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Crystallization behavior and environmental biodegradability of the blend films of poly(3-hydroxybutyric acid) with chitin and chitosan

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Abstract

Crystallization behavior and environmental biodegradability were investigated for the films of bacterial poly(3-hydroxybutyric acid) (PHB) blends with chitin and chitosan. The blend films showed X-ray diffractive peaks that arose from the PHB crystalline component. It was suggested that the lamellar thickness of the PHB crystalline component in the blends was large enough to show detectable X-ray diffractive peaks, but this was too small to show observable melting endotherm in the DSC thermogram and the crystalline band absorption in the FT-IR spectrum. In the PHB/chitin and PHB/chitosan blends, thermal transition temperatures of PHB amorphous region observed by dynamic mechanical thermal analysis were almost the same as that of neat PHB. Both the PHB/chitin and the PHB/chitosan blend films biodegraded in an environmental medium. Several blend films showed faster biodegradation than the pure-state component polymers. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Poly(3-hydroxybutyric acid); Crystallization behavior; Environmental biodegradability

1. Introduction

Microbial poly(3-hydroxybutyric acid) [PHB] is a natural biodegradable plastic with biocompatibility and optical activity (Holmes, 1985; Howells, 1982; Inoue & Yoshie, 1992; King, 1982). PHB has some problems of application to the medical area, such as polymer matrix of drug delivery system because PHB is brittle and it has a poor site for chemical modification.

An approach to overcome these circumstances is to make miscible blends of PHB with another kind of polymer and/or plasticizer. PHB has been reported to be miscible in melt with a few chemically synthesized polymers, such as poly(ethylene oxide) (PEO) (Avella & Martuscelli, 1988), poly(vinyl acetate) (Greco & Martuscelli, 1989), poly(vinylidene fluoride) (Marand & Collins, 1990), poly(vinyl phenol) (Iriondo, Iruin & Fernandez-Berridi, 1996) and highly saponified poly(vinyl alcohol) (PVA) (Azuma, Yoshie, Sakurai, Inoue & Chujo, 1992; Ikejima, Yoshie & Inoue, 1996, 1998; Yoshie, Azuma, Sakurai & Inoue, 1995). Since the PHB/PEO and the PHB/PVA blend systems are composed of fully biodegradable polymer components, these blend systems are biodegradable. The extent and rate of biodegradation of the polymer blend are mainly determined not only by intrinsic degradability of the blend components themselves, but also by the blend composition, phase structure (miscibility, crystallinity of the components) and blend composition of the surface region of bulk materials. Thus, a blending strategy is an important technique for a biodegradable polymer such as PHB, not only to improve the inferior properties of components, but also to control the profile of biodegradation. Immiscible binary blend systems containing PHB are also important to control the profile of biodegradation. Kumagai and Doi (1992) have reported the thermal properties and enzymatic degradation of PHB/poly(propiolactone), PHB/poly(ethylene adipate) and PHB/poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid) blends. These blend systems, which were immiscible systems as judged by the observation of independent glass transition temperatures (T_g), degraded faster than any of the neat component polymer. They concluded that the acceleration occurred due to the phase-separated structures of blends.

The authors have employed chitin and chitosan as new components for fully biodegradable PHB-based polymer blend systems. Chitin is a natural polysaccharide which exists in considerable amounts as the exoskeleton of arthopods and fungi (Muzzarelli, 1977). The chitin which has a high degree of deacetylation is called as chitosan. In recent decades, chitin and chitosan have taken much attention for

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their biodegradability and biocompatibility in vivo (Prudden & Nishihara, 1957). Chitosan shows mild antimicrobial activity that arises from its cationic residue, which is an important property for it to be used as biomedical materials. Since chitin and chitosan have chemically modifiable functional groups, such as hydroxyls, amines and amides, PHB can acquire the ability of chemical modification by blending with chitin or chitosan.

In our previous paper (Ikejima, Yagi & Inoue, 1999), PHB/chitin and PHB/chitosan blend films were prepared and their thermal properties and crystallization behavior were characterized, in order to develop novel functional PHB-based polymer composites. The decrease in the degree of crystallinity of PHB in the blends was found by using differential scanning calorimetry (DSC) and Fourier-transformed infrared spectroscopy (FT-IR). In the PHB blends with chitosan, which was much deacetylated compared with chitin, significant suppression of PHB crystallization was observed in the wider range of blend composition than that in the PHB/chitin blends. The suppression of PHB crystallization found in the blends with chitin and chitosan might be caused by the lowering of mobility of the PHB amorphous chain due to at least two factors. One is the formation of the intermolecular hydrogen bond between the PHB carbonyls and the chitin/chitosan hydroxyls and/or amine. Another is rigid environment that arises from inflexible polysaccaride molecules. These factors were explained on the basis of information obtained from solid-state ^{13}C-NMR.

In this paper, crystallization behavior of the PHB/chitin and PHB/chitosan blends will be further investigated by using wide angle X-ray diffractometry (WAXD). Since chitin and chitosan do not show any DSC melting peaks, WAXD is expected to give information on the crystallization status of chitin and chitosan. Environmental biodegradation profiles of the PHB blend films with chitin and chitosan will be also investigated using biochemical oxygen demand (BOD) method, in relation to the mixing state.

2. Experimental

2.1. Sample preparation

The PHB sample was purchased from Aldrich Chemical (USA, LOT. 06707KN, $M_w = 380\,000$). It was purified by precipitation in *n*-hexane from chloroform solution, subsequently precipitated in methanol from chloroform solution. The samples of α-chitin and chitosan, supplied by Unitika Ltd (Kyoto, Japan), were used without further purification. The degrees of deacetylation of α-chitin and chitosan samples were, respectively, 2 and 70% determined by colloidal titration and 13 and 61% by FT-IR spectroscopy (Domszy & Roberts, 1975).

1,1,1,3,3,3-hexafluoro-2-propanol [HFIP] was supplied by the courtesy of The Central Glass Corp. (Tokyo, Japan). HFIP was used in the draft chamber.

2.2. Preparation of blend films

All film samples were prepared by solution-cast technique using HFIP as the common solvent. PHB, chitin and chitosan were dissolved separately in HFIP before blending, to prevent the chitin and chitosan samples from forming gel and from thermal degradation by heating. The polymer concentration of each solution was 10 g l^{-1}. After the chitin and chitosan solutions were well homogenized, they were mixed with the PHB solution. The mixed solution was dipped on a poly(tetrafluoroethylene) dish, then left at room temperature for 10 days and subsequently dried at 60°C for further 5 days under vacuum to eliminate the solvent completely. The blend composition was denoted as wt.% of PHB through this article.

2.3. Procedure of analysis

2.3.1. Wide angle X-ray diffraction

Wide angle X-ray diffraction (WAXD) patterns were recorded on a Rigaku RU-200 diffractometer with a Rigaku IP R-AXIS-DS3 data processing system. The nickel-filtered CU-Kα X-ray beam (wavelength = 0.15418 nm) with a pinhole graphite monochromater was used as the source. The diffraction intensities were measured in a 2θ range of 5–40° at a scanning speed 1° min^{-1}. The power of the source was tuned to 50 kV/180 mA for the intensity analysis. The photographic diffraction patterns of the films were measured at the radiation time of 30 min. The power of the CU-Kα X-ray source was tuned to 20 kV/35 mA for the photographic analysis.

2.3.2. Viscoelastic measurement

Viscoelastic properties were observed using dynamic mechanical thermal analysis (DMTA) system SEIKO DMS210 with SSC5300 controller at frequencies of 0.1 and 5 Hz. The polymer sample was heated from −50 to 200°C at the rate of 1°C min^{-1}.

2.3.3. Biodegradation in an environmental water

The BOD values were determined using a TAITEC 200-F BOD tester from the amount of oxygen consumption, based on the method reported by Abe and Doi (1996). The water sample of the River Tama (Kawasaki City, Kanagawa, Japan) was employed as an environmental medium. The river water was filtered using 17 G glass filter and saturated by oxygen before BOD test. The BOD medium contained 200 ml of the oxygen-saturated river water, 0.2 ml of mineral salts stock solution (KH_2PO_4 8.50 g l^{-1}, K_2HPO_4 1.75 g l^{-1}, $Na_2HPO_4{\cdot}H_2O$ 33.30 g l^{-1}, NH_4Cl 1.70 g l^{-1}, $MgSO_4{\cdot}7H_2O$ 8.550 g l^{-1}, $CaCl_2$ 27.50 g l^{-1}, $FeCl_3{\cdot}6H_2O$ 0.25 g l^{-1}). Each medium was kept at 25°C throughout the test. The 7×7 mm^2 blend films (within 10 mg of weight, 100 μm of thickness) were immersed into each medium.

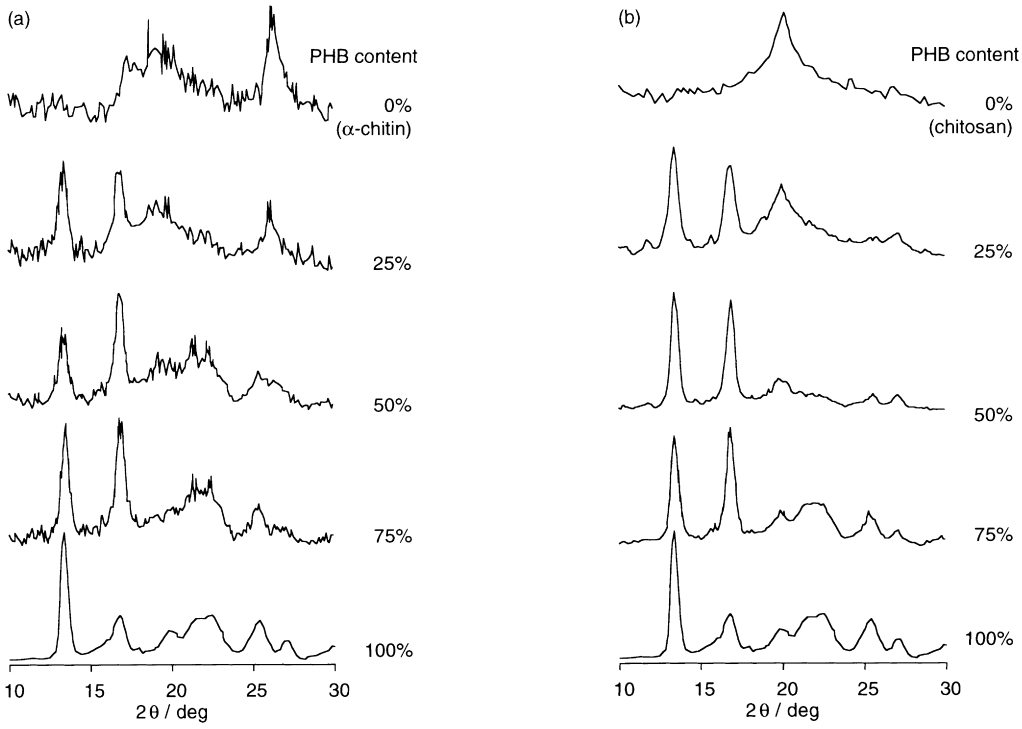

Fig. 1. Wide angle X-ray diffraction of the films of PHB, α-chitin, chitosan, PHB/α-chitin blends and PHB/chitosan blends: (a) PHB/α-chitin; (b) PHB/chitosan.

The BOD biodegradability was defined as follows for polymer samples:

$$\text{biodegradability}\ (\%) = (\text{BOD}_{\text{sample}} - \text{BOD}_{\text{blank}})/\text{ThOD} \times 100$$

Here, $\text{BOD}_{\text{sample}}$ and $\text{BOD}_{\text{blank}}$ are the experimentally observed values of oxygen demand of the sample and the blank medium, respectively. ThOD is the theoretically expected value of oxygen demand of the blend sample, which was obtained by assuming that the film completely degraded into CO_2 and H_2O. Since the degradation of PHB and PVA in the blend film would proceed independently by attacks of different microbes, the value of ThOD of each

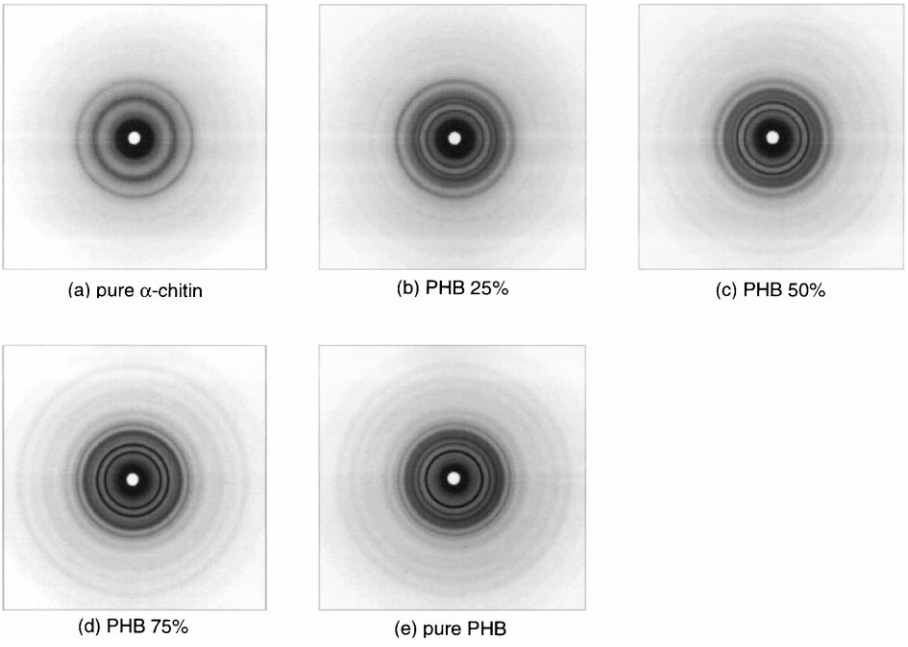

Fig. 2. Wide angle X-ray diffraction patterns of the films of PHB, α-chitin and PHB/α-chitin blends.

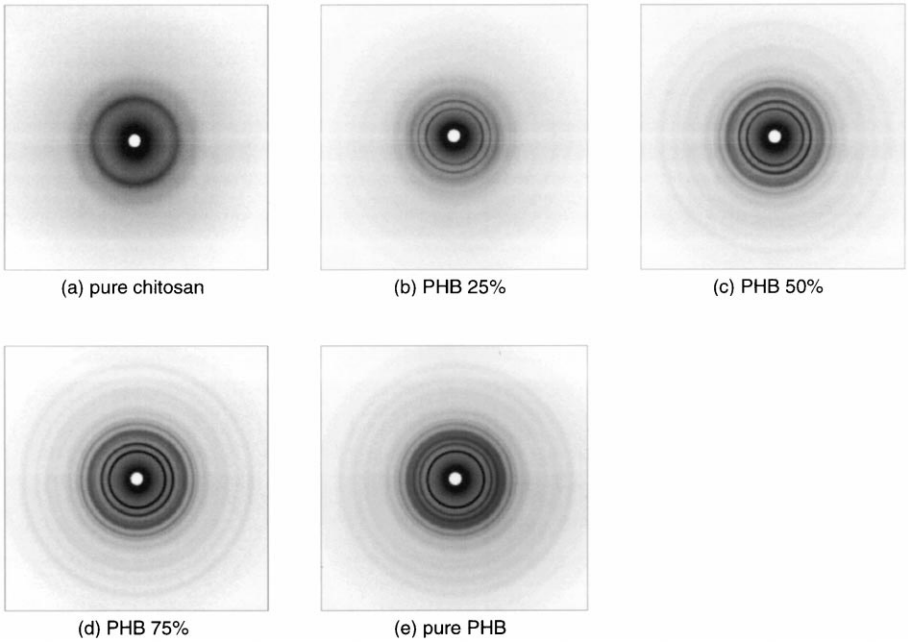

Fig. 3. Wide angle X-ray diffraction patterns of the films of PHB, chitosan and PHB/chitosan blends.

film was assumed as the weight average of the values of oxygen demand of both components as an approximation. The value of biodegradability of each sample was determined as an average of at least two independent experiments. The values of the theoretical oxygen demand of the chitin and chitosan components were calculated with exclusion of the nitrogen.

3. Results and discussion

3.1. Crystallization status of the blend components observed by WAXD

Fig. 1(a) shows the normalized X-ray diffraction intensities of PHB, α-chitin and PHB/α-chitin blend films. The α-chitin films show two well-resolved specific diffractions, i.e. the broader peak centered around 19° and the sharper one around 26°. The normalized WAXD intensities of PHB, chitosan and PHB/chitosan blend films are shown in Fig. 1(b). The chitosan film has a characteristic diffraction centered around 19°. Since all characteristic diffractions of chitin and chitosan overlapped with those of PHB, the crystallization states of chitin and chitosan in the blends are only discussed qualitatively.

In Fig. 1, the films of both chitin and chitosan blend films, and also the films containing only 25% PHB, clearly exhibit the diffractions that arises from the PHB crystalline component, centered around 13 and 17°. In our previous report (Ikejima et al., 1999), for blend samples with the same blend composition, any melting peak (T_m) of PHB was not detected by DSC. From the results obtained from DSC and WAXD experiments, the lamellar thickness of the PHB crystalline component in the both blends is expected to be too thin to be detectable by the DSC melting endotherm, as compared to that in the pure PHB state.

Figs. 2 and 3 show the X-ray diffractions of the PHB/α-chitin and PHB/chitosan blend films. The diffraction rings that arise from the PHB crystalline component were clearly observed for all blends as well as for pure PHB.

One of the probable factors that suppress the T_m of PHB in the blends is the decrease of lamellar thickness of the PHB crystalline. The intermolecular interactions between PHB and the highly rigid chitin and chitosan molecules surrounding the PHB molecules make PHB molecules in the blends inflexible and induce insufficient crystallization as compared to the case of neat PHB. Since the DMTA α-transition of the α-chitin molecules has been reported as 236°C (Kim, Kim & Lee, 1996), which is much higher than the glass transition temperature (T_g) of PHB (around 0°C; Inoue & Yoshie, 1992, the cause of insufficient crystallization of PHB in the blends with chitin and chitosan is supposed to be similar to that of PHB in the PHB/PVA (Azuma et al., 1992; Ikejima et al., 1996; Yoshie et al., 1995) and PHB/cellulose actate butyrate (Pizzoli, Scandola & Ceccorulli, 1994) blend systems. In the PHB/PVA blend system, since the glass transition temperature (T_g) of the conventional PVA sample is around 85°C (Finch, 1992), which is also much higher than the T_g of PHB, the crystallization at room temperature of PHB is supposed to be restricted by the rigid environment constituted by the PVA amorphous component.

3.2. Viscoelastic observation of the blend component

Fig. 4 shows the viscoelastic profiles of the PHB/α-chitin

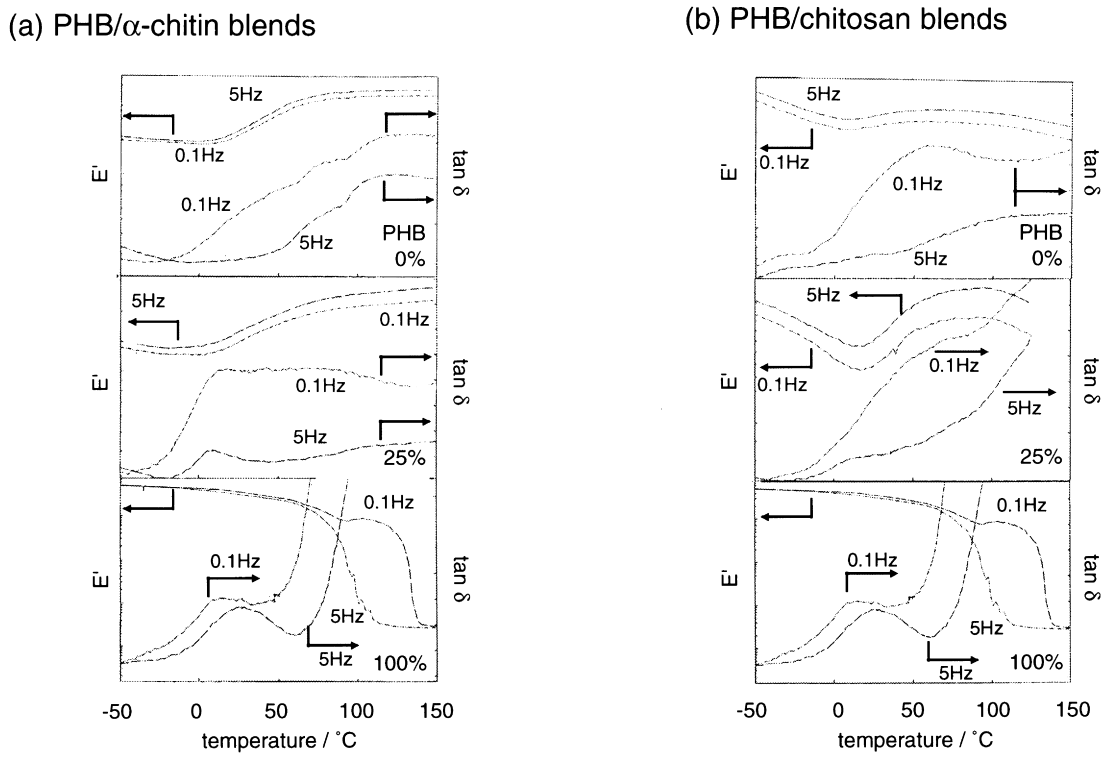

Fig. 4. DMTA viscoelastic profiles of PHB, α-chitin, chitosan and the blends: (a) PHB/α-chitin blends; (b) PHB/chitosan blends.

and PHB/chitosan blend films containing 0, 25 and 50% PHB against the temperature, obtained by DMTA. A conventional method of T_g measurement using DSC cannot be applied to the PHB/chitin and PHB/chitosan blend systems. The DMTA peaks of these blends mean the viscoelastic transition that arises from the amorphous region of the blend components. The pure PHB film exhibits a tan δ transition centered around 15°C, which corresponds to the T_g of the PHB amorphous region. The temperature of tan δ transition detected in the blend was not largely different from that detected in the pure PHB. From this result, the decrease of crystallinity of PHB in the blends is also thought to be caused by the restriction of the chain mobility of the PHB. As mentioned in the earlier section, the molecules of chitin and chitosan are so rigid that their DMTA transition temperatures are very high compared to room temperature. In this work, the DMTA transition temperatures of polysaccharide components

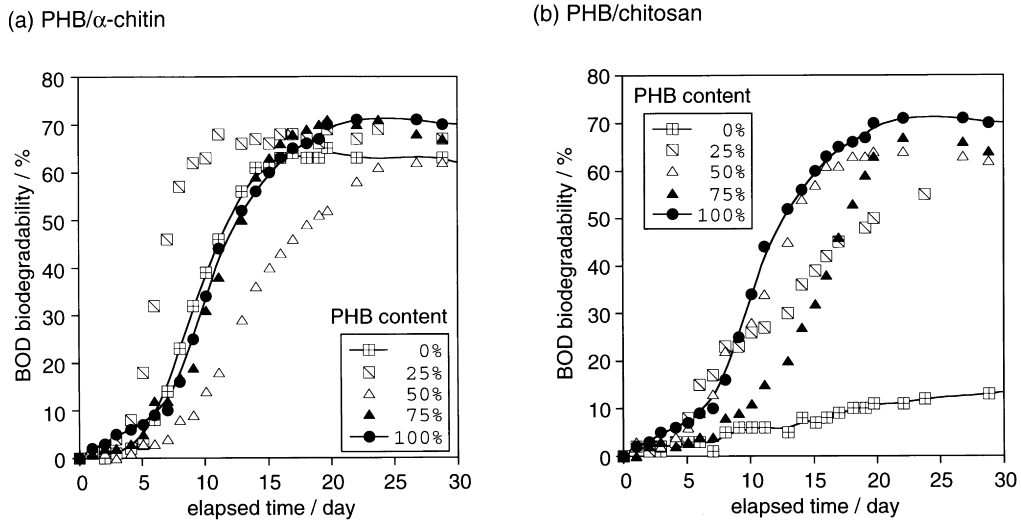

Fig. 5. BOD biodegradation profiles of PHB, α-chitin, chitosan and the blends: (a) PHB/α-chitin blends; (b) PHB/chitosan blends.

Table 1
Average BOD biodegradability of the films of PHB blends with α-chitin and chitosan

PHB content (wt.%)	BOD biodegradability (%)	
	PHB/α-chitin	PHB/chitosan
0	56	15
25	67	49
50	61	60
75	66	63
100	70	70

could not be detected due to the stiffness of the cast films at higher temperature.

3.3. Environmental biodegradation of the blends

Fig. 5(a) and (b) show the BOD biodegradation profiles of PHB and the PHB blends with α-chitin and chitosan, observed in February 1998.

PHB and all blend films showed high biodegradability, over 60%, as shown in Table 1. The PHB/α-chitin blend containing 25% PHB degraded much faster than the pure PHB or pure α-chitin. This acceleration of the biodegradation is supposed to be arisen from the lowered crystallinity of PHB. The crystallinity of PHB strongly affects its degradation rate. It is reasonable that the PHB/α-chitin blend film containing 25% PHB shows accelerated biodegradation, because faster degradation of the PHB component causes an increase in the surface area of the film.

The pure chitosan film showed slow biodegradation compared to the other films. The reason of this is unknown, strictly speaking, but one of the possible reasons is the dependence of the kinds and the activities of the environmental microbes in the season. The biodegradability of the PHB/chitosan system was found to be significantly improved, in spite of the low biodegradability of the pure chitosan.

4. Conclusion

Crystallization behavior and environmental biodegradability of the PHB/chitin and the PHB/chitosan blend films were investigated, in order to obtain the PHB-based composite materials with an improved ability of chemical modification of PHB and controllability of the biodegradation profiles of chitin and chitosan as well as PHB.

The extent of crystallization in the blends of PHB have been studied by using WAXD. Both the blend systems were found to show the X-ray diffraction arising from the PHB crystal lattice; this is even true for the samples, which do not exhibit the melting DSC endotherm and the specific IR absorption arising from the PHB crystalline component, as reported in our previous paper. The results from the WAXD experiments indicated that the lamella of the PHB crystalline component in the blends is too small or thin to be detectable by FT-IR and DSC. The DMTA transition temperatures of the PHB component in the blends were found to be almost the same as that of neat PHB. The biodegradabilities of chitin and chitosan were found to be improved by blending with PHB, especially for chitosan.

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