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Hydrolytic Degradation of Poly(3-hydroxybutyrate), Polylactide and their Derivatives: Kinetics, Crystallinity, and Surface Morphology

A. P. BONARTSEV,[1,2] A. P. BOSKHOMODGIEV,[1]
A. L. IORDANSKII,[1,3,*] G. A. BONARTSEVA,[1] A. V. REBROV,[4]
T. K. MAKHINA,[1] V. L. MYSHKINA,[1] S. A. YAKOVLEV,[1]
E. A. FILATOVA,[1] E. A. IVANOV,[1] D. V. BAGROV,[4]
AND G. E. ZAIKOV[3]

[1]A. N. Bach's Institute of Biochemistry, Russian Academy of Sciences, Leninskiy prosp. 33, 119071 Moscow, Russia
[2]Faculty of Biology, Moscow State University, Leninskie gory 1-12, 119992 Moscow, Russia
[3]General Institute of Chemical Physics, Russian Academy of Sciences, Kosygin str. 4, 119991 Moscow, Russia
[4]A. V. Topchiev Institute of Petroleum Chemistry, Leninskiy prosp. 27, 119071 Moscow, Russia

Hydrolytic degradations of biodegradable poly(3-hydroxybutyrate) (PHB), polylactide (PLA) and their derivatives were explored by kinetic and structure methods at 37 and 70°C in phosphate buffer. It was revealed the kinetic profiles for copolymer PHBV (20% of 3-hydroxyvalerate) and the blend PHB-PLA (1:1 wt. ratio). The intensity of biopolymer hydrolysis depending on temperature is characterized by total weight loss and the viscosity-averaged molecular weight decrement (ΔMW) as well as by WAXS and AMF techniques. Characterization of PHB and PHBV includes both ΔMW and crystallinity evolution (x-ray diffraction) as well as the AFM analysis of PHB film surfaces before and after aggressive medium exposition. The degradation is enhanced in the series PHBV < PHB < PHB-PLA blend < PLA. The impact of MW on the biopolymer hydrolysis is shown.

Keywords AMF; bacterial poly(3-hydroxybutyrate); copolymer PHBV; crystallinity; PHB-PLA blend non-enzymatic hydrolysis; polylactide; role of molecular weight; total weight loss; WAXS

Introduction

The bacterial polyhydroxyalkanoates (PHA)s and their principal representative - poly(3-R-hydroxybutyrate) (PHB) create a competitive option to conventional synthetic polymers such as polypropylene, polyethylene, polyesters et al. The biopolymers are nontoxic and

*Address correspondence to A. L. Iordanskii, Institute of Petroleum Chemistry, Leninskiy prosp. 27, 119071 Moscow, Russia. Phone: +74959397434; Fax: +74959382956; E-mail: aljordan08@gmail.com

renewable. Their biotechnological productions do not based on fossil-fuels as well as their biodegradation intermediates and resulting products (water and carbon dioxide) do not provoke the adverse actions in environmental media or living systems [1–3]. Being friendly environmental and biocompatible [4], the PHB and its derivatives are widely used as the alternative packaging and biomedical materials [5,6].

The copolymerization of 3-hydroxybutyrate entities with 3-hydroxyoctanoate (HO), 3-hydroxyheptanoate (HH) or 3-hydroxyvalerate (HV) fragments modifies the physical and mechanical characteristics of the neat PHB, such as ductility and toughness to depress its processing temperature window and to improve exploitation parameters. Besides, copolymers PHB-HV [7], PHB-HH [8] or PHB-HO [9] et al. have improved thermophysical and/or mechanical properties and hence they expand the spectrum of constructional and medical materials/items. For predicting the behavior of PHB and its copolymers in a aqueous media e.g. in vitro, in a living body or in a wet soil, it is essential to study kinetics and mechanism of hydrolytic destruction.

Despite the history of such-like investigations reckons about 25 years, the problem of (bio)degradation in semicrystalline biopolymers is too far from a final resolution. Moreover, in the literature the comprehensive description of hydrolytic degradation kinetics during long-term period is rarely appearing [10–14]. Therefore, the main object of this paper is the comparison of long-term degradation kinetics for the PLA, PHB and their derivatives, specifically the copolymer of PHB with 3-oxyvalerate (PHBV) and the blend PHB/PLA. The contrast between degradation profiles for PHB and PLA makes possible to compare the general degradation behavior for two most prevalent biodegradable polymers. Besides, a significant attention is devoted to the impact of molecular weight (MW) for above polymer systems upon hydrolytic degradation and morphology (crystallinity and surface roughness) at the physiological (37°C) and elevated (70°C) temperatures.

Experimental

Materials

In this work we have used poly-L-lactide (PLA) with different molecular weights: 67, 152, and 400 kDa (Fluka Germany); chloroform (ZAO EKOS-1, RF), sodium valerate (Sigma-Aldrich, USA), and mono-substituted sodium phosphate (NaH_2PO_4, ChimMed, RF).

PHAs Production

The samples of PHB and copolymer of hydroxybutyrate and hydroxyvalerate (PHBV) have been produced in A.N.Bach's Institue of Biochemistry. A highly efficient strain-producer (80 wt.% PHB in the dry weght of cells), *Azotobacter chroococcum 7*Б, has been isolated from rhizosphere of wheat (the sod-podzol soil). Details of PHB biosynthesis have been published in [15]. Under conditions of PHBV synthesis, the sucrose concentration was decreased till 30 g/L in medium and, after 10 h incubation, 20 mM sodium valerate was added. Isolation and purification of the biopolymers were performed via centrifugation, washing and drying at 60°C subsequently. Chloroform extraction of BPHB or BPHBV from the dry biomass and precipitation, filtration, washing again and drying have been described in our previous work [15]. The monomer-content (HB/HV ratio) in PHBV has been determined by nuclear magnetic resonance in accordance with procedure described previously in [16]. The percent concentration of HV moiety in the copolymer was calculated as the ratio

between the integral intensity of methyl group of HV (0,89 ppm) and total integral intensity the same group and HB group (1,27 ppm). This value is 21 mol.%.

Molecular Weight Determination

The viscosity-averaged molecular weight (MW) was determined by the viscosity (η) measurement in chloroform solution at 30°C. The calculations of MW have been made in accordance with Mark-Houwink equation [17]:

$$[\eta] = 7{,}7 \cdot 10^{-5} \cdot \mathrm{M}^{0,82}$$

Film Preparations of PHAs, PLA and their Blends

The films of neat polymers (PHB, PHBV and PLA) and their blends with the thickness about 40 μm were cast on a fat-free glass surface. We obtained the set of films with different MW = 169 ± 9 (defined as PHB 170), 349 ± 12 (defined as PHB 350), 510 ± 15 kDa (defined as PHB 500) and 950 ± 25 kDa (defined as PHB 1000) as well as the copolymer PHBV with MW = 1056 ± 27 kDa (defined as PHBV). Additionally we prepared the set of films on the base of PLA with same thickness 40 μm and MW = 67 (defined as PLA 70), MW = 150 and 400 kDa. Along with them we obtained the blend PHB/PLA with weight ratio 1:1 and MW = 950 kDa for PHB, and MW = 67 kDa for PLA (defined as PHB+PLA blend). Both components mixed and dissolved in common solvent, chloroform and then cast conventionally on the glass plate. All films were thoroughly vacuum-processed for removing of solvent at 40°C.

Hydrolytic Degradation In Vitro Experiments

Measurement of hydrolytic destruction of the PHB, PLA, PHBV films and the PHB-PLA composite was performed as follows. The films were incubated in 15 ml 25 mM phosphate buffer, pH 7.4, at 37°C or 70°C in a ES 1/80 thermostat (SPU, Russia) for 91 days; pH was controlled using an Orion 420+ pH-meter (Thermo Electron Corporation, USA). For polymer weight measurements films were taken from the buffer solution every three day, dried, placed into a thermostat for 1 h at 40°C and then weighed with a balance. The film samples weighed 50–70 mg each. The loss of polymer weight due to degradation was determined gravimetrically using a AL-64 balance (Acculab, USA). Every three days the buffer was replaced by the fresh one.

Wide Angle X-ray Diffraction

The PHB and PHBV chemical structure, the type of crystal lattice and crystallinity was analyzed by wide angle X-ray scattering (WAXS) technique. X-ray scattering study was performed on device on the basis of 12 kW generator with rotating copper anode RU-200 Rotaflex (Rigaku, Japan) using CuK radiation (wavelength $\lambda = 0.1542$ nm) operated at 40 kV and 140 mA. To obtain pictures of wide angle X-ray diffraction of polymers two-dimentional position-sensitive X-ray detector GADDS (Bruker AXS, Germany) with flat graphite monochromator installed on the primary beam was used. Collimator diameter was 0.5 mm [18].

Atomic Force Microscopy of PHB Films

Microphotographs of the surface of PHB films were obtained be means of atomic force microscopy (AFM). The AFM imaging was performed with Solver PRO-M (Zelenograd, Russia). For AFM imaging a piece of the PHB film ($\sim 2 \times 2$ mm^2) was fixed on a sample holder by double-side adhesive tape. Silicon cantilevers NSG11 (NT-MDT, Russia) with typical spring constant of 5.1 N/m were used. The images were recorded in semi-contact mode, scanning frequency of 1–3 Hz, scanning areas from 3×3 to 20×20 μm^2, topography and phase signals were captured during each scan. The images were captured with 512×512 pixels. Image processing was carried out using Image Analysis (NT-MDT, Russia) and FemtoScan Online (Advanced technologies center) software.

Results and Discussion

The degradation of PHB with different molecular weight (MW) and its derivatives (PHBV, blend PHB/PLA) prepared as films was observed by the control of total weight loss, MW, and morphologies (AFM, XRD) for the period of 91 days.

1 The Hydrolysis Kinetics of PLA, PHB, and their Derivatives

The hydrolytic degradation of biopolymers (PHB, PLA) and their derivatives (the copolymer PHBV and the blend PHB/PLA) has been monitored for 3 months under in vitro condition: the phosphate buffer, pH = 7.4, 37°C. The analysis of kinetic curves shows that the highest rate of weight loss is observed for PLA with the lowest MW $\approx$ 70 kDa and for PHB with the relatively low MW $\approx$ 150 kDa (Fig. 1). On the base of these data it is possible to compare the weight loss for the polymers with different initial MW. In Fig. 1 we clearly see that the samples with the relatively high MWs (300–1000 kDa) are stable against hydrolytic degradation in contrast to the samples with the lowest MW. The total weight of PHB with initial MW = 150 kDa is decreased sharper in comparison with the weight decrease of the other PHB samples. Moreover, during the exposure in 91 days the total weight loss of PHB with MW = 150 kDa reaches 10,5% that is essentially higher than the weight loss of the other PHB samples.

After the MW impact upon the hydrolysis was established, we compared the weight-loss kinetic curves for the PLA and PHB films with the comparative MWs equal 400 and 350 kDa respectively and the same film thicknesses ($\sim$40 μm) for the both polymers. If we have pairwise compared the hydrolysis intensity for PLA and PHB samples having the close MWs, the total weight of the formers decreases with the higher rate than the rate of PHB. The results obtained here are in line with the previous literature data demonstrating the poor stability of PLA relative to PHB [8,15–18].

Comparing the degradation behavior of the homopolymer PHB and the copolymer PHBV, we can conclude that the introduction of hydrophobic units (HV) into the PHB molecule enhances the hydrolytic stability of PHBV molecules. For PHBV the hydrolysis has the longest period of induction in comparison with the other polymer systems. Within 70 days its weight loss was minimal ($<$1% wt) and probable related with desorption of low-molecular impurities presenting in the polymer after biosyntheis and isolation. The kinetic curves in Fig. 1 demonstrate also the decrease of blend hydrolysis in comparison with the rate of initial PHB (MW = 1000 kDa) even if the second component is a readily hydrolysable PLA (MW = 70 kDa).

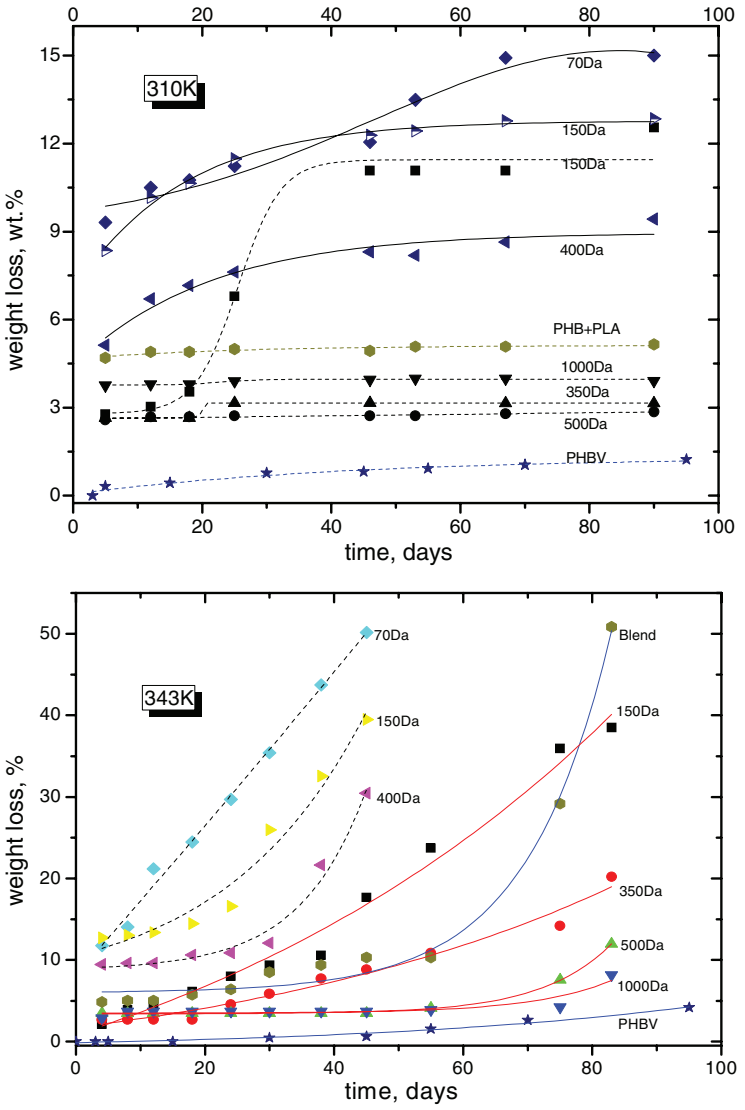

Figure 1. Weight loss in the phosphate buffer for PHB and its derivatives with different MW (shown on the curves in kDa). 37°C (343K), 70°C (310K): ♦, ►, and ◄ are PLA films with MW = 70, 150, and 400 kDa respectively; ■, ▲, ●, and ▼ are PHB samples with 170, 350, 500, and 1000 kDa, respectively; PHBV 1050 (□); and PHB-PLA blend (⬢).

To specify the concept of biopolymer degradation and to accelerate chemical and transport processes in degradable matrix, its contact with aggressive media has been often performed at elevated temperatures [15,19]. To find out a temperature influence on degradation of PHB, PLA, and other polymer systems and intensify hydrolytic reactions, we have kept the temperature in phosphate buffer at 70°C. As one should expect, under such condition the hydrolysis of all samples is enhanced as presented in Fig. 2. By the 45th day of PLA incubation its films turned into the fine-grinding dust with the weight-loss about

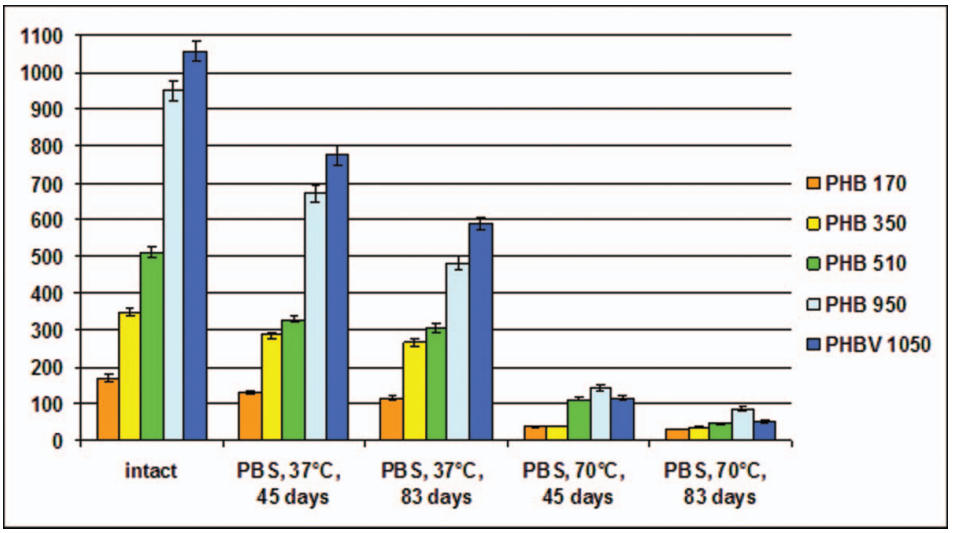

Figure 2. The molecular weight conversion of PHB and PHBV films during hydrolysis in phosphate buffer (PBS), pH = 7.4, 37°C and 70°C.

50% (MW = 70 kDa) and 40% (MW = 350 kDa). Similarly, PHB with the lowest MW = 170 kDa has the weight loss 38 wt.% and the film was markedly fragmented as well, while the PHB samples with the higher initial MWs 350, 500 and 1000 kDa lost the essentially less percents, namely 20, 10 and 15% respectively. Additionally, in 83 days the weight drop in the PHB-PLA blend was about 51 wt.% and hence blend hydrolytic stability observed at 37°C is essentially declined at elevated temperature (cf. Figs. 1 and 2).

Summarizing the behavior of the copolymer at elevated (70°C) and physiological (37°C) temperatures, we have shown again that the PHBV films are most stable, as they lost only 4 wt% in 95 days of exposing. The enhanced stability of PHBV relative to the parent PHB has been confirmed by recent literature data [18]. Here it is worth to note that during biosynthesis of PHBV when the part of the methyl groups in the macromolecules are replaced by the ethyl groups, two effects of water sorption are opposed to each other. On the one side, the total hydrophobicity of the copolymer exceeds the analogous characteristic of PHB and the water activity should decrease. On the other side, such biosynthetic replacement dislocates the regularity of parent polymer molecules and, as a result, it should lead to decrease of crystallinity in the copolymer and hence to a water sorption increase [20]. The interplay between two opposite processes determines a total water concentration in the copolymer and therefore stimulates hydrolytic degradation. In the case of PHBV copolymer (4:1 mol. ratio) the hydrophobization of its chain predominates the effect of crystallinity decrease (from 75% for PHB to ~60% for PHBV) and hence the total water content is decreased twofold (from 1% for PHB to 0,5 wt.% for PHBV).

2. Change of Molecular Weight for PHB and PHBV

The hydrolytic degradation of biopolymers (PHB, PLA) and their derivatives (the copolymer PHBV and the blend PHB/PLA) has been monitored for 3 months under in vitro condition: the phosphate buffer, pH = 7.4, 37°C. The analysis of kinetic curves shows that the highest rate of weight loss is observed for PLA with the lowest MW ≈ 70 kDa and

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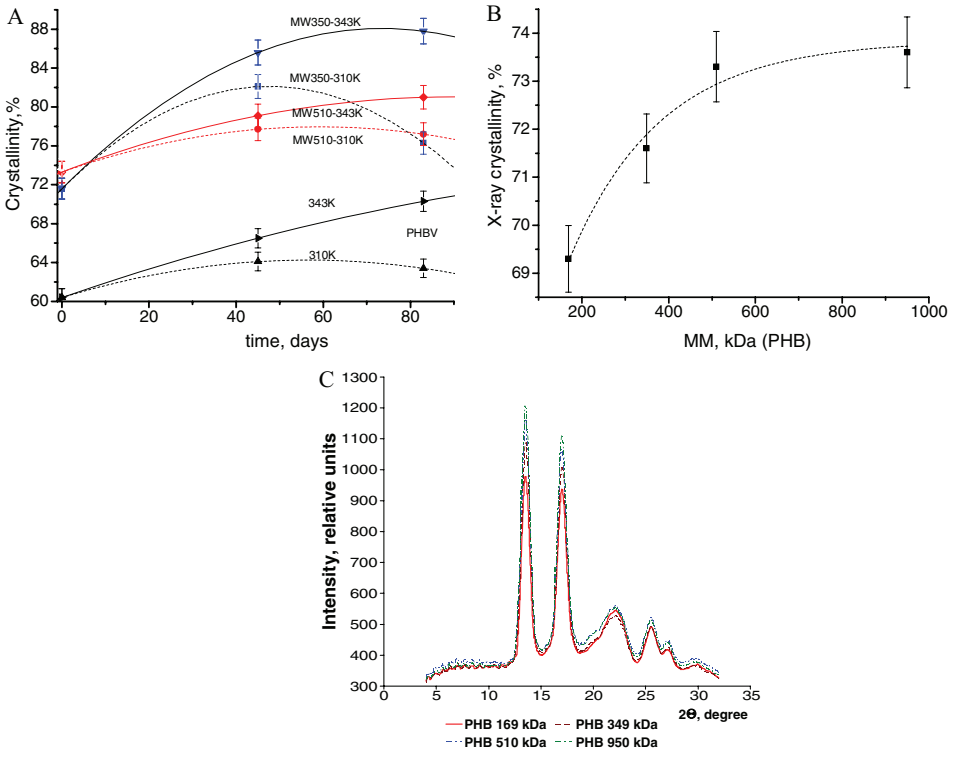

Figure 3. A: Crystallinity evolution during the hydrolysis for PHB and PHBV films (denoted values of temperature and MW). B: Crystallinity as function of initial MW for PHB films prepared by cast method. C: X-ray diffractograms for PHB films with different molecular weight given under x-axis.

water concentration in the copolymer and therefore stimulates hydrolytic degradation. In the case of PHBV copolymer (4:1 mol. ratio) the hydrophobization of its chain predominates the effect of crystallinity decrease (from 75% for PHB to ∼60% for PHBV) and hence the total water content is decreased twofold (from 1% for PHB to 0,5 wt.% for PHBV).

3 Crystallinity of PHB and PHBV

Above we have revealed that during hydrolysis the PHB-based polymers show both the MW and the weight reduction (Section 2 and 1 respectively). Additionally, we measured the crystallinity degrees of PHB and PHBV by the WAXS technique that vary as function of degradation time in the interval 60–80 wt% (see Fig. 3A). In the initial stage of polymer exposing in the buffer (at 37°C for 45 days) the crystallinity degree increases slightly and then, under the following exposition, this characteristic becomes constant or even slightly is decreased showing a weak maximum. If it is taken into account that at 37°C the initial weights of PHB and PHBV with MWs ranging from 350 to 1000 kDa are invariable, a possible reason of the small increase in their crystallinity is recrystallisation described earlier for PLA and PHB [26]. Recrystallization (or additional crystallization) occurs in a number of semicrystalline polymers by mechanism of segmental transport from amorphous phase into the crystallites [22].

At higher temperature of PHB hydrolysis, 70°C, the crystallinity increment is clearly indicated and has a progressive trend. In contrast to recrystallisation mechanism that is more typical at 37°C, the plausible explanation of the MW increase at higher temperature is the hydrolysis propagation in amorphous areas of the biopolymers with the following desorption of hydrophilic degradation products. It is well known that the matrices of PHB and PHBV are composed by alternative crystalline and noncrystalline regions, which determine both polymer morphologies and transport of aggressive medium. Additionally, we have revealed recently by an H-D isotopic exchange technique combined with the FTIR spectroscopy that the functional groups in the PHB crystallites are practically not accessible to water molecules. Therefore, the hydrolytic destruction and the weight decrease are predominantly developed in the amorphous part of polymer [22,27]. Hence, the crystalline fraction in PHB grows because of degradation product desorption from the amorphous phase. This effect takes place under the strong aggressive conditions (70°C) and does not appear under the physiological conditions (37°C).

During the PHBV biosynthesis the appearance of HV entities decreases essentially the characteristics of the parent polymer, PHB, such as crystallinity, melting point, and glass temperatures and hence improve the processing characteristics [14,28,29]. Additionally, we have founded out that the initial crystallinity of PHB films is a monotonically increased function of initial MW (see Fig. 3B). For samples with relatively low molecular weight it is difficult to compose the perfect crystalline entities because of a relatively high concentration of terminal groups that perform crystalline defects.

Thus, at physiological temperature (37°C) the PHB crystallinity measured during degradation has a slightly extreme character. On the initial stage of degradation the crystalline/amorphous ratio was increased owing to additional crystallization. In contrast, at 70°C after reaching the critical MW values (see section 2), the desorption of water-soluble intermediates from polymer matrices occurs. On the second stage, while the degradation was developed, the crystallinity drop should take place as the result of crystallite disruption that was followed by total film disintegration.

4 The Analysis of Film Surfaces for PHB by AFM Technique

Both the surface morphology and the surface roughness of PHB films exposed in the corrosive medium (phosphate buffer) have been studied by the AFM technique. This technique is informative for surface characterization because the state of implanted surface determines not only mechanism of degradation but the protein adsorption and cell adhesion which are responsible for polymer biocompatibility [30]. As the basic sample we have used the PHB film with relatively low MW = 170 kDa. The film cast procedure can lead to distinction in morphology for two opposite surfaces where the one surface of the polymer film is adjacent with a glass plate support and another side is exposed to the air. Really, as it is shown in Fig. 4, the air-exposed surface has a roughness formed by the pores with the depth about 600 nm. The opposite side of the film contacting with the glass support is characterized by minor texture and the pores with the depth as small as 100 nm (Fig. 4b). At higher magnification (here not presented) in certain localities it can see the stacks of polymer crystallites with the width about 100 nm and length 500–800 nm. Thus, the porosity depends on the crystallite morphology, more specifically, by the height dimensions of the crystallite stacks

The difference in two surface morphologies gets clearly evident if we compare quantitatively the parameters of roughness. The statistical analysis of this characteristic has shown that the averaged roughness (R_a) and the root mean square of roughness (R_q) for

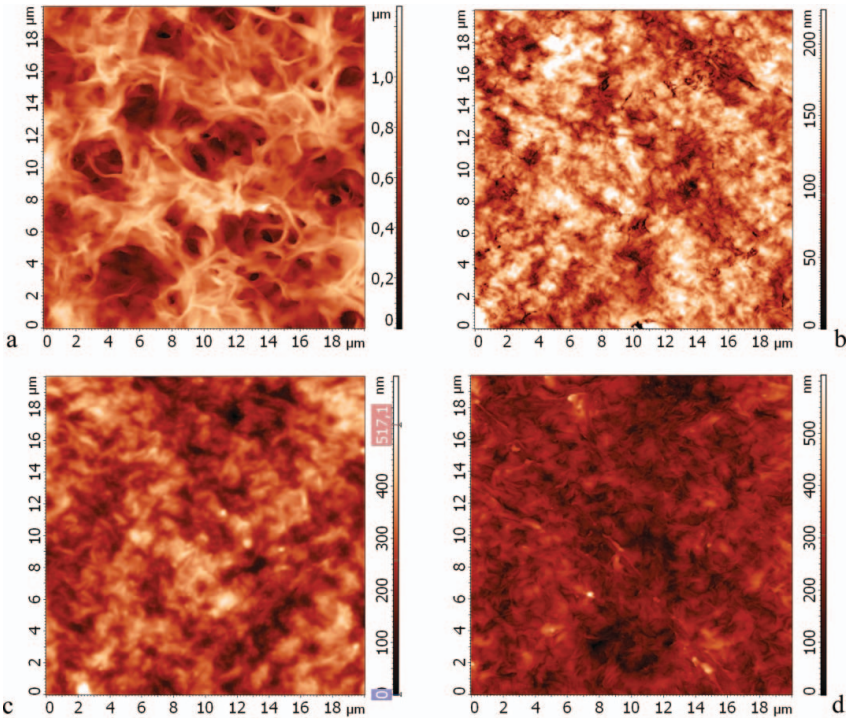

Figure 4. AFM topographic images of PHB films (170 kDa) with a scan size of 18 × 18 μm: the rough surface of fresh-prepared sample (exposed to air) - a; the smooth surface of fresh-prepared sample (exposed to glass) - b; the sample exposed to phosphate buffer at 310K for 83 days - c; the sample exposed to phosphate buffer at 343K for 83 days - d. General magnificence is 300.

film surfaces exposed to the glass or the air differ about ten times (see Table 1). The variance of the surface characteristics are related with solvent removing conditions. During chloroform evaporation through the surface faced to the air, the solvent flux forms additional pore system which is fixed when a film is solidified and crystallized. In contras, the morphology on the opposite side of the film is not subjected by the pore-formation affect of solvent. The morphology of the latter surface depends on interfacial free energy

Table 1. Roughness of PHB 170 kDa films

Sample	Side of film	R_a, nm	R_q, nm
Control	"rough*"	130 ± 10	165 ± 10
Control	"smooth**"	15 ± 2	20 ± 1
PBS buffer, pH 7.4, 37°C	"rough*"	135 ± 5	166 ± 7
PBS buffer, pH 7.4, 37°C	"smooth**"	46 ± 2	59 ± 1
PBS buffer, pH 7.4, 70°C	"rough*"	138 ± 6	167 ± 7
PBS buffer, pH 7.4, 70°C	"smooth**"	41 ± 3	52 ± 3

PBS - acronym of phosphate buffer, * - film surface exposed to air, ** - film surface exposed to glass.

at glass-biopolymer interface predominantly. The exposure of PHB films in the buffer at long-term condition (for 83 days) leads to a threefold growth of roughness characteristics (see Table 1) glass-biopolymer and practically does not affect the air-exposed surface. It is interesting that temperature of film degradation does not influence on the roughness. After treatment at 37°C and 70°C, the surface characteristics have the same values.

Summarizing the AMF data, we can conclude that during degradation the air-exposed rough surface remains stable that probably related with the volume mechanism of degradation (V-type [31,32]). The pores on the surface provide the fast water diffusion into the bulk of PHB. However, under the same experimental conditions, the change of surface porosity (roughness) for glass-exposed surface is remarkable that shows the involving of poor-pore surface into degradation (S-type [31,32]). In the works dealing with PHB destruction, the authors [20,21] have recently reported on domination of surface mechanism. Another point of view states a volume mechanism of degradation [12]. Here, using an advanced method of surface investigation (AMF) we have shown that for the same film under the same exterior conditions the mechanism of degradation could be combined by V and S modes of degradation. The parity of both mechanisms or the domination of anyone of them depend on the specific conditions of polymer preparation.

Conclusion

Analyzing the results on hydrolytic degradation of PLA and PHB as well as their derivatives, we suppose that two consecutive stages of the process are presented as follows. During the initial stage of hydrolysis, the total weight is invariable but the cleavage of macromolecules is observed. Within this time the PHB fragments are too large and hydrophobic to diffuse from the polymer matrix into aqueous media. Because the PHB crystallites are stable, the crystallinity degree is constant or even it may slightly grow up due to additional crystallization. On the second stage of degradation, when the MW of PHB decreases and attains a critical value about 30 kDa, the intermediates can dissolve and diffuse from the polymer into an aqueous medium. Within second period the weight loss and the MW decrement are clearly observed and the hydrolytic process is enhanced in the series PHBV < PHB < PHB-PLA < PLA.

The growth of initial MW (a terminal group content reduction) impacts on the hydrolytical stability probably due to the increase of both crystallite perfection and crystallinity degree. The WAXD data reflect this trend (see Fig. 3B). Additionally, the surface morphology of PHB films explored by AFM depends on the conditions of film preparation. For the cast films there is a great difference in morphologies of surfaces exposed to air and to glass plate. It is known that the mechanism of hydrolysis could include two competitive processes, namely, a) volume degradation and b) surface degradation. Under enhanced pore formation (in the surface layer exposed to air) the V- mechanism prevails. The smooth surface of PHB film contacted during preparation with the glass plate is degraded much intensely than the opposite rough surface.

In conclusion, we have shown that the MW determines the form of a hydrolysis kinetic profile. For acceleration of degradation it should be used the polymers with the low MW values. In this case we affect both the degradation rate and the crystalline degree. By contrast, for a service-time prolongation of biodegradable materials working in living systems or as the packaging barriers it is preferable to use the high-MW PHB or PHBV copolymer as the most stable polymers.

Acknowledgments

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