

Changes in the mechanical properties of compression moulded samples of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) degraded by *Streptomyces omiyaensis* SSM 5670

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ABSTRACT

Streptomyces omiyaensis SSM 5670 was characterized by its ability to use compression moulded samples of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) as its sole carbon source. Biodegradation of PHBV in liquid mineral salts medium was investigated using scanning electron microscopy, gravimetric measurements, capillary viscometry, tensile testing and wide angle X-ray spectroscopy. The biodegradation of PHBV proceeds via surface erosion mechanism, resulting in the formation of pits by microbial attack. PHBV specimens lost about 45% of their original weight after 45 days of exposure. During the degradation process the elastic modulus reduces less than 10%. The formation of pores and microcracks initiated at the degraded pits determines the reduction of the elongation and stress at break. However, the true stress at break is practically independent of the degradation time. No significant changes of PHBV molecular weight or crystallinity were observed during biodegradation. The polymer chain cleavage occurred only at the specimen surface and does not discriminate between crystalline and amorphous states.

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1. Introduction

Polyhydroxyalkanoates (PHAs) are natural polyesters produced by numerous microorganisms under unbalanced growth conditions, that is, enough renewable carbon sources and depletion of an essential nutrient [1,2]. PHAs can be used in packaging, agricultural, medical, veterinary and marine applications [3] because they behave similarly to conventional thermoplastics with the advantage of complete biodegradation [4].

The chemical structure, depicted in Fig. 1, corresponds to over a hundred different types of PHAs made from different monomers. The first PHA discovered in 1926 [5] was poly(3-hydroxybutyrate) (PHB), a extremely brittle polymer because of its high degree of crystallinity. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), a copolymer of PHB with monomers that include two carbons in the side chain, attracts the attention of many researchers because of its better mechanical performance and faster biodegradation. Thus, many studies have been primarily oriented to characterize the production, morphology, mechanical properties and biodegradation kinetics of PHBV [6–8].

Biodegradability of PHBV in different environments has been evaluated not only regarding the weight loss, the kinetics and yield of intermediate products but also by monitoring changes in the morphology and mechanical properties [9–14].

Biodegradation tests of PHBV performed in natural environments, such as soils [15,16], composts [9,16], sludges [17,18] or natural water [10,13,19] were mainly oriented to determine how the degradation kinetics depends on the environmental factors. Biodegradation under laboratory conditions, however, has been useful to evaluate enzymatic or hydrolytic mechanisms [1,20]. Both mechanisms involve polymer chain cleavage and should result in molecular weight reduction. Doi et al. found, however, that the molecular weight of the sample does not change during enzymatic degradation because cleavage occurs only at the specimen surface while it decreases during hydrolytic degradation [21].

Similar results were achieved by Luo and Netravali [9], for bulk PHBV samples biodegraded in composting medium; they found that the molecular weight remained almost unchanged since degradation occurs by surface erosion. Furthermore, since the degree of crystallinity remained virtually unchanged they concluded that degradation during composting does not discriminate between crystalline and amorphous phases. Regarding the mechanical properties, in agreement with previous tests [22], they

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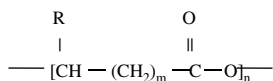


Fig. 1. General molecular structure of PHAs. $m = 1, 2, 3$, ($m = 1$ is most common), n : from 100 to several thousands. R is variable. When $m = 1$, $\text{R} = \text{CH}_3$, the monomer structure is 3-hydroxybutyrate, while $m = 1$ and $\text{R} = \text{C}_2\text{H}_5$ is a 3-hydroxyvalerate monomer.

found that the tensile modulus did not show much change with composting time but the ultimate tensile strength and the elongation at break reduces as the biodegradation proceeds. Since both the molecular weight and the degree of crystallinity do not change while degrading the sample, they presumed that surface defects should be responsible for the decrease in the tensile strength.

Therefore, the aim of this work is to propose a model for the surface erosion that aids in explaining the reduction in the tensile strength of bulk PHBV samples as the degradation proceeds. In order to achieve this goal compressed moulded PHBV samples were degraded by *Streptomyces omiyaensis* SSM 5670 cultivated in liquid mineral salt medium (MSM). Few contributions have been devoted to analyze the morphological changes of PHBV films also degraded by *Streptomyces* sp. [23,24] so this work also provides new insights on the mechanical changes due to the PHBV degradation by this microorganism.

2. Experimental

2.1. Samples

Bacterial PHBV with 12 wt% of hydroxyvalerate, was purchased from PHB Industrial SA, Brazil. PHBV is sold as white granules with a weight average molecular weight 165 kg/mol. This material was used as-received.

PHBV samples for the mechanical and morphological tests were prepared by hot compression moulding. The granules were put between two stainless steel plates separated by a metallic ring 200 μm thick, melted in a hydraulic hot press at 463 K for 10 min and then quenched at 269 K. In this way we got disks around 100 mm in diameter and 200 μm thick. Finally specimens for biodegradation followed by tensile tests were cut with a die according to the ASTM D 1708-84 standard [25]. The weight of each sample was (104 \pm 6) mg.

2.2. Culture of microorganisms

Stock culture of *S. omiyaensis* SSM 5670 (Culture collection SSM of AGRAL FAUBA) was maintained at (30 \pm 1) $^\circ\text{C}$ on Actinomyces agar slants containing, in g/l distilled water: yeast extract 0.2; starch 1.0; agar 1.5, pH 7.2.

2.3. Analysis of biodegradation in liquid medium

Erlenmeyer flasks (150-ml) with 50 ml of the MSM, were inoculated with a 2% v/v fresh spore suspension of *S. omiyaensis* SSM 5670. Each flask contained one piece of surface sterilized (70%, v/v ethanol) compression moulded samples of PHBV polymer. The solution was agitated in a rotary shaker at 100 rpm and kept at (30 \pm 1) $^\circ\text{C}$. Control experiments were carried out without the inoculation of the MSM. After 15 days a set of 5 samples was removed from the solution, washed with distilled water under the ultrasonic action and dried at 60 $^\circ\text{C}$ to constant weight. This procedure was repeated after 30 and 45 days of the first immersion using inoculated solution.

PHBV biodegradation was measured in terms of the percentage weight loss, i.e. $\Delta w/w_0 \times 100\%$, where w_0 and Δw are the initial weight and weight loss of the PHBV sample, respectively.

2.4. Scanning electron microscope observation (SEM)

The aliphatic polyester films were retrieved directly from the culture broths, washed with distilled water under the ultrasonic action and dried at 60 $^\circ\text{C}$ to constant weight. Then, changes in the morphology of the biodegraded surface of the polyester were observed with a Phillips PSEM 500 scanning electron microscope at various stages of cultivation.

2.5. Wide angle X-ray spectroscopy

Wide angle X-ray diffraction was used to determine possible changes in the crystallinity of biodegraded samples. A wide-angle X-ray diffractometer Phillips PW 3710 with a Ni filter to provide a Cu K_α radiation ($\lambda = 0.154248$ nm) was used. Crystalline peaks were well-defined and the degree of crystallinity X_c was obtained from the normalized X-ray diffractogram by the relationship: $X_c = [A_t - A_a]/A_t$, being A_t the integral of the whole diffraction pattern and A_a the area under the background associated to the halo produced by the amorphous structure.

2.6. Weight average molecular weight

The weight average molecular weight (\bar{M}_w) of the control and degraded PHBV specimens was calculated from viscosity measurements according to the equation $[\eta] = 1.18 \times 10^{-4} \bar{M}_w^{0.78}$, being $[\eta]$ the intrinsic viscosity, defined by $[\eta] = \lim_{c \rightarrow 0} ((\eta/\eta_s) - 1)/c$, where η_s is the viscosity of the solvent and η that of a solution with a concentration c of the polymer in a diluted solution [26]. Viscosity was measured at 30 $^\circ\text{C}$ using a glass capillary Ostwald viscometer. Several diluted solutions of PHBV in chloroform were prepared in the range from 0.01 to 0.2 g/dl in order to determine the limiting viscosity at $c = 0$.

2.7. Mechanical properties

Tensile tests were performed according to ASTM D 1708 [25] in an Instron testing machine, model 1122 at a constant strain rate of 0.001 s^{-1} and at 25 $^\circ\text{C}$. The characteristic parameters of the mechanical behaviour of PHBV – evaluated at different stages of the biodegradation process – were the tensile modulus (E , in GPa), that is, the slope at the beginning of the tensile curve, and the ultimate values: the tensile strength (σ_b , in MPa) and the elongation at break (λ_b).

It is noticed that the σ – λ plot is usually considered to represent the mechanical behaviour of elastomers with elongations higher than 100% and this is not the case of PHBV. However, this plot enables a more accurate determination of the tensile modulus from both the slope and the intercept to the origin of a line that approximates the initial portion of the tensile curve. In fact, at low deformations the tensile curve can be described by the linear equation $\sigma = (F/A_0) = E\varepsilon = E(\lambda - 1)$, where F is the applied force at time t , A_0 the initial cross-sectional area, $\lambda = l/l_0$, the ratio between the length (l) of the sample at time t , and the initial length (l_0).

3. Results

3.1. Biodegradation ability of PHBV samples

The fresh suspension of *S. omiyaensis* SSM 5670 was grown in the liquid mineral salt medium with a (104 \pm 6) mg sample of PHBV. The compression moulded PHBV specimens lost about 45% of their original weight after 45 days of exposure to the MSM as shown in Fig. 2.

The microorganism can excrete extracellular enzymes to solubilise the PHBV surface on which they are growing in homogenous

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