# Environmental Degradation of Polyester Blends Containing Atactic Poly(3-hydroxybutyrate). Biodegradation in Soil and Ecotoxicological Impact

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*Received July 20, 2006; Revised Manuscript Received August 22, 2006*

The degradation of poly[(*R*,*S*)-3-hydroxybutyrate], a-PHB, binary blends with natural PHB (n-PHB) and poly(L-lactic acid), PLLA, respectively, has been investigated in soil. In such a natural environment, a-PHB blend component was found to biodegrade. The degradation of a-PHB-containing blends proceeded faster than that of respective plain n-PHB and PLLA. The molecular weight decrease of the n-PHB component was higher, while the same rate of bioerosion of both components was observed for the a-PHB/n-PHB binary blend. For the a-PHB blend with PLLA, the weight loss was accompanied by blend composition changes and the decrease of a-PHB content. However, the PLLA molecular weight decrease was lower in the blend in comparison with the plain PLLA sample. The increase of the number of microorganisms particularly observed for the soil where binary blends were incubated indicates that microbial degradation of a-PHB takes place. The terrestrial plant growth test (cress and barley) demonstrates no environmental toxicity of the materials studied.

## Introduction

Degradable polymers used in agriculture, packaging, or for disposable articles production must satisfy special requirements. Ideally, such polymeric materials should be a part of the natural life cycle of biomass; that is, raw materials should be renewable and should biodegrade in the environment to harmless natural products. Biodegradable polymers, the degradation of which is initiated by microorganisms such as bacteria, fungi, and algae,[1] offer a viable alternative to commodity plastics in a number of bulk applications. Among biodegradable polymers, the best known are poly($\beta$-hydroxyalkanoates),[2] which can be produced on a large scale through bacterial fermentation.[3] The most common representative of this family of biopolymers, poly([*R*]-3-hydroxybutyrate) (n-PHB) with isotactic structure, is synthesized by a variety of bacteria as a reserve energy source[2,4] and possesses a remarkable feature of being totally biodegradable in various environments. Poly($\alpha$-hydroxyalkanoates) such as poly-L-lactic acid (PLLA) also constitute a promising group of degradable materials. PLLA is a chemically hydrolyzable and bioerodible material synthesized from lactic acid derived from renewable sources obtained by microbial fermentation of biomass. PLLA decomposes rapidly and completely in proper compost environment to yield carbon dioxide and biomass.[5−8] Sometimes it is classified as a bioassimilable polymer due to the mechanism of the degradation proposed; that is, at the beginning of the degradation process, the hydrolytic degradation leads to oligomeric lactic acid products, which next can be bioassimilated or mineralized by microorganisms such as fungi or bacteria.[9,10]

Biodegradable polymer blends were developed to enhance the degradation of the final products obtained from the polyesters mentioned above. Blending these polymers with atactic poly[(*R*,*S*)-3-hydroxybutyrate] (a-PHB) opens new opportunities.[11−13] The atactic poly[(*R*,*S*)-3-hydroxybutyrate] (a-PHB), a synthetic amorphous analogue of n-PHB, can be prepared by anionic polymerization of $\beta$-butyrolactone,[14,15] the monomer that could be obtained using synthetic gas derived from coal or waste biomass gasification.[16] Synthetic a-PHB undergoes heterogeneous enzymatic attack (by PHB depolymerse) in the presence of a second crystalline polymer in the form of a component of binary blend or block in a-PHB containing block copolymer. Moreover, the heterogeneous enzymatic hydrolysis of a-PHB occurs both when the crystalline component is susceptible to enzymatic attack itself, as in the case of n-PHB[17] and PHBV,[11] and when it is nonbiodegradable by the PHB depolymerase, that is, in the case of poly($\epsilon$-caprolactone) (PCL), poly(L-lactic acid) (PLLA),[18] and polypivalolactone (PPVL).[19] Enzymatic degradation of a-PHB can be induced by its blending with high glass transition temperature amorphous polymers such as poly(methyl methacrylate) (PMMA) and atactic poly(L,D-lactic acid).[20−22] Recently, it was found that a-PHB, for a long time known as the polyester that in the pure state cannot be hydrolyzed by extracellular PHB depolymerases, could be degraded to the mixture of monomer, dimer, and trimer in the presence of PHA depolymerases purified from *Paucimonas lemoignei* (*PhaZ7*)[23] as well as *Acidovorax* Sp. TP4 ($PhaZ_{aci}$).[24] Thus, the synthetic a-PHB can be degraded also in a pure state by natural PHA depolymerase.[23,24] However, little is known about the environmental biodegradation of materials containing a-PHB, and, to our knowledge, there is no report on their degradation in soil.

The morphology of the polymer blends containing a-PHB was studied before, and it is known that a-PHB/n-PHB blends

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10.1021/bm060708r CCC: $33.50 
Published on Web 09/28/2006

consisting of natural PHB and its synthetic analogue prepared with zinc-based initiator are miscible.[17,25,26] Such melt processed binary blends possess spherulitic and lamellar morphology with a-PHB chains incorporated in the amorphous regions between individual lamellae within the spherulites.[26] The blends of synthetic a-PHB, prepared via anionic ROP, with a natural bacterial PHBV containing 10 mol % of 3HV units are also miscible in the range of compositions investigated (10−50% a-PHB).[11] It was reported that anionically prepared a-PHB forms miscible blends with PLLA from the melt over the whole range of compositions[18] and that spherulites of PLLA that entrap the amorphous a-PHB component develop upon isothermal crystallization. PLLA and a-PHB prepared with zinc-based initiator can form miscible blends depending on the molecular weight of the two polymer components, and the decrease of a-PHB molecular weight improves miscibility.[12] Dynamic mechanical spectra for such binary blends indicated the partial phase separation of two components occurring in the amorphous phase during the isothermal crystallization process. The effect of a-PHB/PLLA blend composition on morphology was evaluated from a structural point of view.[27,28]

For the first time, to our knowledge, we report the degradation of the films of a-PHB binary blends with n-PHB and PLLA, respectively, in soil. The influence of NPKMg fertilizer on the degradation process has been also evaluated. Plain n-PHB and PLLA homopolymers have been used as reference. Moreover, the ecotoxicological impact of the investigated polymeric materials and the products of their degradation has been studied on the basis of the plant growth test as well as the soil pH and salinity measurements. The changes of microbial numbers in soil during the biodegradation experiment have been determined.

## Experimental Section

**Materials.** *Polymers.* Natural poly[(*R*)-3-hydroxybutyrate], n-PHB ($M_n$ = 103 000; $M_w/M_n$ = 2.5), product of BIOMER, and poly-L-lactic acid, PLLA, product of GALACTIC ($M_n$ = 58 000; $M_w/M_n$ = 1.8), were used without additional purification. Poly[(*R,S*)-3-hydroxybutyrate] (a-PHB) was synthesized by bulk polymerization of (*R,S*)-$\beta$-butyrolactone at room temperature, using KOH/18-crown-6 complex as the initiator.[14,29] The extent of the reaction was monitored by FT-IR spectrometry on the basis of the intensities of the carbonyl stretching vibration bands of the lactone monomer (1815 $cm^{-1}$) and of the polyester formed (1760 $cm^{-1}$). After completion of the reaction, crude product was dissolved in $CHCl_3$ and acidified with ion-exchange resin. After filtration of the resin, polymer was precipitated in hexane and dried under vacuum at 40 °C. a-PHB with number average molecular weight (MnGPC) equal to 6100 ($M_w/M_n$ = 1.8) was used for blend preparation.

*Preparation of Polymer Films.* All samples were prepared in the form of disks with diameter equal to 55 mm and thickness of ca. 1 mm. The sample of 0.5 g of respective homopolymer (PLLA or n-PHB) was solubilized in $CHCl_3$ (5% w/v), and the solution was cast on a Petri dish. The solvent was allowed to evaporate at room temperature. Polymer blend films (PLLA/a-PHB and n-PHB/a-PHB, both with composition 50/50 wt %) were obtained in a similar manner using 0.25 g of each respective component. All films obtained were dried at 40 °C under vacuum overnight to eliminate residual solvent.

Films containing mineral fertilizer were prepared as follows: at first, one-half of the solution of respective polymeric material in chloroform was cast on the Petri dish, and after partial evaporation of solvent, 5 g of fertilizer (N:P:K:Mg ratio: 1:0.5:0.9:0.16; dried 24 h under reduced pressure, granularity, 2−3 mm) was placed into a very viscous polymer solution contained in a Petri dish. Next, the remaining part of the polymer solution was used to cover the fertilizer. The solvent was evaporated from the Petri dish at room temperature. The films obtained were dried as described above.

**Measurements.** *The NMR spectra* were recorded using a Varian VXR-300 multinuclear spectrometer. $^1H$ and $^{13}C$ spectra were run in $CDCl_3$ by using TMS as an internal standard. The composition of a-PHB/n-PHB blends film samples was determined on the basis of the intensity of the lines corresponding to the iso and syndio dyads in the $^1H$ NMR signal of the $CH_3$ group at $\delta$ 1.28 ppm.[15b] The iso-to-syndio dyads ratio in the starting blend was 75:25. The a-PHB/PLLA blend composition was determined on the basis of the intensity of signals of the a-PHB and PLLA methyl group, respectively, at $\delta$ 1.28 and 1.58 ppm.

*Gel permeation chromatography* was performed at 30 °C, using a Spectra Physics 8800 gel-permeation chromatograph with two Mixed C Styragel columns in series and a Shodex SE 61 refractive index detector. A volume of 10 $\mu$L of sample solutions in $CHCl_3$ (concentration of ca. 1.5% w/v) was injected. Polystyrene standards with low polydispersity (PL-lab) were used to generate a calibration curve.

*Electrospray mass spectrometry analysis* was performed using a Finnigan LCQ ion trap mass spectrometer (Finnigan, San Jose, CA). The samples of methanol/chloroform extracts of post-degradation soil were introduced to the ESI source by continuous infusion using the instrument syringe pump at a rate of 3 $\mu$L/min. The LCQ ESI source was operated at 4.25 kV, and the capillary heater was set to 200 °C. For ESI-$MS^n$ experiments, mass selected mono-isotopic parent ions were isolated in the trap and collisionally activated at standard He pressure. The experiments were performed in both negative- and positive-ion modes.

*pH and Conductivity*. pH of the soil during the degradation process was determined in a mixture of a 1 M water solution of potassium chloride and the medium in a volume ratio 1:5 using a HI 9318 pH meter (Hanna Instruments). Conductivity was measured in a mixture containing 20 g of soil medium and 100 $cm^3$ of water using an EC 215 conductivity meter (Hanna Instruments).

**Biodegradation in Soil.** *Soil Characteristic*. Soil used in all degradation experiments, based on its composition of 83% sand, 11% dust, and 6% loam, can be qualified as "sandy soil". Properties of used soil, that is, acidity (determined according to standard PN-ISO 10390[30]), salinity (according to PN-ISO 11265 + AC1 standard[31]), and moisture content (according to PN-EN 13040[32]), were determined as pH (KCl) = 5.28, salinity, 84 mg KCl $L^{-1}$, and moisture content, 15%.

*Degradation Experiments.* Biodegradation of prepared polyester films was performed in the above-described sandy soil, according to standard PN-ISO 11269-2, in plastic pots (capacity 1500 $cm^3$) containing 1300 g of soil per each pot.[33] Polyester film disks were buried in the pots 3 cm under the surface of the soil. The soil average humidity and temperature in each pot during all degradation experiments were constant and equal to 15% and 22 ± 2 °C, respectively. Biodegradation tests were carried out for 183 days for n-PHB-containing samples and 730 days for PLLA-containing samples. After a specified period of time (14, 28, 42, 70, 183, and 730 days), molecular weight and molecular weight distribution of polymeric material, sample weight loss, as well as changes of selected soil properties such as pH (KCl) and salinity have been determined. Tests for each period of time were carried out in quadruplicate.

*Changes of Microbial Numbers in Soil, during the Biodegradation.* Changes of the microbial population in soil with PHB were detected by enumeration of culturable microbes, using a number of different selective media. The number of heterotrophic bacteria was estimated on YS medium (soil extract agar supplemented with 1% yeast extract).[34,35] The AGS medium (arginine, glicerol) was used to determine the number of *Streptomyces* populations in the soil.[36] The media were supplemented with cycloheximide and nystatine (from Fluka), both in the concentration 50 $\mu$g $mL^{-1}$, to inhibit the growth of fungi.[36,37] The number of fungi was estimated on peptone, glucose medium with rose bengal (0.025 g $L^{-1}$), and chloramphenicol (0.1 g $L^{-1}$) (from BTL).[34] Microorganisms were extracted from soil using 0.1% Tween 80 in

saline.[36] The microbiological analyses of soil samples were done after 14, 28, 42, and 70 days of the tested polymer incubation in soil.

*Analyses of Soil after Degradation Experiments*. 40 g of soil after 183 or 730 days of degradation experiment (for n-PHB- or PLA-containing samples, respectively) was extracted for 5 h with 200 mL of a methanol/chloroform (50:50) mixture. Filtered extracts after partial solvents evaporation (up to 5 $cm^3$) were analyzed using the ESI-MS technique.

*Plant Growth Test*. Plant growth test was performed with cress (*Lepidium sativum*) and barley (*Hordeum* vulgare), adapting the OECD 208 Terrestrial Plants Growth Test[38] for soil toxicity studies. The medium for the plant growth test was the soil defined above (for control tests) and soil after 28 and 183 days of polymeric films biodegradation process. The test for each medium was performed in quadruplicate.

In all experiments, cress (400 pcs) or barley seeds (100 pcs) were sown into the medium and plants were grown for 2 weeks under controlled conditions, that is, 16 h light/8 h dark, temperature 20/15 °C, relative humidity equal to 55%. According to standard PN-ISO11269-2,[33] the visual evaluation of potential growth inhibition, chlorosis, and necrosis occurring both in the control and in the test (containing post-degradation medium) pots was carried out during all tests. Moreover, after 14 days growing period, the emerged seedlings were counted, and the dry weight of the plants above the soil was determined. The dry weight of the plants was determined after drying at 75 °C until constant weight was achieved.

## Results and Discussion

**Biodegradation of Atactic Poly[*R,S*]-3-hydroxybutyrate Binary Blend Films.** Commonly known biodegradable polymers are able to degrade via enzymatic and/or hydrolytic mechanisms.[39−42] The extent of polymer environmental degradation depends on the kind of environment in which the experiment is conducted, for example, sea and river water, compost, or soil. In such an environment, several factors such as pH, UV, temperature, and the presence of specific microorganisms affect the degradation process.[43−45] In the present work, the degradation of the solvent cast films of 50:50 (wt %) binary a-PHB blends with n-PHB and PLLA, respectively, has been investigated in soil in comparison with plain n-PHB and PLLA reference homopolymers. Solution cast n-PHB films, studied already, exhibit high brittleness and low flexibility. However, a-PHB added acts as a plasticizer, decreasing elastic modulus and increasing elongation at the break of resulting binary blend films.[46] It should be mentioned, however, that the blend films obtained by solvent evaporation may be heterogeneous with the surface enriched in one component. Thus, the comparison of the properties of solvent cast and hot pressed samples may be troublesome.[47]

The results of the weight loss during the degradation process of a-PHB/n-PHB blend and n-PHB film samples are presented in Figure 1a. The higher weight loss was observed for the a-PHB blend film.

The composition of binary blend film samples after each degradation period has been determined via $^1$H NMR spectroscopy (see Experimental Section). As is presented in Figure 1a, no changes in the composition of a-PHB/n-PHB blend films are observed during the degradation process, suggesting that both components biodegrade with the same rate in soil. Molecular weight changes of investigated blend components have been performed using the GPC method. However, for the a-PHB/n-PHB blend, the peaks corresponding to blend components are overlapped (see Supporting Information). Therefore, in the quantification of molecular weight changes presented in Figure 1b, the molecular mass at maximum of GPC curve ($M_p$) was used. As is shown in Figure 1b, the molecular weight of plain n-PHB almost did not change during the degradation process. In the a-PHB/n-PHB blend, an ca. 40% decrease of $M_p$ of the n-PHB component of blend is observed after 183 days of investigated degradation process. A similar accelerating effect of a-PHB on the hydrolytic degradation of a-PHB/n-PHA blend films in phosphate buffer was reported earlier.[46,48] The above presented results indicate that the a-PHB in blend with n-PHB can be degraded (bioeroded) in soil and the process proceeds with the same rate for both blend components, a-PHB and n-PHB.

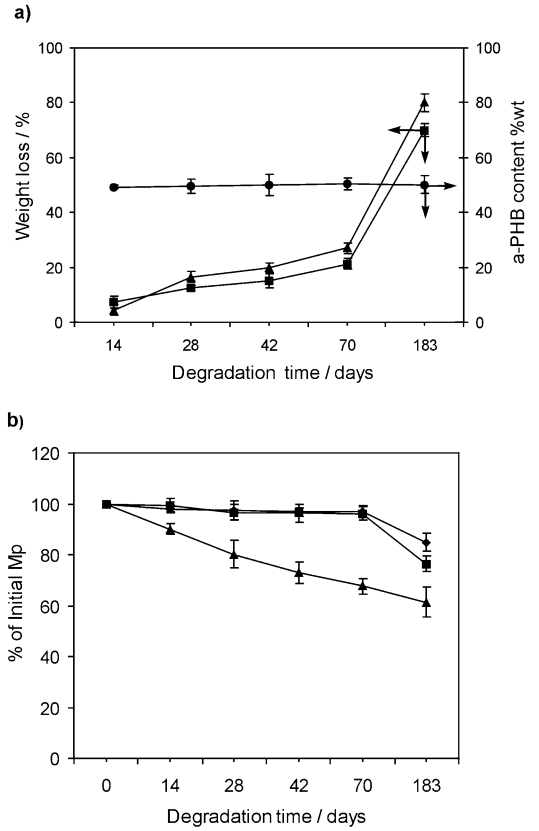


**Figure 1.** Degradation of a-PHB/n-PHB 50:50 binary blend and reference n-PHB homopolymer in soil. (a) Sample weight loss and blend composition changes during incubation (▲, a-PHB/n-PHB blend and ■, n-PHB homopolymer). (b) Number average molecular weight changes (as a percent of initial $M_n$) during degradation process (▲, n-PHB component of blend, ◆, a-PHB component of blend, and ■, pure n-PHB). (Error bars represent the standard deviation of four replicates. The error bars are smaller than the symbols where they are not shown.)

In the case of a-PHB/PLLA as well as plain PLLA films, the weight loss is not observed at the beginning of the degradation process. However, after the 70 days of incubation in soil, a slight weight loss of a-PHB/PLLA blend sample (3.3 wt %) is observed (Figure 2a). After the next period of time, 183 days of degradation, more visible weight loss of both investigated PLLA-based materials is indicated.

The results of the a-PHB/PLLA binary blend composition measurement during the degradation process presented in Figure 2a have shown that the contribution of the a-PHB component is decreasing along the degradation time from 50 to 11.5 wt %, after 730 days of degradation. Thus, a-PHB is a much faster biodegrading component in the blend with PLLA. The results of the molecular weight measurements during the degradation experiment presented in Figure 2b as a percent of initial $M_p$ show the decrease of the molecular weight during the degrada-

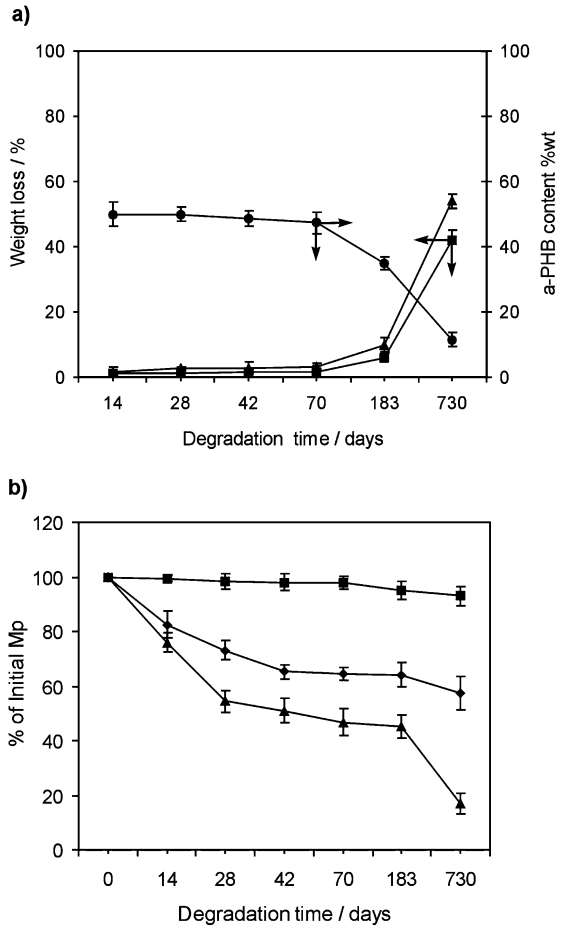


**Figure 2.** Degradation of a-PHB/PLLA 50:50 binary blend and reference PLLA homopolymer in soil. (a) Sample weight loss and blend composition changes during incubation (▲, a-PHB/PLLA and ■, PLLA). (b) Molecular weight changes (as a percent of initial $M_p$) during degradation process (■, a-PHB and ◆, PLLA component of blend, respectively, and ▲, plain PLLA). (Error bars represent the standard deviation of four replicates. The error bars are smaller than the symbols where they are not shown.)

tion process. It is worth noticing that the molecular weight decrease rate of PLLA is higher for the plain PLLA film than for the a-PHB/PLLA blend. It is probably due to the higher hydrophobicity of the blend film caused by the presence of a-PHB in the material. The above results suggest that at the first period of time the hydrolytic degradation plays the main role in the PLLA-based films degradation process; however, after 70 days of degradation in soil, weight loss of plain sample is also observed. After 730 days of degradation, significant disintegration of both samples is indicated and the observed weight loss may be caused by PLLA biodegradation.[49]

The results of the present studies indicate clearly that the PLLA-based films degrade much slower than those containing n-PHB. This observation is in agreement with results obtained by Kim et al., who have found that in various types of soil n-PHB degrades much faster than does PLLA.[50]

It is worth noticing that in the presence of NPKMg fertilizer all investigated samples degraded faster and samples based on n-PHB again disintegrate much faster than those based on PLLA. The measurements of the composition of blends during their incubation in the presence of fertilizer indicate that only the a-PHB/PLLA blend composition is changing during experiment. The rate of the changes is much higher during the process conducted in the presence of fertilizer than that without fertilizer. After 70 h, a-PHB content was 47.3 wt % in the sample degraded without fertilizer, while only 32.6 wt % of a-PHB was found in blend film in its presence. The fertilizer added may act as the nutrient for bacteria, streptomycetes, and fungi present in the soil. Such microorganisms are capable of utilizing the investigated polymeric materials as a carbon source, which may accelerate biodegradation of the polyester films induced by the fertilizer.[51]

The investigations of the molecular weight changes of a-PHB blends and respective plain polymer films degraded in the soil in the presence of fertilizer indicate molecular weight decrease trends similar to those observed for experiments without fertilizer (data not shown). Moreover, it was also found that, in the presence of fertilizer, the samples obtained from a-PHB containing blends disintegrate faster than those prepared using the respective plain polymers.

To check the post-degradation soils for the possible presence of the oligomeric degradation products that might be released during the biodegradation experiments, the electrospray mass spectrometry analysis (ESI-MS) of their methanol/chloroform extracts was performed. The selected ESI-MS method was previously successfully applied for the identification and structural characterization of the products of enzymatic hydrolysis of blends of a-PHB/n-PHBV,[11] a-PHB/PCL,[18] and a-PHB/PLLA[18] by PHB-depolymerase A from *P. lemoignei* as well as for the monitoring of the rate of the enzymatic degradation of amorphous PHB by *PhaZ7* depolymerase of high specificity.[23] It was shown that, in the case of PHA-depolymerase-catalyzed enzymatic degradation of a-PHB/PCL and a-PHB/PLLA binary systems, signals characteristic for 3-hydroxybutric acid and its water-soluble oligomers up to heptamer could be detected as degradation products using the ESI-MS technique.[18] However, no signals corresponding to PCL as well as PLLA degradation fragments were observed in the ESI-MS, proving that these blend components cannot be hydrolyzed by the specific PHB-depolymerase. Moreover, the 3-hydroxybutric acid and its oligomers up to octamer (with the most intensive ESI-MS signal corresponding to pentamer) were identified in the case of enzymatic degradation of amorphous PHB by the recently discovered *PhaZ7* depolymeraze.[23]

In the current studies, no signals related to any degradation products of PHB and PLLA have been found in the ESI-MS spectra acquired for the methanol/chloroform (50/50) extracts of post-degradation soil after degradation of respective binary blends as well as plain polymers. This result may suggest that the low molecular products formed during degradation of the investigated binary blends and respective homopolymer films have been probably assimilated by microorganisms present in soil under the experiment conditions (see microbial part of work). These results are in agreement with data reported previously in the literature, showing that the water-soluble a-PHB oligomers are bioassimilable by bacteria isolated from natural environment.[52] Water-soluble lactic acid oligomers with less than 10 monomers units can also be assimilated more or less rapidly by microorganism[53] and are additionally claimed to possess plant growth stimulation activity.[54]

**Changes of Microbial Numbers in Soil during the Biodegradation.** It is generally known that biopolymers can be degraded in soil by the action of a wide range of microorganisms as gram-negative and gram-positive bacteria, streptomycetes, and fungi. The rate of degradation depends mainly on the temperature and the number of characteristic microbial populations in soil used for experiments.[37,55] In most mineral soils, the activity and growth of the soil microorganisms depend strongly on the availability of substrate, especially carbon.[51]

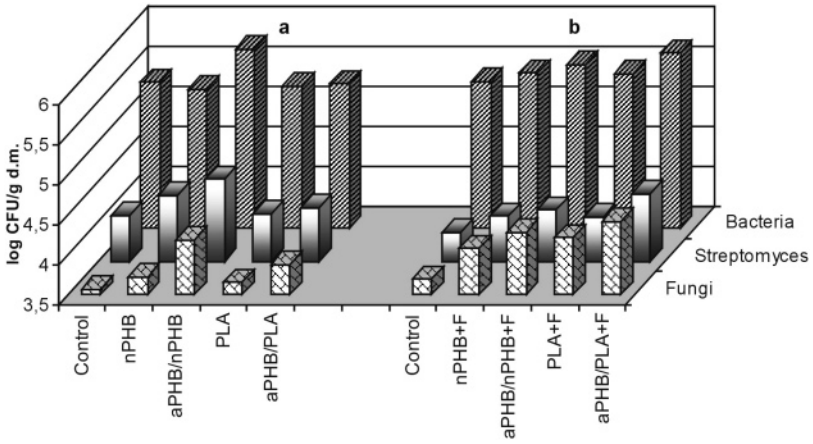


**Figure 3.** Number of selected microorganisms after 70 days of incubation of investigated blend and plain polymer films in (a) soil and (b) soil with fertilizer.

The degradation experiments of a-PHB containing binary blends, as well as control plain polymers, have been conducted in soil with the number of bacteria as $2.3 \times 10^6$ CFU $g^{-1}$ of dry soil, *Streptomyces* as $2.3 \times 10^5$ CFU $g^{-1}$ of dry soil, and fungi as $8.8 \times 10^4$ CFU $g^{-1}$ of dry soil. During the experiment, the number of all tested microorganisms decreased slowly with the time of degradation, probably due to the decreasing level of nutrients in soil.[35,51] However, in the soil with a-PHB/n-PHB blend after 42 and 70 days of degradation process, an increased number of bacteria has been observed, in comparison with control soil. In the soil with n-PHB, there has been only a slight increase of the bacteria number observed (Figure 3). Moreover, after 42 and 70 days of degradation, the increase in *Streptomyces* number has been detected in soil after degradation of both a-PHB/n-PHB blend as well as n-PHB films. It indicates that *Streptomyces* can take part in these polymeric materials degradation at this stage of the process. Savenkova et al. observed the increase in *Streptomyces* number in soil after n-PHB degradation after just 14 days; however, it can be due to the difference in both the composition of the microorganism population and the pH of the soil.[56] *Streptomyces* favors neutral or alkaline pH of the soil rather than an acidic pH.[53] The number of the fungi in the soil samples after 70 days of degradation of both films versus control soil has increased; however, it is higher in the post-blend degradation soil.

In the soil with PLLA as well as a-PHB/PLLA films, there have been no changes observed in bacteria population during incubation time as compared to control. In the literature, there is little information about bacteria degrading PLLA and only two thermophilic and one mesophilic strains degrading PLLA homopolymer have been isolated so far.[57] The *Streptomyces* number has not been increased during a-PHB/PLLA and PLLA films degradation; however, the number of fungi after 70 days of degradation process was increased when compared to the control experiment. The addition of mineral salts into the system (degradation in the presence of NPKMg fertilizer) results in an increase of all microorganisms in all experiments, although it was higher in the soil after blend degradations. It is probably due to the better utilization of the investigated polymeric materials as a carbon source in the presence of nitrogen and microelements in soil microorganism growth. The results presented above suggest that microorganisms can take an active part in the degradation process starting from 42 to 70 days of experiment. Moreover, a higher number of microorganisms has been observed in soil where a-PHB blend films have been incubated. It is probably due to the fact that the a-PHB oligomers constitute the nutrient for operating microorganisms, and, in the presence of a second crystalline blend component, the biodegradability of a-PHB is enhanced, as was described previously.[11,17−19]

**Evaluation of Toxicity of Post-degradation Soil Samples.** Toxicity of post-degradation soil has been investigated by the measurement of changes in soil acidity and its salinity as well as by the plant growth test. The investigation of the changes in soil properties after a specified period of time of polymeric samples degradation indicates a slight increase in both acidity (e.g., pH (KCl) changes in the range 5.28−5.05 for both n-PHB-based samples and 5.28−5.02 and 5.28−4.97 for plain PLLA and a-PHB/PLLA, respectively) and salinity (84−180 mg KCl/L and 84−110 for n-PHB- and PLLA-based materials, respectively) during the degradation process. However, after comparison of these data with the changes in the control soil properties (pH (KCl) changes in range 5.28−4.82 and salinity in the range 84−150 mg/L), it can be stated that there is almost no polymeric sample effect on the measured soil parameters. Because the optimal pH range for most plants is about 5.5,[58] the post-degradation soil possesses pH close to the value optimal for plant growth (5.28−5.05).

These results, that is, pH and salinity changes, are in agreement with the data obtained by Rosa et al.[45,59] for n-PHB and by Tuominen et al.[60] for lactic acid-based polyurethanes degradation in soil and compost, respectively. However, for degradation in soil of other PHAs such as poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) copolymer, the substantial decrease of pH was observed,[45] whereas in the degradation of this copolymer in compost the pH of substrate slightly increased.[60] Such a difference in the influence of the polymer degradation on the acidity of substrate depends probably not only on the type of degraded polymer but also on the substrate used as well as the type and activity of microorganisms present in the substrate.

To exclude the ecotoxicological effect of investigated a-PHB-containing blends and plain PLLA and PLLA films as well as the products of their degradation, respective plant growth tests have been carried out.[38] In the performed tests, the growth of cress and barley has been examined. The growth medium consisted of post-degradation substrate (soil after 28 and 183 days of degradation) and control soil (see Experimental Section). After 14 days of growth, the visual evaluation of seedlings (cress and barley) growing in all media indicates that the average amount of emerged plant ranged from 85% to 86% in both background soil and post-degradation media. Moreover, no changes in cress and barley quality, as compared to plants seedlings in the control substrate, have been observed (Figure

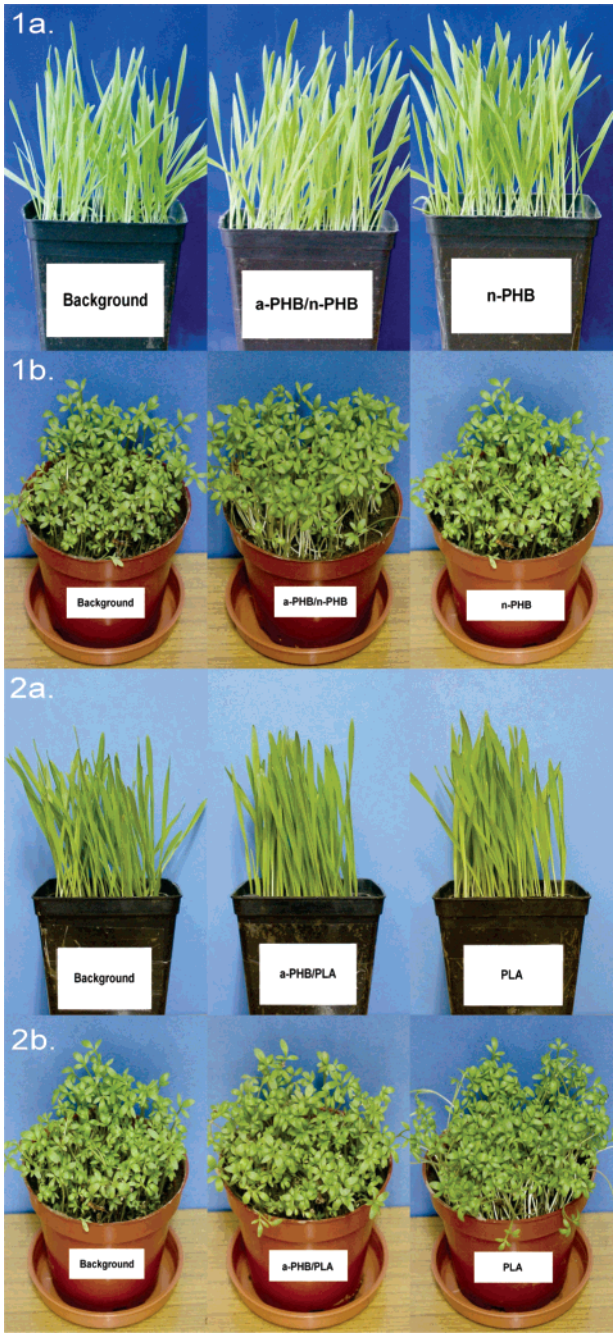


**Figure 4.** Growth of (a) barley and (b) cress in soil after 183 days of degradation of (1) a-PHB/n-PHB and n-PHB, and (2) a-PHB/PLLA and PLLA films.

4) regardless of the substrate sample used, that is, obtained respectively after 28 and 183 days of degradation of films of a-PHB-containing binary blend as well as n-PHB and PLLA reference plain polymers.

Additionally, after 14 days of growth, plants have been harvested, dried, and gravimetric measurements have been made. The obtained results presented in Figure 5 indicate that there are no differences in dry matter content between the plants growing on tested media (obtained after degradation of all polymeric films) and the control substrate.

The above results of ecotoxicological evaluation of post-degradation soil suggest that the biodegradation products of the investigated polymeric materials are totally nontoxic to the natural environment.

## Conclusions

The results of the present study demonstrate for the first time, to our knowledge, the biodegradation in soil of a-PHB in binary blends with n-PHB and PLLA, respectively. In the case of the a-PHB/n-PHB blend, progressive bioerosion of both components proceeds with the same rate (no composition changes have been observed with blend sample weight loss during the incubation). Moreover, the addition of a-PHB accelerates the process of degradation of natural PHB. The biodegradation of a-PHB in blend with PLLA also proceeds. The blend weight loss is accompanied by blend composition changes (50 wt % in starting blend up to 11.5 wt % after 730 days of incubation) with only a slight alteration in a-PHB molecular weight. In the presence of mineral fertilizer, the erosion of all samples occurs faster than that without fertilizer. The increase of the number of microorganisms has been observed in soil during the degradation process, especially for the soil where binary blends of a-PHB have been incubated. Moreover, the degradation of a-PHB-containing blends proceeds faster than that of the respective reference plain n-PHB and PLA films. Therefore, if acceleration of the degradation of natural PHB or PLLA in soil is desired, the addition of a-PHB seems to be an effective method.

The evaluation of ecotoxicity by using the terrestrial plant growth test as well as gravimetrical measurements of harvested and dried plants shows no effect of the post-degradation soil on the average amount of emerged plants, seedlings appearance (outlook, design), and dry matter yield. These results suggest that investigated polymeric materials as well as the products of their degradation possibly present temporarily in the soil are not toxic to the environment.

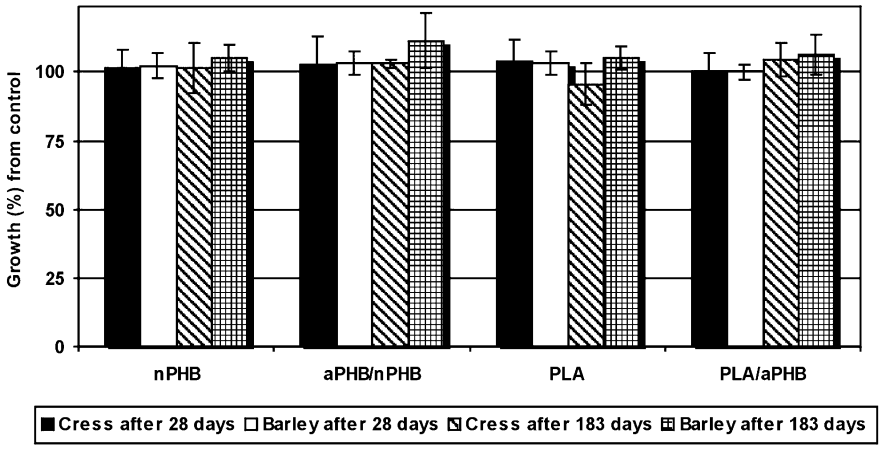


**Figure 5.** Growth of cress and barley measured as percent of dry weight against the control. (Error bars represent the standard deviation of four replicates.)

**Acknowledgment.** This research project has been supported by a Marie Curie Transfer of Knowledge Fellowship of the European Community's Sixth Framework Program under contract number MTKD-CT-2004-509232. Financial support by the Polish Ministry of Science and Higher Education grants no. PBZ-KBN-070/T09/2001/7 and Eureka! 3064 is acknowledged. We are indebted to Professor S. Łabużek from Silesian University, Poland, and Dr. I. Radecka from the University of Wolverhampton, UK, for stimulating discussions.

**Supporting Information Available.** GPC traces of a-PHB/n-PHB and a-PHB/PLLA binary blend films after specified times of incubation. This material is available free of charge via the Internet at http://pubs.acs.org.

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BM060708R