Introduction

Poly(hydroxyalkanoates), PHAs, constitute a family of biopolymers synthesized by microorganisms under conditions of nutrient limitation and used as a carbon and energy reserve.1,2 Due to their biodegradability and biocompatibility, they have attracted much attention as application polymers and in medical pharmaceutics, mainly as controlled release drug carriers.3 Poly(3-hydroxybutyrate), PHB, is the most prominent of this family of polymers, while poly(3-hydroxybutyrate-co-3-hydroxyvalerate), PHB/V, represents the first attempt to prepare a PHA with improved physical properties compared to PHB. Careful control of the fermentation conditions and the carbon source used has led to the bacterial synthesis of almost 100 different PHA homopolymers and co-polymers, exhibiting diverse physical properties.4 Laboratory synthesis5 and biotechnologically modified agricultural pathways6 to large scale PHA production are also intensively explored.

The most exciting feature of PHAs is their ability to be degraded under a wide variety of environments, under anaerobic or aerobic conditions, in activated sludge, public wastefields, etc. Their degradation is due to microorganisms that secrete extracellular depolymerases to degrade environmental PHB and use it as a nutrient. Several of these PHB depolymerases have been isolated, purified and characterized in recent years.7,8 Pseudomonas lemoignei, a soil bacterium, was found to possess six different PHB depolymerases.7,9 However so far, most of the degradation studies on the PHB-P. lemoignei system have used either depolymerase A or depolymerase B. It was reported that the two enzymes exhibit similar behavior toward PHB degradation to one another, as also to depolymerases from different microorganisms,10,11 including Alcaligenes faecalis.

It is usually assumed that the various PHB depolymerases attack preferentially the amorphous regions of the semicrystalline PHB, citing as evidence the well known inverse dependence of degradation rate on PHA crystallinity.12 This preference has also been associated with the characteristic texture observed in optical and scanning electron micrographs of the film surface of PHB and various PHAs degraded by depolymerases from P. lemoignei13 and A. faecalis.12 Recently Nobes et al. provided evidence that the enzymatically degraded PHB film texture is due to splintering of radial lamellar spherulitic ribbons.14 However, the fact that degradation rates depend on a large number of different variables, e.g., polymer chemical composition, physical state (i.e. granule suspension, solvent or melt cast films, single crystals), crystallinity and crystal size perfection, type and concentration of enzyme, and temperature, makes the evaluation of the degradation characteristics of bacteria or enzymes a very challenging goal. For example Hocking et al. reported the complete degra-
degradation in PHAs, 1H NMR imaging, and demonstrate a new method for the monitoring of crystalline plus amorphous techniques used so far, total weight loss measurements and turbidimetric or titrimetric methods follow the total, crystalline material has been the lack of a technique that can probe selectively the degradation of only one of the two physical states of the polymer. Indeed the techniques used so far, total weight loss measurements and turbidimetric or titrimetric methods follow the total, crystalline plus amorphous, consumption of PHA by the degrading enzyme or bacteria. In the present report, we introduce a new method for the monitoring of degradation in PHAs, 1H NMR imaging, and demonstrate its applicability to the degradation of thin PHA polymer films. In recent years NMR imaging has gained increased acceptance as a tool for the characterization of materials and the study of physical processes such as thermal aging and stress distribution in polymers. The main advantages of this technique are its nondestructive character and the availability of several different NMR parameters that can be used for generating image contrast.

PHAs are semicrystalline polymers with a wide range of crystallinity values depending on the chemical structure of the monomer unit or the mole percentage of the two monomer units in PHA copolymers, such as PHB/V. PHB and the PHB/V copolymer used in this study have glass transition temperatures around 0 °C, so it is possible to record high-resolution 1H NMR spectra in the solid state and at room temperature without having to resort to CP/MAS techniques. At more elevated temperatures 13C NMR relaxation has been used to study the polymer dynamics in the amorphous phase for a series of P3/4HB copolymers. In the NMR imaging experiments presented here, the quantity that is monitored during degradation is the proton spin density of the amorphous regions of the polymer in two-dimensional space (for thin films). Under suitable experimental conditions, the 1H spin density is directly correlated to the amount of amorphous material present in the film. The crystalline regions of the polymer do not contribute to the 1H signal under the "high-resolution" type imaging experiments presented in this study, because of the large dipolar coupling of the proton nuclei in the crystal regions. The technique will be applied to PHB and PHB/V melt cast films, degraded by depolymerase B isolated from P. lemoignei. Evidence for preferential consumption of polymer in the amorphous phase, at the initial stages of degradation, will be presented. Also we report that, during later stages of degradation, the enzyme does not appear to differentiate between amorphous and crystalline regions, degrading them with equal rates. These results will be discussed in relation to the mechanism of degradation of PHA films by bacterial depolymerases.

Experimental Section

PHB and PHB/V were purchased from Aldrich. The HV content of PHB/V was found to be approximately 23% HV units of the monomer unit or the mole percentage of the two monomer units in PHA copolymers, such as PHB/V. PHB and the PHB/V copolymer used in this study have glass transition temperatures around 0 °C, so it is possible to record high-resolution 1H NMR spectra in the solid state and at room temperature without having to resort to CP/MAS techniques. At more elevated temperatures 13C NMR relaxation has been used to study the polymer dynamics in the amorphous phase for a series of P3/4HB copolymers. In the NMR imaging experiments presented here, the quantity that is monitored during degradation is the proton spin density of the amorphous regions of the polymer in two-dimensional space (for thin films). Under suitable experimental conditions, the 1H spin density is directly correlated to the amount of amorphous material present in the film. The crystalline regions of the polymer do not contribute to the 1H signal under the "high-resolution" type imaging experiments presented in this study, because of the large dipolar coupling of the proton nuclei in the crystal regions. The technique will be applied to PHB and PHB/V melt cast films, degraded by depolymerase B isolated from P. lemoignei. Evidence for preferential consumption of polymer in the amorphous phase, at the initial stages of degradation, will be presented. Also we report that, during later stages of degradation, the enzyme does not appear to differentiate between amorphous and crystalline regions, degrading them with equal rates. These results will be discussed in relation to the mechanism of degradation of PHA films by bacterial depolymerases.

Enzymatic Degradation. Degradation was performed in buffer solutions of double-distilled water containing 50 mM Tris–HCl, pH 8.0, 1 mM CaCl2, and a depolymerase B concentration of 3 µg/mL, without shaking. Each degradation step lasted for 20 h, after which the film was washed three times with water, dried in vacuo to constant weight, and imaged. After imaging, a fresh enzymatic solution was used for the next 20 h degradation interval.

1H NMR Imaging. Imaging experiments were performed on a Bruker DSX 400 spectrometer operating at 9.4 T (400 MHz proton resonance frequency) equipped with a microimaging probe of 1 cm diameter, and actively shielded gradient coils. The films were held in position with the help of a Teflon holder inserted in the probe head. The standard spin-echo 1H imaging sequence used is illustrated in Figure 1. Due to the small thickness of the film, slice selection was not necessary, and a "hard" nonselective 180° refocusing pulse was used for the echo formation. The parameters of typical 1H images used in the following calculations are as follows: FOV 3 × 3 cm2; pixel matrix 128 × 128, zero filled to 256 × 256; echo time 0.737 ms; acquisition time 1.074 ms; recycle delay 100 ms; number of scans 4K; phase gradient 71.6 mT/m; read gradient 97.9 mT/m; total experiment time 15.5 h. All 1H images were recorded under identical experimental conditions and with exactly the same set of imaging parameters, to ensure the quantitative nature of the results. The 1H spectrum of both PHB and PHB/V consisted of a single broad peak with a linewidth of about 2 kHz at room temperature. To improve the signal/noise ratio, imaging experiments were performed at a temperature of 60 °C for PHB and 50 °C for PHB/V.

Results

a. PHB. 1H images of a PHB film recorded as a function of degradation time by depolymerase B from P. lemoignei at 37 °C are presented in Figure 2. A small piece of never degraded PHB film is placed to the right of the degraded one, and serves as an internal reference standard with respect to amorphous polymer concentration. The loss of amorphous polymer during degra-
Integration results in loss of $^1$H image intensity for the degraded film piece. This is particularly evident in the $^1$H images of the film at later stages of degradation. It must be stressed that the images of Figure 2 represent the spatial distribution of amorphous proton intensity, and do not imply any loss of film integrity, since the film retained its rigidity throughout the series of experiments.

Integration of the parts of the images of Figure 2 that contain the degraded polymer film provides a quantitative measure of the amount of amorphous material remaining in the film after each 20 h degradation interval. The results of the integration of the degraded PHB film image as a function of degradation time, along with the integral of the internal standard are shown in Figure 3a. The internal standard integral remains constant during the course of the experiments, assuming a value of $2.12 \pm 0.12$ au. This indicates that the integrals of the film’s $^1$H images are representative of the amount of amorphous material present. The integral of the degraded PHB film displays a complex behavior as a function of degradation time. Up to 120 h the integral decays exponentially, indicating an exponential-type loss of amorphous material by enzymatic degradation. After 120 h, and up to a total of 220 h, of degradation, the loss of amorphous material is

Figure 2. $^1$H NMR images of a PHB film (large piece of film on the left), as a function of degradation time indicated at the top right of each image (FOV $3 \times 3$ cm$^2$). A smaller but identical never degraded piece of PHB film, used as a standard, can be seen at the right of the degraded one in all images. The small intensity appearing at the bottom of the images comes from the Teflon sample holder.
It is interesting to note that the exponential curve does not decay to zero, but to a much larger value. This is a consequence of the nature of the PHB sample, since it is expected that the core of the film is not available for degradation.

To compare the amorphous PHB loss with the total polymer loss during degradation, the data of Figure 3a are plotted in Figure 4 together with the weight loss data. The integrals are first normalized with respect to the standard, never degraded film integral, and then both integrals and weights are normalized to 100% for the film at $t = 0$ h (no degradation). Total weight loss is linear with time, giving an overall rate of degradation of 0.0327 mg cm$^{-2}$ h$^{-1}$, which is consistent with previous reports on PHB degradation by depolymerase B$^{10,11,16}$ and A$^{8,11,16}$ from P. Lemoigne. It is clear from Figure 4 that the overall consumption of amorphous material is greater than that of crystalline material. If the depolymerase did not differentiate between the two polymer phases, the imaging data should coincide with the weight loss data. Instead, Figure 4 shows that after 220 h of degradation the weight loss is about 50%, while almost 70% of the amorphous PHB $^1$H image integral has been lost.

Another striking feature of the graph of Figure 4 is the exponential loss of proton spin density over the first 120 h of degradation, which represents exponential loss of amorphous material. It appears that during the initial stages of degradation, i.e., the first 40–60 h, practically only amorphous polymer is consumed. The degraded PHB film was examined under crossed polars in a polarizing microscope at different degradation times. In Figure 5a a micrograph of the PHB film before degradation started is presented, showing the large (0.5–1 mm diameter) spherulite structure representing the high crystallinity of the film. Parts b and c of Figure 5 show the appearance of the film after 20 and 40 h of degradation, respectively, and present characteristics well-known from previous studies, such as the central holes from spherulites having their center on the film surface$^{18}$ and the circular erosion pattern characteristic also of degradation of PHB by depolymerase A from P. lemoigne. $^{24}$ The above characteristics are usually associated with preferential erosion of the amorphous interlamellar phase in semicrystalline PHB films.

The exponential-type loss of the degraded PHB $^1$H image integral in Figure 4 does not reach an apparent plateau value. Instead, after 120 h of degradation the pattern of integral loss changes and becomes linear with time. The slopes in Figure 4 for total and amorphous (120–220 h) degradation of PHB are $-0.224$ and $-0.237$, respectively, so effectively the rate of amorphous material loss in the time window between 120 and 220 h is the same as that of total weight loss. This implies that in this interval equal amounts of amorphous and crystalline PHB are consumed by the enzyme as a function of time. We believe this might be due to the amorphous material deeper in the film not being as easily accessible to the enzyme as that on the film surface.

DSC examination of the PHB film before and after 220 h of degradation gave crystallinity values of 65 ± 5% and 90 ± 5% respectively. $^{25}$ This result is in accord with the NMR imaging results presented in Figure 4 that show the preferential erosion of amorphous PHB at the initial stages of degradation and indicate that after 220 h of degradation a total weight loss of 50% was actually accompanied by an amorphous material loss of 70%.

b. PHB/V. Figure 3b depicts the $^1$H image integral of a PHB/V film as a function of degradation time by PHB depolymerase B using the same experimental protocol as with PHB, along with the integral of a never degraded PHB/V standard film. Similar behavior to that observed with PHB is noted, i.e., a fast initial loss of amorphous material in an exponential manner, followed by an almost linear loss at later stages of degradation.
degradation. The comparison between total weight loss and amorphous material loss for PHB/V from the 1H NMR imaging data is reported in Figure 4. The change from exponential to linear behavior for PHB/V takes place at shorter degradation time, approximately after 80 h of degradation. The total weight loss rate for PHB/V was found to be similar to that of PHB, and equal to 0.037 mg cm$^{-2}$ h$^{-1}$. It is evident from Figure 4 that a large loss of amorphous PHB/V takes place at the initial stages of degradation, similar to that observed for PHB. Examination of the PHB/V film under polarized light before degradation showed no evidence of spherulitic structure at a magnification of 50:1. Thus, the fast initial loss of amorphous material observed by 1H NMR imaging cannot be associated with the presence of large (0.5 – 1 mm diameter) spherulites in the PHB film but appears to be a more general phenomenon. As with PHB, at the late stages of degradation amorphous polymer loss becomes linear with time, and has the same rate as the overall weight loss. The slope of the linear fits in Figure 4 for PHB/V are $-0.230$ for the total weight loss and $-0.226$ for the amorphous polymer loss (80 to 140 h). After 140 h of degradation the total weight loss for PHB/V was 32%, while the amorphous polymer loss was 46%. The DSC thermogram of the PHB/V sample before and after degradation consisted of broad and multiple melting peaks in the range 80 – 150 °C, some of them possibly produced by recrystallization during the DSC scan. After 140 h of degradation an increase from 29.2 to 37.95 J/g was measured for the enthalpy of fusion $\Delta H_f$ over the above mentioned temperature range. Thus, the increase in crystallinity indicated by the NMR imaging results for PHB/V could also be confirmed qualitatively by DSC.

**Discussion**

**Amorphous Degradation Modeling.** The data of Figure 4 can be analyzed in order to extract numerical values for the degradation rate of the amorphous polymer regions. Two models have been proposed as suitable for the description of PHB total degradation kinetics. Mukai et al. published a heterogeneous kinetic treatment that assumes that the binding and active site of PHB depolymerases is different. With this model, data on different PHB depolymerases were fitted successfully, accounting for the peculiar dependence of the enzymatic hydrolysis reaction on enzyme concentration $E$ at high $E$. Recently a kinetic model that can account for both enzyme and substrate concentration dependences has been proposed. In the following we will assume that the degradation of amorphous PHB can be described, in analogy with total PHB degradation, by a simple two-step reaction scheme, involving the formation of a complex ES$_n$ between the polymer substrate $S_n$ and enzyme $E$, and the subsequent cleavage of a monomer (or oligomer) unit $M$ from the end of the polymer chain in a stepwise manner:

$$E + S_n \xrightarrow{k_1} [ES_n] \xrightarrow{k_2} [ES_{n-1}] + M$$

Tomasi et al. showed that for depolymerases A and B from *P. lemoignei* and at high enzyme concentrations (> 3 mg/mL) the degradation rate of a PHB film reaches a plateau and remains constant, in contrast to previous observations for PHB depolymerases from other bacterial species. Consequently at sufficiently high enzyme concentrations, the concentration of ES$_n$ can be approximated by the concentration of $S_n$. Assuming for simplification that the rate-limiting step is the hydrolysis of the polymer chain, the degradation of amorphous PHB or PHB/V can be described by the empirical equation

$$C_a^t = C_{a0}^e + C_0^e e^{-kt}$$

where $C_a^t$ is the % amount of amorphous material remaining after $t$ hours of degradation, $C_0^e$ is the fraction of amorphous polymer initially available for degradation, $C_{a0}^e$ is the amorphous material in the inner part of the polymer film, which is not initially...
Table 1. DSC Crystallinity, Total Degradation Rate, Amorphous Polymer Not Available for Degradation, C^endo, and Amorphous Polymer Degradation Rate, k_2, for PHB and PHB/V Degraded by PHB Depolymerase B from P. lemoignei

<table>
<thead>
<tr>
<th></th>
<th>crystallinity (%)</th>
<th>tot. degradation rate (mg cm^{-2} h^{-1})</th>
<th>C^endo (%)</th>
<th>k_2 (h^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHB</td>
<td>65</td>
<td>0.033</td>
<td>51</td>
<td>0.020</td>
</tr>
<tr>
<td>PHB/V</td>
<td>~55</td>
<td>0.037</td>
<td>67</td>
<td>0.049</td>
</tr>
</tbody>
</table>

available for degradation, and k_2 is the apparent enzymatic hydrolysis rate of the amorphous polymer.

Fitting of the data of Figure 4 with eq 1 provides the parameter values presented in Table 1 for the PHB and PHB/V films. The rate of initial hydrolysis of amorphous material k_2 is found to be about twice as large for PHB/V compared to PHB, although the total degradation rate of both polymers is similar. Further studies are needed to examine if this difference is related to the real "chemical" preferences of the enzyme, or is mainly a matter of polymer film surface texture and characteristics. The values of C^endo in Table 1 indicate that 49% and 33% of the total amorphous material, for PHB and PHB/V, respectively, was available to the enzyme at the early stages of degradation. This represents the surface amorphous material plus the material made available through penetration of the enzyme under the film surface by means of preexisting fissures, pits, and microscopic air bubbles. The lower value for PHB/V is in agreement with observations regarding the differences in surface texture between PHB and PHB/V melt cast films, where it was reported that the presence of fissures in melt-crystallized PHB films was responsible for rapid degradation below the film surface. PHB/V films lacked any fissures and pits, and thus no degradation below the immediate film surface was observed for this polymer. Thus the different values of C^endo for PHB and PHB/V can be attributed to more surface amorphous material being available for PHB, due to the presence of fissures and pits on the film surface.

A second effect that takes place during degradation is the progressive "dissolution" of the film by the enzyme, that leads to the reduction of the film thickness. A 50 µm loss in film thickness was measured for both films at the end of the degradation period. This dissolution affords an extra quantity of amorphous polymer to the enzyme and could be responsible for the similar time dependence of amorphous and total polymer consumption at the late stages of degradation. It must be stressed however that since consumption of the surface reservoir and dissolution are events that are not clearly separated in time and at some point could both take place simultaneously, more work is needed to clarify the relative role of each procedure in the degradation process.

One important aspect stemming from the results presented so far is the demonstration of the preference of depolymerase B from P. lemoignei toward the degradation of amorphous polymer. This preference has been established for both PHB and PHB/V by the large loss of 1H image intensity at the early stages of the degradation procedure. Inspection of Figure 4 reveals that during the first 60 h of degradation both polymer films have lost significant amounts of amorphous polymer, compared to crystalline. Thus, the present data suggest that the depolymerase B enzyme degrades first any amorphous material available, and then it proceeds with the degradation of crystalline regions. This is in accord with a recent study of bacterial degradation in PHB blends, where it was suggested that the enzyme attack to the lamellar PHB crystalline phase is conditioned by prior consumption of some interlamellar amorphous material. However, the present results do not imply that depolymerase B has any specificity toward amorphous PHB. Indeed, it is clear in Figure 4 that the overall degradation rate (amorphous plus crystalline) for both polymers remains unchanged during the course of the degradation, although during this same time the enzyme degrades mainly amorphous (initial stages), then mainly crystalline (apparent plateau region) and then equal amounts of both at the last stage. This observation leads to two important points suggested by the present data: First, the rate of degradation of amorphous and crystalline PHB regions by depolymerase B cannot be very different. If such a huge difference existed, the total degradation rate would change depending on whether amorphous or crystalline material is degraded. Second, once some interlamellar material has been removed, the enzyme degrades both amorphous and crystalline regions indiscriminately (late stages of degradation in Figure 4). Since depolymerases A and B show similar behavior, such a mode of operation would also explain the versatility of P. lemoignei toward amorphous and crystalline degradation. It is well known that in several cases very efficient degradation of PHB single crystals of very high crystallinity has been effected by the P. lemoignei PHB depolymerase A enzyme. The above points leave an important question unanswered: If the amorphous and crystalline PHB degradation rates by depolymerase B are similar, why does then the PHB total degradation rate scale inversely with crystallinity, as has been convincingly shown for PHB using a variety of enzymes and bacterial species? Recently it was shown that the extracellular depolymerases of P. lemoignei possess two distinct domains, a catalytic active domain and a binding domain situated at the C-terminal end of the protein. Truncated PHB depolymerase that lacked the binding domain was unable to degrade PHB, although the catalytic domain remained fully active toward butyrate ester bond cleavage. This indicates that the binding domain characteristics can have a severe effect on the total PHA degradation rate, the effect of the catalytic domain being somewhat smaller, except when the chemical structure of the PHA ester bond is very different than that of PHB. One explanation offered then could be that crystallinity affects the total degradation rate by changing the characteristics of the amorphous/crystalline interphase and the film surface characteristics, thus modifying the binding ability of the enzyme. Support for this argument comes from recent work that showed that the crystal size perfection has also a significant effect on the PHB degradation rate at constant crystallinity. Also recent work has shown that PHB blends with cellulose acetate butyrate (CAB) as high as 80% rich in PHB failed to degrade at all after 1 year in activated sludge, while pure PHB completely degraded in 20–25 days. This was attributed to inaccessibility of the amorphous PHB/CAB regions to the bacteria, possibly due to changes in surface hydrophobicity.

Recently Gazzano et al. produced evidence that in melt cast PHB films with small thickness compared to the average spherosite size, the crystallographic axis a lies quasi-parallel to the film surface. The crystallographic axis a is the axis of chain folding in the crystalline lamellae, and this means that the radial
lamellar regions in the PHB film have a preferential orientation with respect to the film surface. This preferential orientation is absent in the PHB/V film of the present study, which lacks any large spherulite structure, and consequently possesses a more or less random distribution of lamellar regions with respect to the film surface. It is possible that this difference also plays a role in the ease with which enzymes are able to penetrate and erode subsequent regions of amorphous and crystalline polymer in the two films.14

Finally, in a recent paper12 it was reported that melt-crystallized films of several PHA copolymers (including PHB/V films of different composition) were degraded with a rate several times higher than that of PHB films by an extracellular depolymerase from A. faecalis, when crystallinity was taken into account. Faster degradation of PHB/V melt-cast films was also reported by bacterial cultures of P. lemoignei.24 Comamonas sp.24 and Penicillium funiculosum.31 The depolymerase B enzyme from P. lemoignei used in the present study also degraded PHB/V with a slightly larger total rate (see Table 1), although the two values are too close to allow definite conclusions. Interestingly, it is the initial amorphous phase degradation rate of PHB/V that was found to be twice as fast as that of PHB in the present study. It is not clear why in solution cast films the opposite trend is observed, with PHB being degraded faster than PHB/V for films of similar crystallinity.32 In view of the results presented in this report, one can postulate that the effect of the film texture on the binding domain characteristics of the depolymerase is responsible for the opposite trend observed for solution and melt-cast films.

It is worth noting that if the behavior of depolymerase B proves to be shared by depolymerases from other bacteria also, it will represent an extra, and hitherto unknown, factor that might complicate the interpretation of degradation studies done on semicrystalline PHA films. If the consumption of amorphous or crystalline material by the enzyme changes with time depending on what material is available, the length of the degradation period becomes relevant when comparing for example the effect of film crystallinity on degradation.

Conclusions

The results presented in this work demonstrate for the first time in a quantitative manner that depolymerase B isolated from P. lemoignei shows a clear preference for amorphous PHB material during the initial stages of degradation of thin PHA films. An equally important finding was that this preference disappears at later stages of the degradation procedure, when amorphous and crystalline regions are degraded indiscriminately. The overall linear weight loss of polymer material as a function of time for both PHB and PHB/V establish clearly that despite this preference, depolymerase B does not display any specificity toward amorphous PHB material. This result corroborates earlier work that demonstrated the high efficiency of depolymerase A from P. lemoignei toward the degradation of PHB single crystals.5,15 It is concluded that the amorphous and crystalline degradation rates are not too different for this enzyme. Also, support for an argument17 requiring some interlamellar amorphous degradation before crystalline PHB regions become accessible to the enzyme was indirectly offered.

More generally, this work has demonstrated that 1H NMR imaging is an efficient new tool for the study of degradation of biologically synthesized polymers. The main importance of the NMR imaging method stems from the fact that it can selectively trace the degradation of amorphous polymer material. In combination with weight loss measurements, NMR imaging, unlike other techniques, allows the evaluation of the specificity or selectivity of the degrading enzyme toward the physical state of the PHA polymer.

Finally, as the whole degradation experiment can be performed with the same film sample, this technique requires the minimum amount of depolymerase enzyme and polymer. Although this application described work done using enzymes to degrade the polymers, the 1H NMR imaging methodology developed can be easily extended to the study of PHA chemically induced degradation as well.33 Experiments using solvent-cast PHB and PHB/V films, different depolymerase enzymes, and also different PHAs are currently in progress, in an effort to further elucidate the mechanism of enzymatic degradation in PHAs.

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References and Notes


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