Biodegradation of Poly(Butylene Succinate) in Compost

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ABSTRACT: The biodegradation of poly(butylene succinate) (PBS) was studied under controlled composting conditions. The ultimate biodegradation percentage revealed that the powder-formed sample showing the best biodegradability may be ascribed to the largest specific surface. The biodegradation process of PBS under controlled composting conditions exhibited three phases. The biodegradation in the first phase was slow, got accelerated in the second phase, and showed a leveling-off in the third phase. The degradation of PBS film after composting was further characterized by gel permeation chromatography (GPC) and scanning electron microscopy (SEM). Four strains were isolated from the compost and identified as *Aspergillus versicolor*, *Penicillium*, *Bacillus*, and *Thermopolyspora*. Their degrading abilities to PBS powder in liquid medium were different. Among them, *Aspergillus versicolor* was the best PBS-degrading microorganism. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 97: 2273–2278, 2005

Key words: biodegradation; composting; microorganism; poly(butylene succinate); screening

INTRODUCTION

The large use of synthetic nondegradable polymers has led to serious environmental pollution, and one solution to the problem is to use biodegradable polymers.[1] Aliphatic polyesters are one type of promising biodegradable polymers because of their complete degradation by microorganisms.[2,3] Three representative commercially produced aliphatic polyesters, poly(lactic acid) (PLA), poly(β-hydroxybutyrate) (PHB), and poly(ε-caprolactone) (PCL), have been extensively investigated with respect to their biodegradability.[4–6] However, their commodity application is limited because of the high production cost or poor mechanical properties. Poly(butylene succinate) (PBS) is another aliphatic polyester synthesized by condensation polymerization, which has a high melting point of 115°C, low production cost, and good mechanical properties similar to polypropylene.[7] Furthermore, conventional processing used for polyolefins can also be applied to PBS.[8] Therefore, PBS is expected to be a promising alternative material to ordinary plastics.

In recent years, research on the biodegradation of aliphatic polyesters has attracted much interest. Some work has been done on the microbial degradation of PBS by a single strain in a liquid medium.[9,10] However, these studies cannot reflect the biodegradation of PBS in the real natural environment. As an environmentally friendly material, it is necessary to study the degradation behavior of PBS by microorganisms in the natural environment. Compost is a complex biological environment, which has a high microbial diversity and shows a potential degradation capacity for polymer materials. There is an increasingly acceptable opinion that composting is one of the most promising technologies for the management of plastic wastes.[11] The biodegradation of PLA, PCL, and PHB has been extensively investigated in compost.[12–14] However, the biodegradation of PBS in compost and the effect of sample form on the degradation are not clear; knowledge of this is helpful to recycle PBS in the management of environmental pollution. On the other hand, the isolation and screening of the PBS-degrading microorganisms from the compost is good for further understanding the biodegradation of PBS.

In this research, the biodegradation of PBS under controlled composting conditions was studied. The effect of sample form on the biodegradation was considered. Changes of molecular weight and surface morphology of PBS film after composting were further characterized. In addition, the isolation and screening of PBS-degrading microorganisms from the compost were also discussed.

METHODS

Materials

PBS was synthesized by polycondensation of 1,4-butanediol and succinic acid.[15] The molecular structure

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of PBS is shown in Figure 1. Three forms of PBS, namely, granule, powder, and film, were used in this study. PBS granule with an average particle size of 3mm was obtained by an extruder and a pelletizer. PBS powder with an average particle size of 42μm was prepared in the sequence of being dissolved in chloroform, precipitated in methanol, ground in a mortar, and sieved through a screen. PBS film with an average thickness of 40μm was prepared by the heat-processing method at 150Mpa and 150°C, and then was cut into pieces of about 1cm × 1cm. The physical properties of the three PBS samples are listed in Table I.

TABLE I
Physical Properties of PBS Samples with Different Forms

PBS sample	A[a] ($cm^2 \cdot g^{-1}$)	$M_w \times 10^{-4}$ ($g \cdot mol^{-1}$)	T_m (°C)	ΔH_m ($J \cdot g^{-1}$)	X_c[b] (%)
Powder	1134	8.3	115.1	67.0	60.7
Film	400	8.0	114.8	66.1	59.9
Granule	16	8.1	115.0	66.9	60.6

[a] The calculation of the specific surface area (A), PBS powder and granule: $A = 4\pi r^2/(4/3\pi r^3\, p)$; PBS film: $A = 2(l_1 l_2 + l_1 h + l_2 h)/(l_1 l_2 hp)$, where l_1, l_2, and h were the length, width, and height; $p = 1.26 g/cm^3$.

[b] Calculated by dividing the observed heat of fusion by the theoretical value, using $110.3 J \cdot g^{-1}$ as the melting enthalpy of 100% crystalline PBS.[17]

Biodegradation test

The compost, derived from a properly operating aerobic composting of municipal solid waste, supplied by Nangong Compost Factory (Beijing, China), was used as the inoculum. Three PBS samples were mixed with the compost on a 1 : 6 dry weight basis, respectively. Composting was performed according to ISO 14,855[16] at 58 ± 2°C. Three replicates of each sample and a background control (compost without sample) were aerated throughout the experiment. CO_2 in the exhaust air was trapped with barium hydroxide solution and measured by titration with hydrochloric acid.

The biodegradation percentage (D_t) was calculated as

$$D_t = \frac{(CO_2)_t - (CO_2)_b}{ThCO_2} \times 100$$

where $(CO_2)_t$(g) is the accumulated amount of carbon dioxide released by each composting vessel containing test material, $(CO_2)_b$(g) is the accumulated amount of carbon dioxide released by the control, and $ThCO_2$(g) is the theoretical amount of carbon dioxide of the test material in the test vessel.

Measurements of PBS film

The molecular weights of PBS film before and after composting were determined by gel permeation chromatography (GPC). The instrument was equipped with a Waters 1515 pump, a Waters 2414 refractive index detector, and two Shodex K-805L gel linear columns (filling styrene/divinylbenzen copolymer gels, in-column solvent chloroform, exclusion limit 4×10^6, particle size 10μm, pore size 50nm, column size 8mmID × 300mmL) connected in series. Chloroform was used as the mobile phase. The flow rate was 1.0 mL/min. Polystyrene standards from Shodex were used for calibration.

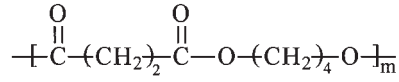


Figure 1 The molecular structure of PBS.

The surface structures of PBS film before and after composting were observed with a HITACHI S-4300 scanning electron microscope using an acceleration voltage of 15kV. The samples were gold-palladium sputtered for 50s.

Isolation of compost microorganisms

The compost samples were diluted $1/10^3$ with sterilized water. A 0.2 mL aliquot was spread on plates with agar/Luria-Bertani (LB) medium, potato dextrose agar (PDA) medium, and Ganoderma complete medium (GCM), respectively. Agar/LB medium was composed of 1.0% (wt/vol) peptone, 0.5% yeast extract, 1.0% NaCl, and 15% agar (pH 7.0). PDA medium was composed of 3.0% potato juice, 2.0% glucose, and 15% agar (pH 7.0). GCM medium was composed of 1.0% soluble starch, 0.2% yeast extract, and 15% agar (pH 7.0). The plates were incubated at 30°C. Five days later, various microorganisms were picked out and purified with corresponding culture medium until a single strain colony in a plate was obtained.

Screening of PBS-degrading microorganisms

The isolates above were diluted with sterilized water and inoculated, respectively, into the basal medium where PBS powder was the sole carbon source. The inoculum volume was calculated to obtain a concentration of 10^7 microorganisms per milliliter medium. The cultures were incubated at 30°C for 25 days. The growth status of various microorganisms was observed, and the best PBS-degrading strain was screened.

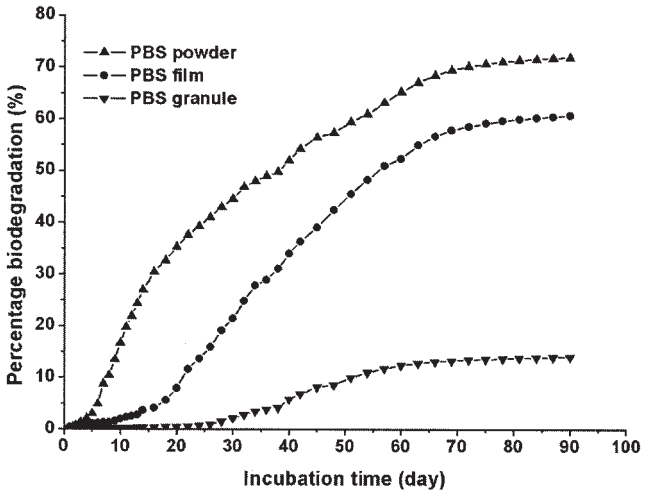


Figure 2 Biodegradation of PBS samples with different forms under controlled composting conditions.

RESULTS AND DISCUSSION

Biodegradation in compost

The biodegradation curves of PBS samples with different forms are shown in Figure 2. After incubation for 90 days, the biodegradation percentage is 71.9%, 60.7%, and 14.1% for powder, film, and granule form sample, respectively (see Table II). This result reveals that PBS with the form of powder shows the best biodegradability under controlled composting conditions. However, the change of sample form had little effect on the molecular weight, melting temperature, and crystallization degree except for the specific surface area (see Table I). It seemed that the fastest degradation of PBS powder was attributed to its largest specific surface area.

Three phases were observed in the biodegradation curves of all PBS samples. On the base of the degradation process, the three phases may be defined as lag phase (0 ~ 5 days), biodegradation phase (6 ~ 66 days), and plateau phase (67 ~ 90 days), as shown in Table II.

TABLE II
Ultimate Biodegradation Percentage and Three Degradation Phases of PBS Samples under Controlled Composting Conditions

PBS sample	D_{90}[a] (%)	Degradation rate (%/day)		
		Lag phase (0 ~ 5d)	Biodegradation phase (6 ~ 66d)	Plateau phase (67 ~ 90d)
Powder	71.9	0.60	1.07	0.14
Film	60.7	0.22	0.91	0.17
Granule	14.1	0.03	0.21	0.04

[a] D_{90} was the biodegradation percentage of the PBS sample for 90 days under controlled composting conditions.

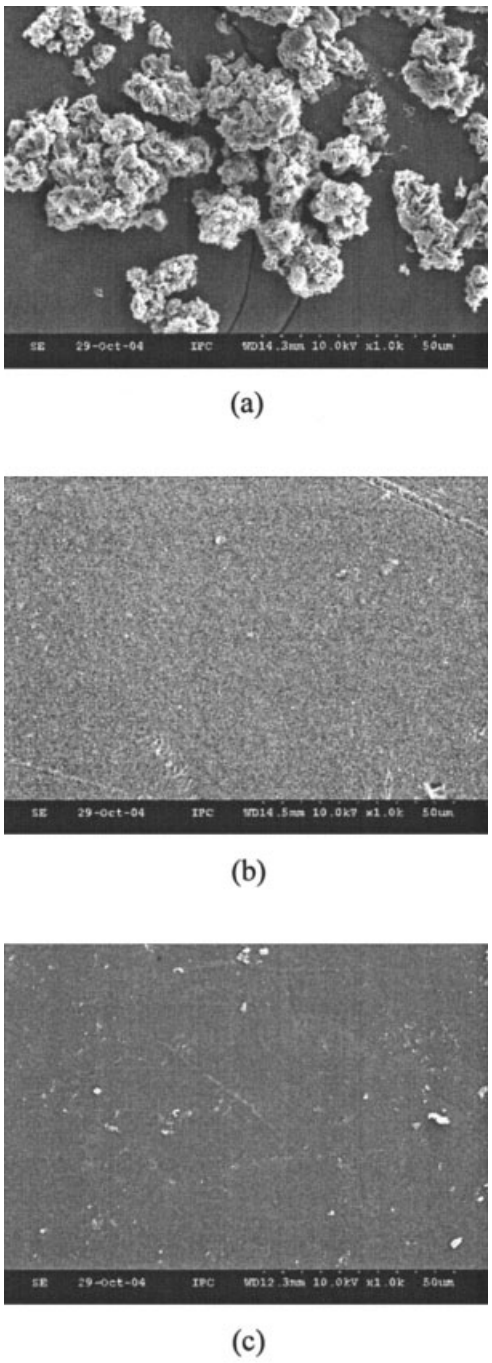


Figure 3 SEM micrographs of the surfaces of three PBS samples with different forms (×1000): (a) powder, (b) film, and (c) granule.

In the lag phase, the degradation of PBS was slow. However, the powder-formed sample degraded much faster than both the film-formed and granule-formed samples, maybe because of their different surface. PBS powder from dissolution and precipitation showed a rough and large surface, which favored the adherence of microorganisms (see Fig. 3a). As a comparison, a smooth surface was obtained from the film or granule

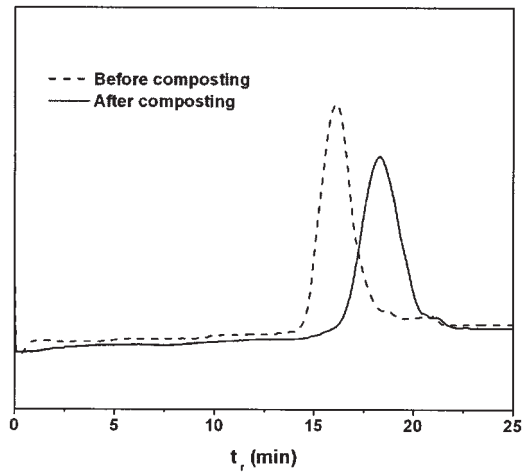


Figure 4 Gel permeation chromatography curves of PBS film before composting and after composting for 90 days.

form sample prepared by heat-processing, and microbial attack was difficult (see Figs. 3 b and c). Compared with the PBS granule, PBS film exhibited a faster degradation, maybe because of its larger specific surface. It is supposed that the biodegradation of PBS began on the sample surface. According to Pitt,[18] a small amount of random hydrolytic cleavage of ester linkages takes place within the polymer bulk in this phase, and this cleavage is nonenzymatic. But most of the fragments formed in this stage were large and remained relatively immune to microbial attack.

Following the lag phase, there was a rapid increase in the biodegradation rate. It is possible that the molecular weight of the polymer decreases to a point where the scission produces fragments small enough to diffuse from the polymer bulk and be attacked by microorganisms. The powder or film sample showed a much faster degradation than the granule sample, because of their much larger specific surface areas.

When entering the plateau phase, the biodegradation leveled off. This indicates the ultimate biodegradation percentage of PBS in the test. Under such conditions, the ultimate biodegradation percentage of the polymer cannot reach 100%, because a small portion of polymer will be incorporated into the microbial biomass, humus, and other natural products.[19]

GPC analysis

Figure 4 shows the GPC curves of PBS film before and after composting. Larger elution time represented lower molecular weight. The peak obviously shifted to the low molecular weight side after composting. A broadening of the peak was also observed. The molecular weight and polydispersity of PBS film before and after composting are shown in Table III.

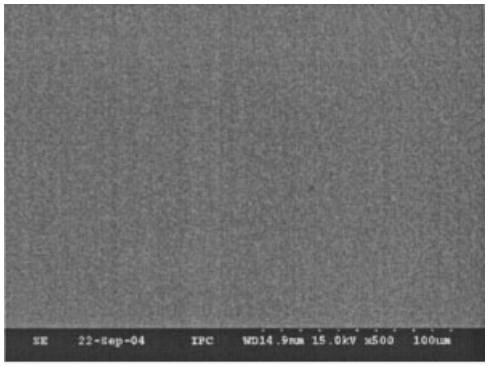
TABLE III
Average Molecular Weight and Polydispersity of PBS Film Before and After Composting

Composting time (day)	$M_n \times 10^{-4}$ ($g \cdot mol^{-1}$)	$M_w \times 10^{-4}$ ($g \cdot mol^{-1}$)	M_w/M_n
0	4.7	8.0	1.8
90	0.6	1.4	2.4

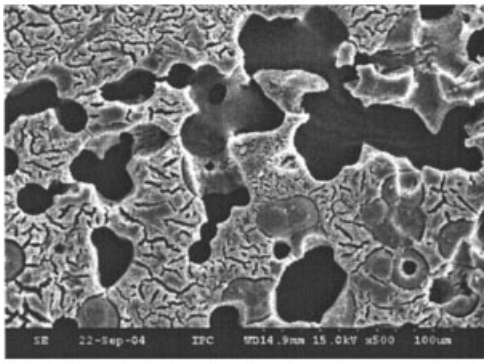
The remarkable decrease in molecular weight and the obvious increase of polydispersity reflected that the long polymeric chain has been cut short and produced small molecular fragments through hydrolysis mediated by microorganisms during composting.

SEM observations

Figure 5 shows the scanning electron micrographs of the surfaces of PBS film. Before composting, the sur-



(a)



(b)

Figure 5 SEM micrographs showing the surface changes of PBS film (×500): (a) before composting, and (b) after composting for 90 days.

face of PBS film was smooth. However, the surface of the sample subjected to microorganisms was significantly eroded, and a lot of cracks and holes in various sizes were formed after composting. This observation indicated that the majority of the polymer film had been degraded by microorganisms after composting. In addition, some slight-eroded regions round in form may be the foregoing stage of the spherical holes as shown in the picture.

Isolation and screening of PBS-degrading microorganisms

The biodegradation of PBS under controlled composting conditions is the result of the synergic effect of the microorganisms in the compost. Screening of the best PBS-degrading strain among them is helpful for further understanding the microbial degradation of PBS. Four main strains, namely, S1, S2, S3, and S4, isolated from the compost, were identified as *Aspergillus versicolor*, *Penicillium*, *Bacillus*, and *Thermopolyspora*[20−22] according to their physiological properties, respectively, as shown in Figure 6. To evaluate the abilities of the strains to decompose PBS, liquid cultures were grown with PBS powder as the substrate within a broad substrate concentration range (Table IV). Strain S1 grew fast and most of the substrate was assimilated except for growing in high substrate concentration. Moderate growth of strains S2 and S3 was observed in low substrate concentration. With the increase of the substrate concentration, the growth was restrained. It is possible that more acid degradation products were produced in high substrate concentration and prevented the strain from growing. By comparison, strain S4 showed the lowest growth rate, and it could hardly assimilate PBS. These results revealed that strain S1, namely, *Aspergillus versicolor*, was the best PBS-degrading strain in the compost.

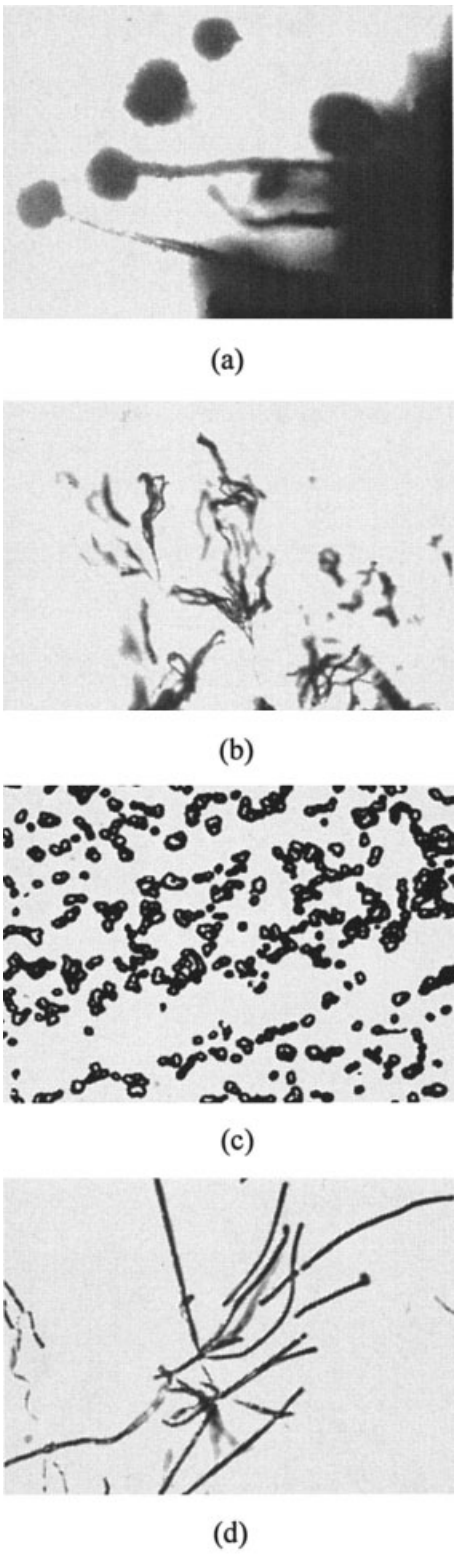


Figure 6 Optical microscopic photographs of four strains isolated from compost (×800): (a) strain S1, (b) strain S2, (c) strain S3, and (d) strain S4.

CONCLUSIONS

PBS with the form of powder showed the best biodegradability under controlled composting conditions. The biodegradation rate was influenced by the sample form. The powder-formed sample showed the fastest degradation, because of its largest specific surface area, then followed by film-formed and granule-formed PBS in sequence. The biodegradation process of PBS exhibited three phases with different degradation speed. The biodegradation in the first phase was slow, got accelerated in the second phase, and leveled off in the third phase. After composting, the molecular weight of PBS film decreased distinctly and the polymer surface was badly eroded. Four strains isolated from the compost were identified as *Aspergillus versicolor*, *Penicillium*, *Bacillus*, and *Thermopolyspora*, which showed different decomposing abilities to PBS. Among them, *Aspergillus versicolor* was the best PBS-degrading microorganism.

TABLE IV
Isolates and Their Abilities to Assimilate PBS Powder

Isolate	Organism	C_{PBS} (wt/vol) 0.1%	0.2%	0.3%	0.4%	0.5%	0.6%
S1	*Aspergillus versicolor*	+++	+++	+++	+++	++	+
S2	*Penicillium*	++	++	+	−	−	−
S3	*Bacillus*	++	++	++	+	−	−
S4	*Thermopolyspora*	+	−	−	−	−	−

All the strains were cultivated in 100ml basic medium containing PBS powder as the sole carbon source on rotary shakers (130rpm) at 30°C for 25 days. "+++" denoted the strain grew fast and most of PBS was assimilated; "++" denoted the strain grew moderately and some of PBS was assimilated; "+" denoted the strain grew slowly and a small amount of PBS was assimilated; "−" denoted the strain didn't grow and no PBS was assimilated.

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