Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

Blends of Bacterial Poly(3-Hydroxybutyrate) with Cellulose Acetate Butyrate in Activated Sludge

Gianpaolo Tomasi[a]; Mariastella Scandola[a]
[a] Dipartimento di Chimica 'G. Ciamician' dell'Università di Bologna and Centro di Studio per la Fisica delle Macromolecole del C.N.R., Bologna, Italy

To cite this Article Tomasi, Gianpaolo and Scandola, Mariastella(1995) 'Blends of Bacterial Poly(3-Hydroxybutyrate) with Cellulose Acetate Butyrate in Activated Sludge', Journal of Macromolecular Science, Part A, 32: 4, 671 — 681
To link to this Article: DOI: 10.1080/10601329508010280
URL: http://dx.doi.org/10.1080/10601329508010280

J.M.S.—PURE APPL. CHEM., A32(4), pp. 671–681 (1995)

BLENDS OF BACTERIAL POLY(3-HYDROXYBUTYRATE) WITH CELLULOSE ACETATE BUTYRATE IN ACTIVATED SLUDGE

GIANPAOLO TOMASI and MARIASTELLA SCANDOLA*

Dipartimento di Chimica 'G. Ciamician' dell'Università di Bologna
and Centro di Studio per la Fisica delle Macromolecole del C.N.R.
Via Selmi 2, 40126 Bologna, Italy

ABSTRACT

Blends of bacterial poly(3-hydroxybutyrate) (PHB) with cellulose acetate butyrate (CAB) were prepared by compression molding followed by different thermal treatments. The morphology of the blends, which are totally miscible in the melt, was investigated by differential scanning calorimetry, x-ray diffraction, and optical and scanning electron microscopy. Depending on composition and thermal treatments, the blends are either single-phase amorphous PHB/CAB mixtures or partially crystalline materials composed of space-filling spherulites, where a constant fraction of the PHB present in the blend (about 65%) constitutes the lamellar crystalline phase, while the interlamellar amorphous phase is formed by the remaining PHB mixed with CAB. PHB/CAB blends over the whole composition range show no weight loss after 12 months of exposure to activated sludge. In the same experimental conditions, pure PHB quickly biodegrades at a rate which is found to be affected—at a constant degree of crystallinity—by small morphological variations. The results suggest that crystalline PHB lamellae can be attacked only after some interlamellar amorphous material has been removed. In the blends, nondegradability of interlamellar PHB/CAB mixtures prevents enzyme action toward the pure PHB lamellar crystals.

INTRODUCTION

Bacterial poly(3-hydroxybutyrate), PHB, and related microbial poly(hydroxyalcanoates), PHA, have attracted much attention as biodegradable and biocompatible thermoplastic polymers [1, 2]. However, wide-scale applications of bacterial polymers are still prevented by high production cost and some undesirable properties (brittleness, for example). A widespread practice in polymer science to modify physical properties is polymer blending. In the case of PHAs, blending also affects biodegradability, and a number of biodegradation studies on PHA-based blends have been recently published [3–8]. It is generally found that when PHAs form miscible blends with nonbiodegradable polymers, biodegradability substantially decreases [4–7]. However, when the two blend components are immiscible, phase separation occurs and in some cases an increase of PHA biodegradation rate has been found [3–5, 8]. In the presence of water-soluble polymers as second blend components, i.e., poly(ethylene oxide) [3] and starch [8], accelerated degradation was attributed to an increase of surface area exposed to enzymatic attack.

Recently, partial biodegradation of miscible blends of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) with cellulose acetate butyrate (PHBV)/CAB in the range of PHBV-rich blends have been reported [7]. The blends were in the form of films obtained by solvent casting, a technique which was shown to induce partial segregation of the components and allows one surface to enrich in the biodegradable PHBV component. Biodegradation was found to occur preferentially on that surface. The present work investigates the biodegradability of a similar system, PHB/CAB, but the blend preparation technique is different. Compression molding instead of solution casting is used on account of good reproducibility, absence of any sidedness, and better representation of manufactured materials. Phase behavior identical to that of melt mixed and injection molded blends is obtained. It is important to point out that most results presently available in the literature on biodegradability of PHB-based blends refer to solvent-cast films.

It is known that the rate of enzymatic degradation of PHB decreases with increasing crystallinity [9, 10]. In this paper the effect of small morphological variations on PHB biodegradation is also investigated in order to show that rather subtle changes—not affecting the overall degree of crystallinity—significantly alter the rate of biodegradation of PHB.

EXPERIMENTAL

Materials

Bacterial poly(3-hydroxybutyrate), PHB, was an ICI product (G08, M_w = 5.39×10^5, $M_w/M_n = 4.11$). Cellulose acetate butyrate was an Eastman Kodak product (EAB 500-1) whose degree of substitution ($DS_{Bu} = 2.58$, $DS_{Ac} = 0.36$) and molecular weight ($M_w = 1.30 \times 10^5$, $M_w/M_n = 2.12$) were kindly determined by Dr. C. Buchanan, Eastman Chemical Co., Kingsport, Tennessee.

Blend Preparation

In order to obtain PHB/CAB blends, weighed amounts of PHB and CAB (weight ratio 80/20, 60/40, 40/60, and 20/80) were dissolved in chloroform (5% w/v solutions). Blend films were obtained by solvent evaporation in glass Petri

dishes followed by vacuum drying overnight. The solution cast blends, inserted between two aluminum plates containing a Teflon spacer, were compression molded using a Carver C12 laboratory press by heating at 190°C for 1 minute under a pressure of 2 tons/m^2. The molten blends were then subjected to one of the following thermal histories: 1) cooling to room temperature (RT), where crystallization was allowed to occur over a period of at least 2 weeks (RT blends); 2) same as in 1) followed by annealing in an oven at 90°C for 63 hours (RT + 90 blends); 3) rapid transfer into an oven at 90°C where PHB was allowed to melt-crystallize isothermally for 63 hours (IC90 blends).

Pure CAB films were obtained as described for RT blends (solvent casting, compression molding, and RT quenching). As regards PHB, the original powder was directly compression molded at 195°C for 1.5 minutes under a pressure of 2 ton/m^2, and the molten sample so obtained was subjected to the same thermal treatments (RT, RT + 90, IC90) described for the blends. The thickness of the compression-molded films was 0.11 ± 0.01 mm for blends and CAB and 0.26 ± 0.02 mm for pure PHB.

Biodegradation Experiments

The compression-molded films were cut into squares 2 × 2 cm^2. Four replicate samples of each type were carefully weighed and fastened to a fishing net (mesh size 2 × 2 mm^2) by means of a thin nylon fishing line (diameter 0.16 mm). The net was folded and sewn to form a bag which was tied to an aluminum frame. An aluminum scaffolding, holding several frames, was used to suspend the sample-containing bags in activated sludge at the municipal wastewater treatment plant of Bologna (Italy). At selected times the bags were recovered, the samples were removed, carefully washed in running water using a soft paint brush, and finally dried to constant weight under vacuum. Sterile controls (samples sterilized overnight in an atmosphere saturated with formaldehyde) were run in analogous conditions using sterilized sludge in sealed bottles. The biodegradation results are reported as weight loss divided by sample surface area ($\Delta m/S$). On account of the thinness of the films, the lateral area was neglected and S was taken as 8 cm^2. No estimate was attempted of possible surface area changes during biodegradation.

Experimental Methods

Differential scanning calorimetry was performed with a DuPont 9900 Thermal Analyzer in the temperature range −80/220°C at a heating rate of 20°/min. The temperature scale was calibrated with high purity standards. Wide-angle x-ray diffraction measurements (WAXS) were carried out with a Philips PW1050/81 diffractometer controlled by a PW1710 unit, using nickel-filtered CuKα radiation (λ = 0.1542 nm, 40 kV, 30 mA). The average crystal size was determined by the Sherrer equation from the 020 reflection, using Warren's correction for instrumental line broadening [11]. A polarizing optical microscope (Zeiss Axioscop) was used to observe sample morphology. Film surfaces were examined before and after biodegradation experiments using a scanning electron microscope (Phillips 515); samples were sputter coated with gold.

RESULTS AND DISCUSSION

Sample Characterization

Owing to the absolutely regular structure resulting from biosynthesis, PHB is a highly crystallizable polymer which develops a crystalline fraction of 60–70% in normal conditions. As commonly found in melt-crystallized polymers, PHB adopts a spherulitic morphology upon solidification from the melt. Optical microscope observations of the three PHB samples used in this work (RT, RT + 90, IC90) show spherulites of a more homogeneous and larger size in IC90 PHB (isothermally crystallized at 90°C) than in the other two samples (crystallized at room temperature). In line with previous evidence [12], this result indicates that with decreasing crystallization temperature the nucleation density increases and the spherulite size is reduced. The crystallinity degree (X_c) of the three PHB samples was obtained from DSC by comparison of the enthalpy (ΔH_m) associated with the melting endotherm with the literature value for 100% crystalline PHB [12]. The X_c values, reported in Table 1, show no variations (within the accuracy limits of this type of estimate) as a consequence of the thermal history applied to the samples. The only appreciable difference regards quality better than quantity of the crystalline phase: WAXS measurements show that the average crystal size is enhanced by annealing and by an increase of crystallization temperature. The crystal size data, reported in Table 1, are seen to increase in the order (RT) < (RT + 90) < (IC90). It is quite clear that annealing or crystallizing closer to the melting temperature of PHB (T_m = 175°C) would have sensibly increased the crystallinity degree, but this was out of the scope of the thermal treatments applied to the samples.

In previous papers [13–16] it was shown that bacterial PHB and a number of cellulose esters—including the CAB used in the present work—are miscible in the melt over the whole composition range. In the course of this investigation it was observed that all the compression-molded (RT) PHB/CAB films were transparent immediately after quenching from the melt to room temperature. Only the 80/20 and 60/40 blends turned opaque upon room storage due to PHB crystallization. The reason for this behavior has to be sought in the glass transition temperature (T_g) of the blends [13]. When the amount of PHB is lower than 60%, T_g is higher than room temperature and the blends are stable glassy mixtures; on the contrary, when PHB content is $\geq$ 60%, T_g lies below RT and the PHB chains have enough mobility to rearrange and crystallize. PHB-rich blends are therefore partially crys-

TABLE 1. Degree of Crystallinity (X_c) and Average Crystal Size (C.S.) of PHB with Different Thermal Histories

Sample	X_c [a]	C.S., Å [b]
RT	0.65	208
RT + 90	0.67	252
IC90	0.66	290

[a]From DSC by comparison with the enthalpy of fusion of 100% crystalline PHB from Reference 12. $\sigma = \pm 2\%$.

[b]From WAXS using the (020) reflection. $\sigma = \pm 5\%$.

talline, being composed of a crystalline pure PHB phase and a mixed amorphous PHB/CAB phase. DSC measurements were carried out on all PHB/CAB blends. As regards the RT blends, a melting endotherm in the DSC curve confirms the visual observations, i.e., the presence of a PHB crystalline phase in both 60/40 and 80/20 blends. The enthalpy associated with the melting process (ΔH_m) is reported in Fig. 1(a) together with the value for pure PHB with the same thermal history (RT). The phase behavior of RT blends is identical to that of blends obtained by melt mixing and injection molding [13]. When RT blends are subjected to annealing at 90°C, i.e., RT + 90 blends, the 40/60 blend also turns opaque, indicating that some PHB has crystallized during permanence at 90°C. Crystallization of PHB occurs because the blend T_g, higher than room temperature, is lower than the annealing temperature. The ΔH_m values of RT + 90 and IC90 blends are reported in Figs. 1(b) and 1(c), respectively, as a function of composition. The three plots of Fig. 1 show a linear dependence of ΔH_m on composition, irrespective of the thermal treatment applied. The results indicate that a constant fraction of the PHB present in each blend segregates as a pure crystalline phase. This fraction corresponds to the X_c of pure PHB, i.e., 65–67% (see Table 1). The remaining bacterial polymer enters the mixed amorphous phase with the cellulose ester, a phase obviously richer in CAB than the overall blend composition.

X-ray diffraction measurements confirmed the calorimetric results: the crystalline phase which is seen to melt by DSC is clearly identified as PHB from the typical x-ray pattern [17, 18]. The morphology of PHB/CAB films was examined by means of polarizing optical microscopy, a technique also used to study the isothermal crystallization from the melt of the blends [19]. As expected in the absence of a crystalline phase, no birefringence was shown by any of the 20/80 blends nor by the RT (40/60) blend. In the remaining RT and RT + 90 blends, birefringent space-filling spherulites of small dimension could be better observed with higher PHB content. The two IC90 blends showed well-developed impinging spherulites.

Biodegradation

Biodegradation experiments in activated sludge of PHB/CAB blends and of the pure components were carried out over a period of 12 months. As expected, the

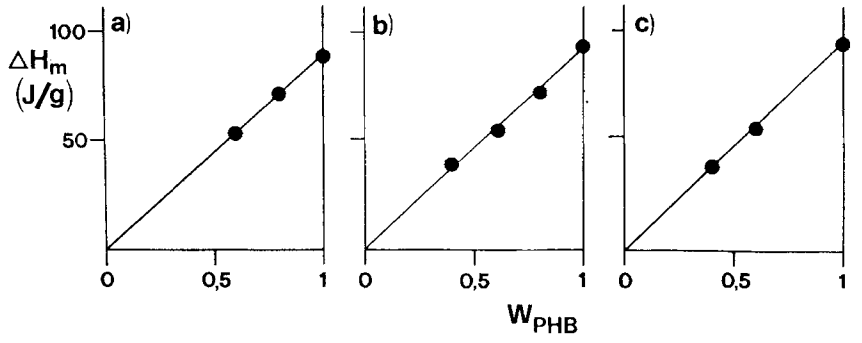

FIG. 1. Melting enthalpy as a function of PHB weight fraction of PHB/CAB blends subjected to different thermal histories (see text): a) RT; b) RT + 90; c) IC90.

pure bacterial polyester degraded quickly, no film samples being recovered after 20–25 days of exposure. Figure 2 shows the biodegradation results of the different types of PHB (RT, RT + 90, and IC90), reported as weight loss divided by surface area ($\Delta m/S$). Upon sample recovery after any given exposure time, weight loss was found to increase invariably in the order (IC90) < (RT + 90) < (RT). Since sterile controls, examined after a period of 7 months in sterilized sludge, do not show appreciable weight loss, the observed degradation is not simple chemical hydrolysis but is an enzymatically mediated process. The results of Fig. 2 show that in PHB film samples having practically the same degree of crystallinity, small morphological variations, consisting in an increase of the average crystal size and—in the case of sample IC90—in larger and more regular spherulites, are effective in altering the rate of biodegradation. Linear regression of the weight loss data yield biodegradation rate values of 0.88, 0.72, and 0.64 mg/(cm^2·day) for (RT), (RT + 90), and (IC90) PHB, respectively.

It is well known that biodegradation of PHB is a surface phenomenon which proceeds via hydrolysis and dissolution [20]; moreover, when the polymer adopts a spherulitic morphology, degradation starts from the polymer chains in the disordered amorphous phase and proceeds to the exposed lamellar crystals [9]. In the course of the present investigation, SEM observations on degraded films of pure PHB after different times of exposure to activated sludge have confirmed that degradation proceeds sequentially and that upon erosion of the exposed amorphous phase, naked crystalline lamellae irradiating from the spherulite center clearly appear on the surface. Figure 3 shows the effect of 4 days of exposure to activated sludge of a compression-molded (RT) PHB sample. An estimate of the degree of crystallinity (by DSC) at various stages of the degradation process (up to a weight loss of 70%) shows no appreciable X_c changes, suggesting that the surface layer where the amorphous phase is preferentially degraded is thin. From SEM observa-

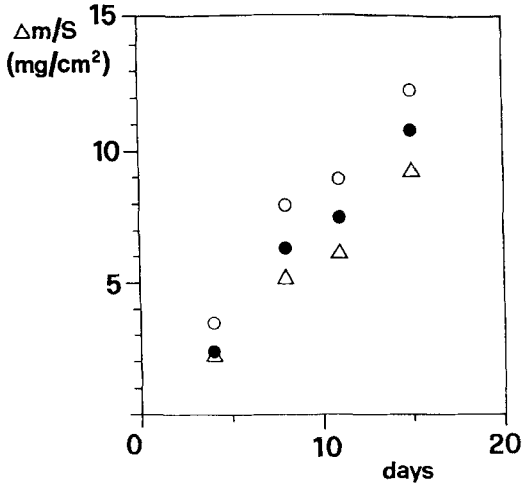

FIG. 2. Weight loss in activated sludge as a function of time of PHB films: (○) RT; (●) RT + 90; (△) IC90.

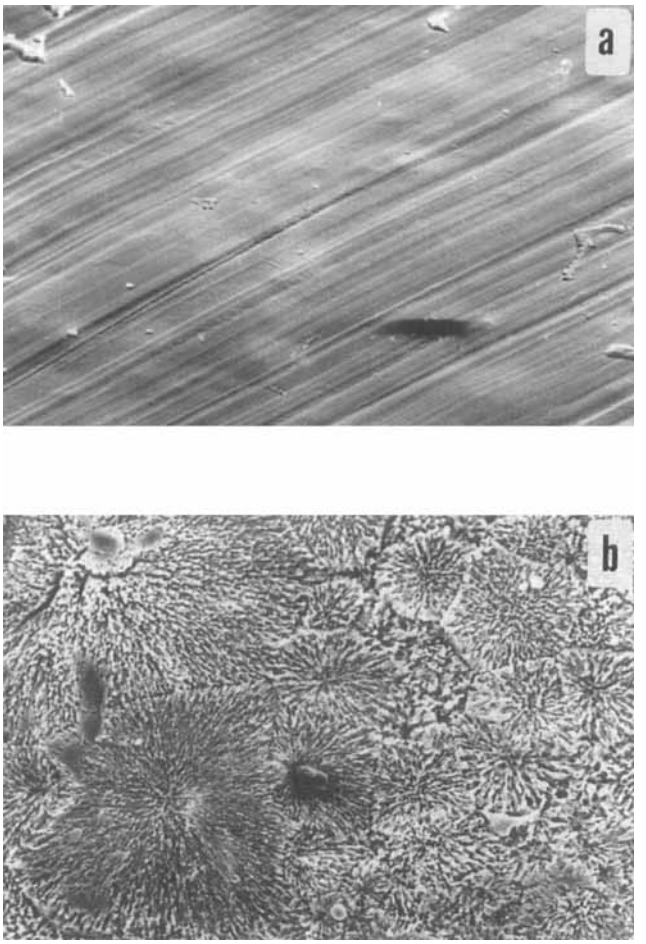

FIG. 3. Scanning electron micrographs of the surface of a PHB (RT) film: (a) before and (b) after 4 days in activated sludge; bar = 0.1 mm.

tions of freeze-fractured biodegraded samples, the layer thickness is estimated as 5–10 μm.

The cellulose ester used in this work was an almost totally substituted cellulose (DS_{Bu} = 2.58; DS_{Ac} = 0.36), which showed no weight loss during twelve months of immersion in activated sludge. It was recently demonstrated that while films of cellulose acetate with DS $\leq$ 2.5 substantially degrade in both wastewater activated sludge [21] and aerobic composting bioreactors [21, 22], in the same experimental conditions cellulose triacetate shows no biodegradation in terms of weight loss [21]. Along these lines, a decrease of biodegradability with increasing DS in acetic esters of starch, cellulose, and xylan was recently reported by Glasser et al. [23]. It was also suggested [21] that the first step in cellulose acetate degradation is hydrolysis of the acetyl groups; only afterward do the cellulases become effective in degrading the polysaccharide main chain. As regards substituents bulkier than acetate, recently Komarek et al. [24] showed that cellulose propionate with DS = 1.8 undergoes

biodegradation in an in-vitro aerobic culture system. It is reasonable to suppose that the problems connected with increasing degree of substitution in cellulose acetate will be enhanced the longer the substituent. In keeping with such considerations, the highly substituted CAB used in this work showed no weight loss during 1 year of immersion in activated sludge.

After the same period of time, no weight loss was observed in PHB/CAB blends, irrespective of the type of thermal treatment applied prior to exposure (RT, RT + 90, or IC90). For the sake of simplicity, let us consider RT blends. In the range of CAB contents of 50–100%, they are composed of a single homogeneously mixed amorphous phase, which is glassy at room temperature. The absence of weight loss in these blends indicates that the attack to PHB macromolecules by PHB depolymerases, whose existence in the environmental conditions employed is demonstrated by fast biodegradation of pure PHB, is totally inhibited by the presence of intimately mixed cellulose ester chains. When the amount of CAB component decreases below 50%, part of the bacterial polymer present in the blends segregates through crystallization and constitutes a crystalline pure PHB phase, coexisting with an amorphous mixture of varying composition. Quite surprisingly, PHB-rich blends showed no biodegradation at all, the surface of the exposed films remaining smooth (by SEM). Figure 4 illustrates the results obtained with the 80/20 RT blend together with weight loss data relative to pure (RT) PHB for the sake of comparison. A result relative to a 80/20 blend prepared by solvent casting is also included in Fig. 4 to show the effect of sidedness: a layer of practically pure PHB forms upon evaporation at the solution-air interface, which biodegrades, causing weight loss. The melting enthalpy data of Fig. 1 can be used to estimate the amount of bacterial polymer present as a segregated crystalline phase in the 80/20 RT blend. The calculations show that as much as 52% of the total blend weight is represented by crystalline PHB. It was already mentioned that in PHB-rich blends prepared by compression molding the bacterial polymer crystallizes according to a spherulitic

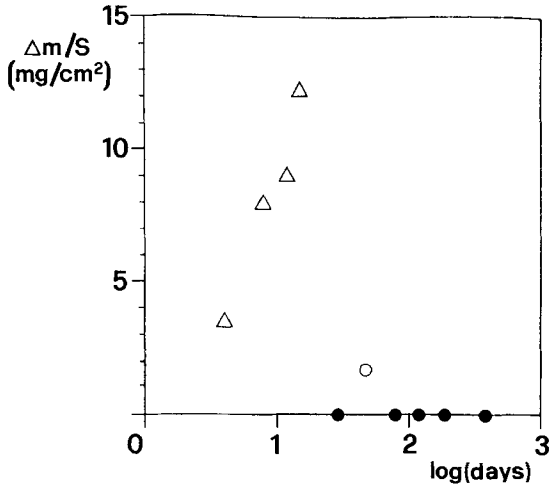

FIG. 4. Weight loss in activated sludge as a function of time of (△) RT PHB and of 80/20 PHB/CAB blend; (○) compression molded (RT type); (●) solvent cast.

morphology: the lamellae are formed of pure PHB and the amorphous PHB/CAB mixture is located in the interlamellar space. If 52% of the blend weight in the 80/20 sample is crystalline PHB, it is reasonable to assume that the sample surface will expose some PHB crystals to enzymatic attack. Experimental evidence of the total absence of enzymatic activity toward accessible phase-segregated PHB is puzzling and deserves some comment.

Work by Saito and coworkers [25–27] dealing with a poly(3-hydroxybutyrate)-depolymerase from *Alcaligenes faecalis* T1 proposes different mechanisms of action of the enzyme toward different substrates. In particular, it is suggested that while water-soluble 3-hydroxybutyrate oligomers are enzymatically hydrolyzed in an *endo* manner [25], a solid sample of the hydrophobic polymer is degraded in an *exo* fashion through attack to the free hydroxyl chain ends and the release of dimers [26, 27]. In the case of a PHB sample with spherulitic morphology, the *exo* degradation mechanism requires that enzymatic attack starts from the hydroxyl chain ends which are located (together with loops and trapped disordered chain segments) in the interlamellar amorphous phase, in agreement with extensive experimental evidence of initial interlamellar biodegradation. Although extension of the mechanism proposed for the *Alcaligenes faecalis* T1 depolymerase to other PHB-degrading enzymes is highly arbitrary, it is interesting to note that the present results seem to fit an analogous picture. Let us consider blend 80/20, where no biodegradation occurs during 1 year of exposure to activated sludge, notwithstanding a large—and supposedly partly accessible—pure crystalline PHB fraction. In this partially crystalline blend the hydroxyl PHB chain ends—hypothetically required by depolymerases to initiate hydrolysis—reside in a mixed interlamellar phase. With the reminder that no evidence of biodegradation is obtained in amorphous PHB/CAB blends, it appears that in blend 80/20 the lack of enzymatic attack to exposed PHB crystals is dictated by chain terminal inaccessibility. It is not yet clear why PHB in the amorphous mixed phase does not undergo enzymatic hydrolysis.

One hypothesis contemplates a dramatic decrease of chain mobility with respect to pure PHB. An alternative reason might be a detrimental change of surface hydrophobicity brought about by the partner polymer in the blend. In this respect it was demonstrated [28, 29] that enzymatic degradation of solid PHB requires depolymerases containing a hydrophobic domain which provides the binding site to the substrate. The cellulose ester intimately mixed with PHB might modify the hydrophobic character of the surface, thus preventing enzyme attachment. Both possibilities (loss of amorphous phase mobility and change of hydrophobicity of the polymeric substrate) need to be tested by selecting appropriate polymers to be blended with PHB. Work is in progress along these lines.

CONCLUSIONS

The present work has shown that rather subtle morphological changes in spherulitic bacterial PHB—varying the average crystal size at constant overall crystallinity—affect the rate of biodegradation in activated sludge. This result calls for attention when comparing data from different sources in the absence of a detailed morphological characterization. The lack of any experimental evidence of biodegra-

dation in all PHB/CAB blends exposed for 12 months to activated sludge raises a number of questions. Most striking in the absence of enzymatic attack to blend 80/20, where pure crystalline PHB amounts to more than 50% of the total blend weight. In pure spherulitic PHB, the depolymerases attack the accessible interlamellar amorphous phase at first, but eventually the crystalline lamellae also undergo biodegradation. The surface layer being sequentially degraded is thin and does not change much in the course of biodegradation, indicating that once the lamellae are cleared of the interlamellar material, they are easily attacked by the enzymes. Apart from possible effects on enzyme–substrate interaction due to changes in surface hydrophobicity upon blending, the results of the present work seem to suggest that attack to the lamellar PHB crystalline phase in crystalline PHB/CAB blends is conditioned by prior consumption of some interlamellar amorphous material. Nondegradability of the mixed interlamellar phase seems to be responsible for inaccessibility to the enzyme of the pure bacterial polymer phase represented by the crystalline lamellae.

REFERENCES

[1] P. A. Holmes, in *Developments in Crystalline Polymers* (D. C. Basset, Ed.), Elsevier, New York, 1988, Vol. 2.

[2] Y. Doi, *Microbial Polyesters*, VCH Publishers, New York, 1990.

[3] Y. Kumagai and Y. Doi, *Polym. Degrad. Stab., 35*, 87 (1992).

[4] Y. Kumagai and Y. Doi, *Ibid., 36*, 241 (1992).

[5] Y. Kumagai and Y. Doi, *Ibid., 37*, 253 (1992).

[6] P. Sadocco, C. Bulli, E. Elegir, A. Seves, and E. Martuscelli, *Makromol. Chem., 194*, 2675 (1993).

[7] D. F. Gilmore, R. C. Fuller, B. Schneider, R. W. Lenz, N. Lotti, and M. Scandola, *J. Environ. Polym. Degrad.*, In Press.

[8] B. A. Ramsay, V. Langlade, P. J. Carreau, and J. A. Ramsay, *Appl. Environ. Microbiol., 59*, 1242 (1993).

[9] Y. Kumagai, Y. Kanesawa, and Y. Doi, *Makromol. Chem., 193*, 53 (1992).

[10] M. Parikh, R. A. Gross, and S. P. McCarthy, *Polym. Mater. Sci. Eng., 66*, 408 (1992).

[11] H. P. Klug and L. E. Alexander, *X-Ray Diffraction Procedures for Polycrystalline and Amorphous Materials*, Wiley, New York, 1974.

[12] P. J. Barham, A. Keller, E. L. Otun, and P. A. Holmes, *J. Mater. Sci., 19*, 2781 (1984).

[13] M. Scandola, G. Ceccorulli, and M. Pizzoli, *Macromolecules, 25*, 6441 (1992).

[14] N. Lotti and M. Scandola, *Polym. Bull., 29*, 407 (1992).

[15] G. Ceccorulli, M. Pizzoli, and M. Scandola, *Macromolecules, 26*, 6722 (1993).

[16] M. Pizzoli, M. Scandola, G. Ceccorulli, and U. Piana, *Book of Abstracts of the 4th European Symposium on Polymer Blends*, Capri, 1993, p. 309.

[17] K. Okamura and R. H. Marchessault, in *Conformation in Biopolymers* (Ramachandran, Ed.), Academic Press, London, 1974, pp. 709–720.

[18] S. Brückner, S. V. Meille, L. Malpezzi, A. Cesàro, L. Navarini, and R. Tombolini, *Macromolecules, 21*, 967 (1988).
[19] M. Pizzoli, M. Scandola and G. Ceccorulli, *Ibid.*, Submitted.
[20] Y. Doi, Y. Kanesawa, M. Kunioka, and T. Saito, *Ibid., 23*, 26 (1990).
[21] C. M. Buchanan, R. M. Gardner, and R. J. Komarek, *J. Appl. Polym. Sci., 47*, 1709 (1993).
[22] J.-D. Gu, D. T. Eberiel, S. P. McCarthy, and R. A. Gross, *J. Environ. Polym. Degrad., 1*, 143 (1993).
[23] W. G. Glasser, G. Ravindran, G. Samaranayake, R. K. Jain, J. S. Todd, and J. Jervis, Paper Presented at the 206th ACS National Meeting, Division of Cellulose, Paper and Textile, Chicago, 1993.
[24] R. J. Komarek, R. M. Gardner, C. M. Buchanan, and S. Gedon, *J. Appl. Polym. Sci., 50*, 1739 (1993).
[25] Y. Shirakura, T. Fukui, T. Saito, Y. Okamoto, T. Narikawa, K. Koide, K. Tomita, T. Takemasa, and S. Masamune, *Biochim. Biophys. Acta, 880*, 46 (1986).
[26] T. Tanio, T. Fukui, Y. Shirakura, T. Saito, K. Tomita, T. Kaiho, and S. Masamume, *Eur. J. Biochem., 124*, 71 (1982).
[27] J. J. Jesudason, R. H. Marchessault, and T. Saito, *J. Environ. Polym. Degrad., 1*, 89 (1993).
[28] T. Fukui, T. Narikawa, K. Miwa, Y. Shirakura, T. Saito, and K. Tomita, *Biochim. Biophys. Acta, 952*, 164 (1988).
[29] K. Mukai, K. Yamada and Y. Doi, *Int. J. Biol. Macromol., 15*, 361 (1993).