprolactone copolymer (PBS-co-CL), binary copolymer of PBS and 1,4-cyclohexane dimethanol (PBS-co-CHDM), ternary copolymer of PBS, PCL, and 1,4-cyclohexane dimethanol (PBS-co-CL-co-CHDM) are synthesized in Laboratory.²⁰ Solvent: toluene, tetrahydrofuran (THF), and chloroform (CHCl₃) are analytically pure and are purchased from Xi'an Chemical Reagent. Immobilized *Candida* lipase (CA: Novozym435) 10,000 plu/g were purchased from Japan Vinorelbine. All the reagents were used directly without further purification.

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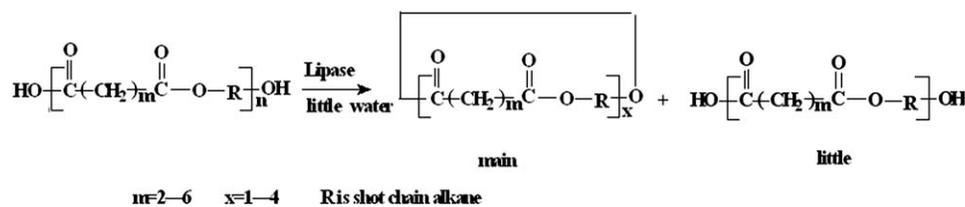


Figure 1 Enzyme catalysis polyester degradation reaction schemes.

Equipments

The Bruker AVANCE 200 NMR (USA Bruker Corporation) and Nicolet NEXUS-470 IR spectrometer (USA Nicolet Corporation) were used to determine the chemical structure of polymer degradation products; HT3-515GPC (U.S. Waters Corporation) was used to determine molecular weight of polymer before and after degradation; MALDI-TOF-MS (Micromass, UK) was used to analyze degradation products.

Copolymer degradation conditions

THF (20 ml), distilled water, CA enzyme (0.08 g), and PBS (0.5 g) were added into a 100-ml three-necked flask. THF dissolves the polymers and stirred at 60°C for 12 h under atmospheric pressure, then toluene were added. The 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 purified further by THF (Fig. 1). Other polyesters were degradation by the same method as mentioned above.

Polyester degradation yield calculated

Polyester degradation yield is calculated as following: the oligomer of degradation products are with strong polarity and soluble (volume fraction) in THF contained 5% water, while polymer with high molecular weight and weak polarity was not dissolved in THF with 5% (volume fraction) water at room temperature. So this character can be used to remove polyester with high molecular weight and to calculate the degradation yield. If the substrate, such as mass of PBS is M (g), and mass of the resulted oligomer is m (g), the polymer consumption can be calculated with the following equation:

$$\text{Recovery} = m/M \times 100\%$$

RESULTS AND DISCUSSION

GPC results of polyester and the degradation

Molecular weight of polyester and the degradation products was analyzed by GPC, the results was shown in Table I. Table I shows that, after being cat-

alyzed and hydrolyzed by enzymes, polyesters with the molecular weight more than 100,000 degraded to a series of low-polyesters whose molecular weight is below 1000. Copolymers had been easily degraded than PBS; the degradation products of PBS/CHDM have the smallest molecular weight about 380 (M_n), which is mainly because of CHDM containing six-membered ring, enlarged the gap between molecules, giving rise to much easier molecular chain stretch, and more favorable exposure of ester bond to enzyme attack; polymer modified by caprolactone (CL) is well degradable, which is mainly due to the increase of plasticity of chain segment caused by adding a small amount of CL, the rotation speed of chain segments are increased in the solvent which results in easy access to the enzyme's active side.

Analysis of polyester degradation yield

P(BS-co-CL-co-CHDM) is more conducive to degradation by the enzyme (Table II), it has a highest degradation yield among the four substances that were investigated, and the best recovery was 54%. Comparing series polymer of PBS and its copolymers, the modified copolymer of PBS have better degradation effect. Degradation yield of polymer modified by CL are slightly increased, which is mainly due to the increase of plasticity of chain segment by adding a small amount of CL, the rotation speed of chain segments is increased in the solvent which results in easy access to the enzyme's active side. Degradation yield of polymer modified by CHDM are much higher, which is mainly because of CHDM containing six-membered ring, enlarged the gap between molecules, giving rise to much easier molecular

TABLE I
The Molecular Weight of Polyesters

Sample	Mass ratio	Polymer molecular weight ($M_n \times 10^4$)	Molecular weight of product (M_n)
P(BS)	—	10.00	847
P(BS-co-CL)	90 : 10	9.67	530
P(BS-co-CHDM)	90 : 10	13.80	380
P(BS-co-CL-co-CHDM)	90 : 5 : 5	8.39	572

TABLE II
Degradation Yield of Polymer

Polymer	PBS	P(BS-co-CL)	P(BS-co-CHDM)	P(BS-co-CL-co-CHDM)
Degradation yield	40%	43%	53%	54%

chain stretch, and more favorable exposure of ester bond to enzyme attack.

Degradation yield of CHDM-modified copolymers and terpolymers of P(BS-co-CL-co-CHDM) have a little difference, but P(BS-co-CL-co-CHDM) has a highest degradation yield, which is due to merging the two advantages above and speeding up rotation of chain segments by CL molecular chain and full stretch of the molecular chain by six-membered ring, which facilitate the polymer to have more effective degradation.

MS analysis of polyester degradation products

Table III shows the monomer structure, Table IV and Figure 2 shows the MS analysis of degradation products of copolymer and PBS. The results confirmed that the lipase N435 has a high activity on catalyzing hydrolysis of PBS and its copolymers. The degradation productions are almost monomers, 1-4 cyclic oligomers, and liner oligomers. Cyclic oligomer is at least tripolymer. Among the four kinds of copolymer, only the degradation product of P(BS-co-CHDM) has BDO single ions peak after enzymatic hydrolysis, which indicates that there are copolymer product of P(BS-co-CL-co-CHDM) after enzymatic hydrolysis, the BDO single ion peak cannot be checked out, indicating that when the content of CHDM ($m = 10\%$) is higher, six-membered ring steric effect of the molecular chain reduces the twist of molecular chain, so the stretch of polymer chain is good in the solvent, which benefits the attack of lipase activity and increases the degradation activity, so degradation products of P(BS-co-CHDM) have BDO single ion peaks, but the product cannot be detected the SA monomer peaks, it is because the SA monomer has higher activity and can be esterified with alcohol hydroxyl at 60°C in organic solvents.

In the report about the enzyme catalytic degradation of PBA-co-PBS by Okajima, the cyclic products decreased with the increase of water content in the degradation condition: PBS (1 mg), toluene(100 ml), water (0.1 mg), and the immobilized lipase CA with the mass fraction of 300%, reacted at 85°C for 24 h, PBS turns into cyclic oligomer.¹¹ But in our study, water content was consistent in each degradation system, 60°C, THF(0.1 ml) with 1 ml of water content, it is because THF have stronger hydrophilic, the system water is easily to solvate, so water content was increased to ensure the surface water film of enzyme are not been absorbed by solvent.

The cyclic oligomer of PBS degradation products is not detected, neither for other degradation products of polyester contained cyclic oligomer, which shows that water content is not the only factor related to the cyclic oligomer content, but also other factors such as reaction temperature, the molecular chain length, and flexibility. Short molecular chain of polyester is better yielding, which not only provide space for molecular chain rotation, but also results in a higher contact chance between terminated hydroxyl, and ensure the reacting activity of molecular chain in the terminated group. With the increase of water content, hydrogen bonds were formed through the combination of hydrolysis product of terminated hydroxyl and water, activity of terminated hydroxyl decreases, but its activity increases when the temperature increases to 85°C and hydrogen bonds are destroyed, which increases the probability of molecular lactonization, therefore cyclic oligomer can be formed at 85°C.

In this study, there is no cyclic oligomer detected in degradation products of PBS at 60°C, which indicates the water content is the leading factor among the multiple impact factors. However, the degradation products of P(BS-co-CHDM) and P(BS-co-CL-co-CHDM) contains the cyclic BS, which is because the

TABLE III
Structure and M_n of Raw Material Monomer

Monomer	Structure	Symbol	M_n
Butanediol	OHCH ₂ (CH ₂) ₂ CH ₂ OH	BDO	90
Succinic acid	HOOC(CH ₂) ₂ COOH	SA	118
Poly(butylene succinate)	HOOC CH ₂ CH ₂ COO(CH ₂) ₄ OH	BS	190
1,4-Cyclohexynol	HOCH ₂ -  -CH ₂ OH	1,4-CHDM	144

TABLE IV
The Results of M_s Analysis About Copolymer Degradation on Products (1) PBS, (2) P(BS-co-CL), (3) P(BS-co-CHDM), and (4) P(BS-co-CL-co-CHDM)

No.	m/z	Structure	Symbol
1	290	HOOC CH ₂ CH ₂ COO(CH ₂) ₄ OOCCH ₂ CH ₂ COOH	BS ₁ SA ₁ ⁺
	362	HO[OC CH ₂ CH ₂ COO(CH ₂) ₄] ₂ OH	BS ₂ ⁺
	518	HO[OC CH ₂ CH ₂ COO(CH ₂) ₄] ₃ OH	BS ₃ ⁺
	714	HO[OC CH ₂ CH ₂ COO(CH ₂) ₄] ₄ OH	BS ₄ ⁺
2	291	HOOC CH ₂ CH ₂ COO(CH ₂) ₄ OOCCH ₂ CH ₂ COOH·H	BS·SA·H ⁺
	342	$\left[\text{CH}_2(\text{CH}_2)_4\text{CO}-\text{OCH}_2(\text{CH}_2)_4\text{CO} \right]_2$	Cyclic CL ₃ ⁺
	400	$\left[\text{O}(\text{CH}_2)_4\text{OOC}(\text{CH}_2)_2\text{CO}-\text{OCH}_2(\text{CH}_2)_4\text{CO} \right]_2$	Cyclic BS·CL ₂ ⁺
	590	HO[O(CH ₂) ₄ OOC(CH ₂) ₂ CO-OCH ₂ (CH ₂) ₄ CO] ₂ OH	BS ₂ ·CL ₂ ⁺
3	90	OHCH ₂ (CH ₂) ₂ CH ₂ OH	BD ⁺
	300	HOO(CH ₂) ₄ OOC(CH ₂) ₂ CO-OCH ₂ -  -CH ₂ OH	BS ₁ CHDM ₁ ⁺
	484	$\left[\text{O}(\text{CH}_2)_4\text{OOC}(\text{CH}_2)_2\text{CO} \right]_3$	Cyclic BS ₃ ⁺
4	190	HO[O(CH ₂) ₄ OOC(CH ₂) ₂ CO] ₂ OH	BS ⁺
	286	HO[O(CH ₂) ₄ OOC(CH ₂) ₂ CO-OCH ₂ (CH ₂) ₄ CO] ₂ OH	BS·CL ⁺
	382	HO-O(CH ₂) ₄ OOC(CH ₂) ₂ CO-OCH ₂ (CH ₂) ₄ CO] ₂ OH	BS·CL ₂ ⁺
	688	$\left[\text{O}(\text{CH}_2)_4\text{OOC}(\text{CH}_2)_2\text{CO} \right]_4$	Cyclic BS ₄ ⁺

big six-membered ring of CHDM has good hydrophobic, when the polyester hydrolysis started from the adjacent ester of CHDM, under the protection of hydrophobic six-membered ring, terminated

hydroxyl can be formed to cyclic oligomer. For cyclic trimer (CL₃) that exist in P(BS-co-CL), caprolactone molecule are yielded, this is due to the facile rotation of long and plasticity of carbon chain segment,

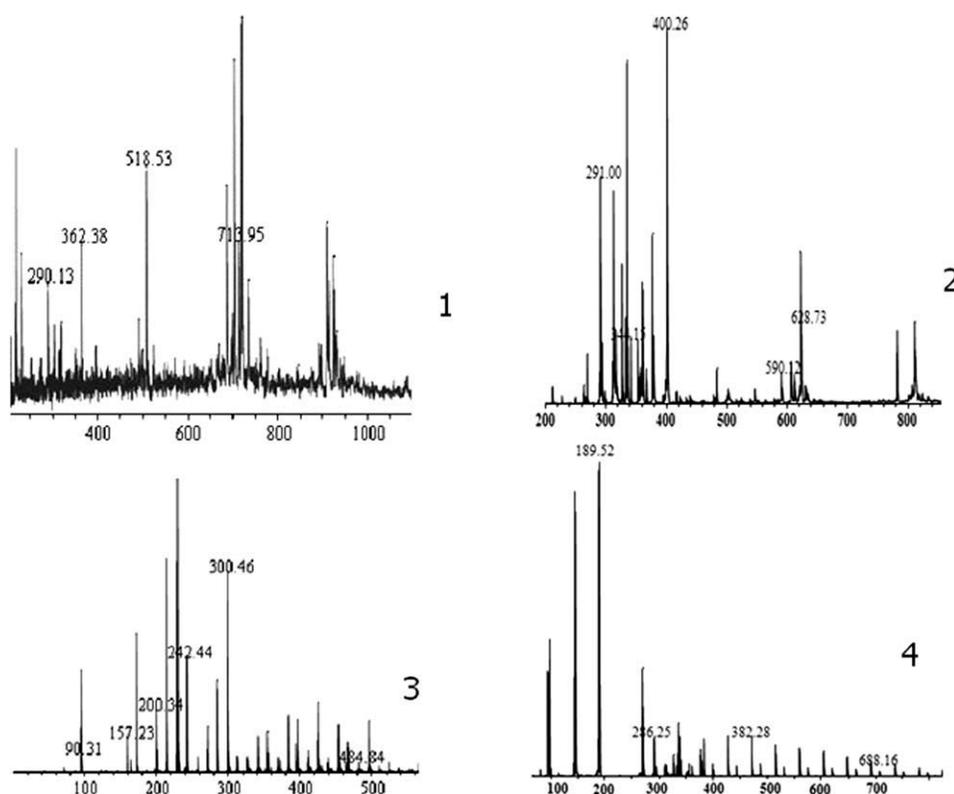


Figure 2 MS spectra of biodegradation product of PBS and copolymer catalyzed by lipase (1) PBS, (2) P(BS-co-CL), (3) P(BS-co-CHDM), and (4) P(BS-co-CL-co-CHDM).

TABLE V
NMR Structural Analysis of P(BS-co-CL)
Degradation Products

	ppm	Structure fraction
a	1.28	HO-CO-(CH ₂) ₂ -CH ₂ -CH ₂ -C-O...
b	1.60–1.68	OHCH ₂ (CH ₂) ₂ CH ₂ O...
c	2.68	...O-OCCH ₂ CH ₂ CO-O...
d	4.13	...OCH ₂ (CH ₂) ₂ CH ₂ O...

resulting in easy contact between the terminated hydroxyl and formation of rings, thus the degradation product of cyclic BS are obtained.

NMR analysis

Positions of NMR peaks are related to the ester bond (Table V, Fig. 3), and displacement of hydrogen peak on the chain segment corresponds to butanediol, succinic acid, and BS molecular chain, which proved that degradation products are polyester with low molecular results; the degradation reaction of polymers mainly are ester key fracture.

FT-IR spectra of polyester degradation products

Figure 4 showed that the ester bond of PBS has an absorption peak in the 1850–1650 cm⁻¹, but absorption peaks of PBS and modified copolymer degradation products were narrow, which indicates that their carbonyl content decreased and a large number of ester bonds of PBS and modified copolymer were hydrolyzed and destroyed.

The peak at 2925cm⁻¹ referred to C-H in the methyl and methylene, the peak of C-H of degradation products was sharper than PBS; this is 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.

The absorption peak in the 3400~3200 cm⁻¹ could be observed, because the content of low polyesters of the degradation products was increased, thus increasing the concentration of hydroxyl, arousing

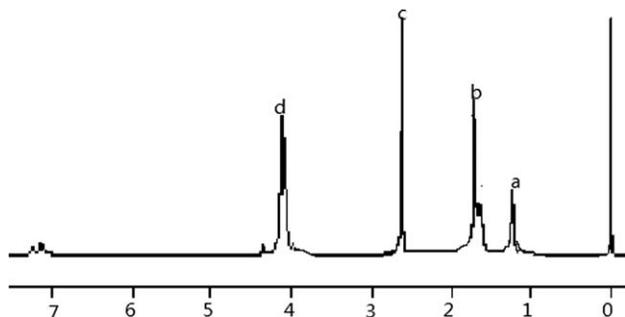


Figure 3 NMR spectra of degradation product of PBS-co-PCL.

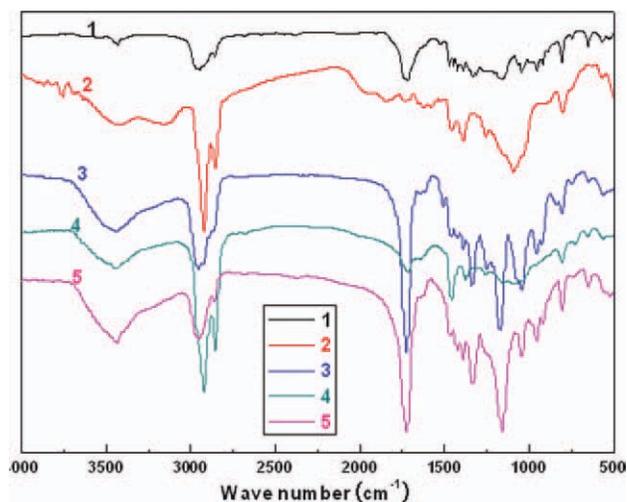


Figure 4 FT-IR spectra of PBS and its copolymer after degradation. (1) Raw material PBS, (2) degradation products of PBS, (3) degradation products of P(BS-co-CL), (4) degradation products of P(BS-co-CHDM), and (5) degradation products of P(BS-co-CL-co-CHDM). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the association of carbonyl compounds, also the stretching vibration absorption peak of O-H shifted to lower wave numbers, all these led to a wide and strong absorption peak.

CONCLUSION

Lipases have catalytic activity on various polyesters. PBS and its modified copolymer are aliphatic polyester, and their degradation products all include cyclic oligomer and linear oligomer, but only the degradation products of P(BS-co-CHDM) contained BDO monomer, and the other PBS copolymer only has trimer or tetramer. Degradation yield of all kinds of polyester is slightly different, the degradation yield of PBS is 40% and P(BS-co-CL-co-CHDM) is 54%.

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