Biodegradable Polymeric Films Based on Microbial Poly(3-Hydroxybutyrate). Effect of Gamma-Radiation on Mechanical Properties and Biodegradability

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Summary: Two samples of microbial poly(3-hydroxybutyrate) (PHB) having different molecular weight were used for the preparation of films to be exposed to gamma radiation. The effect of radiation on those samples with high molecular weight increased the fragility of the film. Biodegradability increased with time and reached about 95% after 18 days. Weight-loss of both samples (irradiated and non-irradiated) after 23 days were 100%, for those films with molecular weight of 265 kD.

Keywords: biodegradation; bioplastic; γ-irradiation; mechanical properties; poly(3-hydroxybutyrate)

Introduction

Polyesters based on β-hydroxy alkanoic acid (PHAs) are produced by a variety of bacteria as an intracellular storage of carbon and energy when nutritional components such as nitrogen, phosphorus, oxygen or magnesium are limited in the presence of excess carbon source. PHAs can be considered as good substitutes in some applications of petroleum derived synthetic polymers because of their similar physico-mechanical properties, biocompatibility and biodegradability [1]. In the last decade they have intensively attracted attention as biobased thermoplastic polymers [2, 3].

Bacterial synthesized poly(3-hydroxybutyrate) (PHB) is a thermoplastic polymer with high degree of crystallinity and comparable physical and mechanical properties to isotactic

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poly(propylene) [4, 5]. A close similarity is observed in melting point, glass transition temperature, and stiffness. PHB can be processed by the same current technology and is holding a great potential in biomedical consumable product applications and due to its resistance to water, and low permeability to oxygen it can be used in food packaging, as well [6-8].

Since the last 15 years a great deal of attention has been devoted to the development of ecocompatible biodegradable polymeric materials derived from fossil fuel feedstock as well as from renewable resources [9, 10].

In the present paper we report on the use of two PHB samples having different molecular weight in the production of films whose physico-chemical and mechanical properties including also biodegradation will be monitored secondly to γ-irradiation. The preparation of the PHB sample having higher molecular weight is as described.

Experimental

Bioreactor experiments. Batch cultures were inoculated with a 1% (v/v) preculture of *Azotobacter chroococcum* 6B grown for a period of 36 h. Two bioreactors equipped with two-six bladed impellers and capacity of 1 liter (Bioflo I, New Brunswick Sci. Co, USA) and 4 liters (Gallemkamp Sanyo, England), were used. A modified Burk medium was used as substrate of selected bacterial strain according to a procedure formerly described [11]. Temperature was controlled automatically at 30°C and initial pH was adjusted to 7.0 and maintained by controlled addition of 10N KOH solution. The aeration rate was 0.5 l/min at a stirring speed of 100 rpm. During fermentation, a glucose concentration of 10 g/l was used. The adopted procedure led to a PHB sample characterized by an average weight molecular weight of 1,150 KD.

The PHB sample with average weight molecular weight 265 KD was kindly supplied by Copersucar Co. (Brazil). The two polymer samples prior uses were purified by reprecipitation into cold methanol by drop wise addition to solutions of the polymers sample in chloroform.

Molecular weights were determined by a Jasco PU-1580 HPLC connected to a Jasco 830-RI detector and a Perkin-Elmer LC-75 spectrophotometric detector.

Thermal characterization was performed by means of a Mettler TA 4000 System equipped with a differential scanning calorimeter DSC-30 and a TA72 GraphWare software.

Polymer films (65 μm) were prepared by casting from solutions and exposed to γ-irradiation treatment by using ^{60}Co source from a semi industrial irradiation plant (Centro Atómico Ezeiza) at room temperature. Each sample was irradiated at a dose rate of 32 $kGy \cdot h^{-1}$.

Tensile properties of PHB films were measured at 25 °C by means of an Instron Tensile Testing Machine, Model 1122. Test specimens were cut under the specification of ASTM D 1708-84.

Biodegradation tests were performed on film samples of 25 mg and thickness of 65 μm. They were buried in a 350 cm^3 test reactors containing 250 g of earth worm compost (pH 7.0) maintained at a 60 % water holding capacity and incubated in a controlled environment chamber at 30 °C. Test films were removed from containers at definite time interval and after cleaning by sonication, were dried and weighted.

Results and Discussion

PHB Preparation

The PHB with high molecular weight was prepared by fermentation in the presence of *Azotobacter chroococcum* 6B at variance of ammonium sulfate concentration (with different initial C/N ratios) and glucose feeding up to a maximum concentration of 10 g/l. Data were obtained on fed-batch culture in a 4 liter fermentor. The synthesized polymer displayed an average weight molecular weight of 1,150 kD. The maximum PHB yield was 60% of the dry cell mass after 40 h of culture and with glucose fed, upon reaching the stationary phase. At 40 h incubation the PHB yield decreases because of the action of depolymerases (Fig. 1). In most bacteria, PHB is a reserve material for carbon and energy when the carbon source is depleted and those enzyme related to degradation metabolic action are induced.

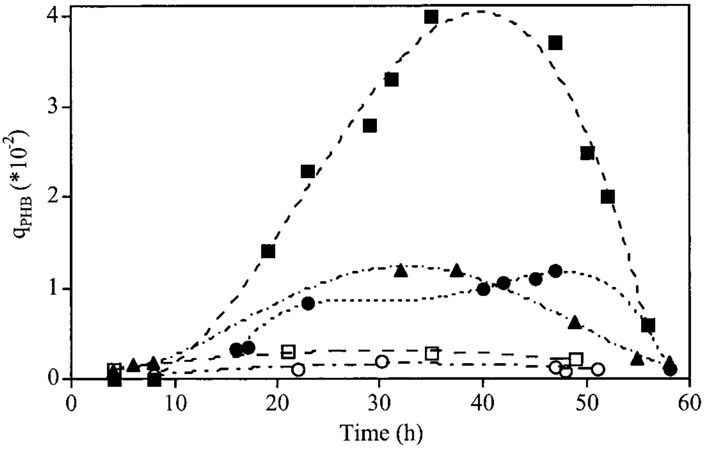


Figure 1: Specific velocities of PHB production (q_{PHB}) at different C/N molar ratios in the feed *A. chroococcum* was grown in a 4 liter fermentor, at 30°C , pH 7.0. The aeration rate 0.5 l/min at 100 rpm stirring speed. Ammonium sulfate was used as nitrogen source at the following concentration in mg/l. ●: 138, ■: 69, ▲: 29, O: 7.3, □: 3.7.

Effect of γ-irradiation

Table 1 summarizes GPC results of non-irradiated and irradiated PHB samples. The molecular weight substantially decreased after γ-irradiation at a dose rate of 32 kGy·h^{-1}. The effect was more significative for the sample of higher molecular weight.

Table 1. Effect of γ-irradiation on PHB molecular weight

Sample	Mw Non-irradiated	(kD) irradiated
PHB Copersucar	265	250
PHB A. *chroococcum*	1,150	584

Tensile properties were measured on chloroform cast films of PHB with central length of 15.9 mm and transversal section 4.8 mm^2. The elongation is $\lambda=l/l_o$, that is the ratio between the actual (l) and the initial (l_o) length of the sample. In Figure 2 (PHB 265 kD) and 3 (PHB 1,150 kD) is reported a comparison in terms of stress-strain tensile curves between the PHB samples prior and after γ-irradiation. In both cases the irradiation results in a substantive increase in the rigidity of the sample, the effect being higher in the case of the higher molecular weight sample.

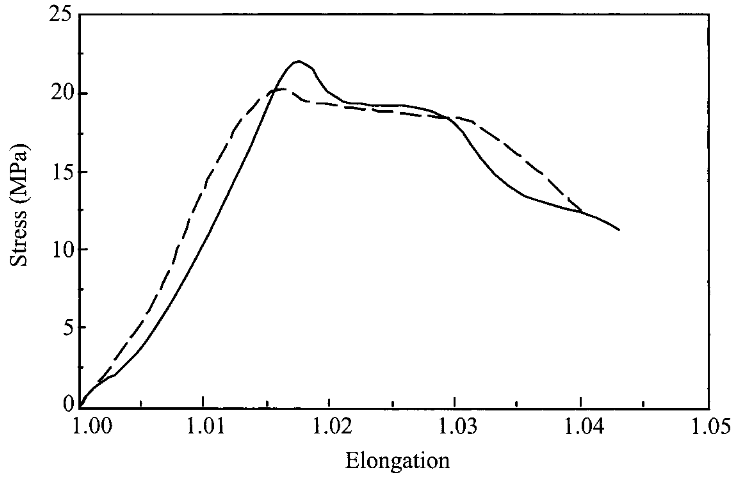


Figure 2. Tensile curves for 265 kD PHB films (–) Non-irradiated. Irradiated at 32 kGy (- -).

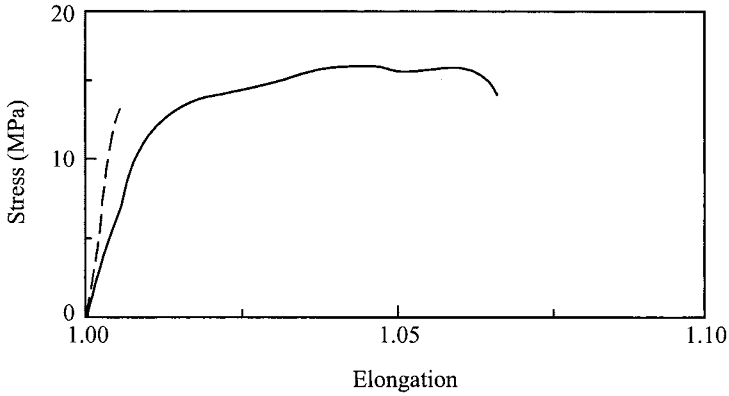


Figure 3. Tensile curves for 1,150 kD PHB films (–) Non-irradiated. Irradiated at 32 kGy (- -).

It is observed that the Young modulus of irradiated samples increases with respect the non-irradiated counterparts, while the tenacity, the elongation and the maximum tension decreased abruptly.

The relevant thermal parameters of PHB samples prior and after irradiation are reported in Table 2. The melting enthalpy of 146 $J \cdot g^{-1}$ [12] was used to determine the degree of crystallinity. DSC results displayed that both glass transition and melting temperatures of both PHB's decreased as a consequence of irradiation. The γ-irradiation effect on glass transition temperatures of both PHB's was relatively equivalent observing a decrease of about 3 °C after irradiation. Conversely, a decrease of ca. 7 °C was observed on melting temperature of higher molecular weight PHB. The resulting crystallinity indicates that only 265 kD show a decreasing after γ-irradiation.

Table 2. Effect of γ-irradiation observed on PHB thermal parameters.[a]

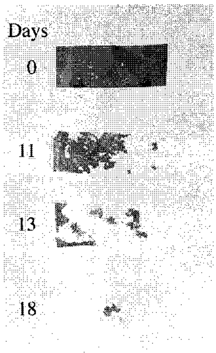
Sample	T_g (°C)	T_{cc} (°C)	T_m (°C)	ΔH_m ($J \cdot g^{-1}$)	X_c (%)
265 kD non-irradiated	2.4	45.9	174.4	82.5	56.5
265 kD irradiated	-0.4	43.1	171.2	73.8	50.5
1,150 kD non-irradiated	2.9	41.0	173.8	66.8	45.7
1,150 kD irradiated	0.7	52.4	166.9	70.1	48.0

[a] T_g (glass transition), T_{cc} (cold crystallization), and T_m (melting) temperatures, H_m (melting enthalpy) X_c (degree of crystallinity) recorded in the second heating run after a quenching.

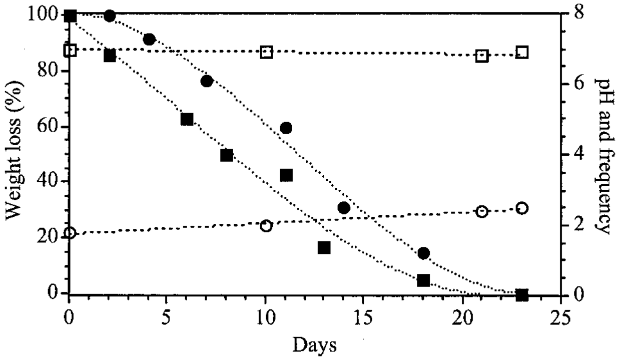
Biodegradability

Figure 4 reports the weight loss recorded within the time for solvent-cast 265 kD PHB films exposed to a controlled microbial medium such as that of stabilized earth worm-compost. The biodegradability tests were carried out by counting the ufc of mesofile microorganisms able to grow on mineral salt solution with PHB as the only carbon source. These counts were carried out at different exposure times of the PHB films with the stabilized earth worm-compost.

The weight loss of the incubated sample increased with time and reached about 95% after 18 days. After 23 days, no fragment of both samples (irradiated and non-irradiated) could be retrieved from the incubation medium. At this last time of exposure, irradiated and non-irradiated film samples with higher molecular weight 1,150 and 584 kD, respectively, experienced a weight loss of 60 and 72 %. On the other hand, the pH of the system remains constant and the total quantity of microorganisms that could grow diminishes. However, the ratio of microorganisms able to degrade PHB increases with increasing the time of exposure of the PHB films to the microbial colonies.



A)



B)

Figure 4: A) Photographs of the non irradiated PHB film (265 kD, 25 mg/65 μm thickness) taken at different incubation times. B) PHB film (265 kD, 25 mg/65 μm thickness), incubated at 30 °C in stabilized earth worm-compost. (□) pH, (O) frequency, (●) non-irradiated, (■) irradiated.

Conclusion

Microbially produced poly(3-hydroxybutyrate) when exposed to γ-irradiation at a dose rate of 32 $kGy{\cdot}h^{-1}$ experience a drop in the average molecular weight that is much higher in the case of the sample with higher molecular weight (1,150 kD). Minor effect is recorded for the PHB sample having a molecular weight about one fifth of the former one.

Irradiation of the samples slightly influences the thermal and crystallinity properties of the samples, while it affects substantially the mechanical properties of film specimens.

The γ-irradiated and non-irradiated PHB samples when exposed to a microbial inoculum deriving from earth worm compost tend to be degraded at fairly high rate, the major effect being recorded for the irradiated PHB samples and samples having a lower molecular weight.

Acknowledgements

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