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Enzymatic hydrolysis of poly (lactic acid) (PLA) and thermoplastic starch (TPS)

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Abstract

The use of biodegradable polymers is one of the strategies used to minimize environmental problems caused by the inappropriate disposal of plastic articles. Thus, biodegradation is an extremely important phenomenon and needs to be clearly elucidated because the rate of biodegradation of the same sample may vary from one method to another, due to the different environmental conditions and the degrading factor to which the same is exposed. Such factors may be biotic or abiotic. In addition, characteristics such as crystallinity, morphology and the surface profile of the materials may influence its degradation. In this context, the aim of this work is to understand the enzymatic hydrolysis process for two biodegradable polymers with adverse behavior, thermoplastic starch (TPS), poly (lactic acid) (PLA) and their blends. The materials were characterized by contact angle (CA) and scanning electron microscopy (SEM). The samples were submitted to the enzymatic hydrolysis assay in the presence of the α-amylase and proteinase K enzymes. The results obtained allow us to conclude that the incorporation of TPS in the matrix of the PLA confers greater roughness to the mixtures, making them more susceptible to hydrolysis and consequent biodegradation. With respect to the enzymatic hydrolysis, it was possible to conclude that the samples showed higher affinity with the proteinase-K enzyme.

Keywords: *enzymatic hydrolysis; proteinase-K; α-amylase; biodegradation; poly(lactic acid); thermoplastic starch..*

1. Introduction

The use of polymers for the manufacture of plastic products has become more frequent. Its properties, easy processability and low cost make them to be applied in the most diverse segments of the productive sector, among which we can highlight the one of packaging [1]. However, an issue that has been worrying society in recent times is related to the short shelf life of these products, a factor that contrasts with the durability of the materials that constitute them. This characteristic has caused serious environmental problems after its inadequate disposal due to the great time needed for its degradation [2].

One of the strategies that have been used to minimize the effects caused by the inappropriate disposal of polymeric materials is the use of biodegradable polymers, which have chemical structures that allow them to be degraded through the action of biological systems such as bacteria, fungi, enzymes and algae, generating CO_2, CH_4, cellular components and other products, depending on the conditions to which they are submitted [3,4].

Studies to improve the suitability and reproducibility of laboratory methods for evaluating polymer biodegradation are in continuous progression. Several methodologies are applied with the purpose of characterizing a biodegradable polymer in different environments, such as soil, water or fertilizer. However, the rate of biodegradation of a same polymer sample may vary from one method to another due to the different environmental conditions and the degrading factor to which it is exposed. In addition, certain characteristics of the polymers may influence the degradation of the polymeric materials [5].

Within this context, the analytical procedures are being assigned individually, taking into account the special needs of the polymer and the (biotic and abiotic) phases that constitute the biodegradation process, as well as the applications and the conditions to which it is submitted.

According to Briassoulis *et al.* [6], the biodegradation kinetics of a material subjected to evaluation under terrestrial conditions can be influenced by the concentration of the material in the solid medium as well as by the nature of the microbial populations, whereas the results of these evaluations can vary significantly according to the duration of the test and when compared to a reference material as defined in the standard test specifications.

Biodegradation depends on the crystallinity of the material, and amorphous regions are more vulnerable to enzymatic hydrolysis and that abiotic hydrolysis is critically influenced by temperature. In addition to the nature and morphology of the polymer, as well as the environment to which it is exposed, it is important to note that the biotic or abiotic conditions or factors involved in the biodegradation process must be considered. The factors defined as abiotic include appropriate temperature range, presence of H_2O, nature and level of salts, presence or absence of oxygen, trace metals, pH, environmental stability, flow or pressure [2]. The biotic factors are related to the action of specific enzymes, which are catalytic proteins that decrease the activation energy level of the

molecules, favouring to the occurrence of chemical reactions that lead to the biodegradation process.
In this context, the aim of this work is to evaluate the enzymatic hydrolysis (biotic) of poly(lactic acid), thermoplastic starch and their blends promoted by enzymes with specificity for each of the studied polymers.

2. Materials

Were used the poly (lactic acid) (PLA) Ingeo 3801-X 653-89-01) from Cargill Agricultural S.A. (Minnesota, USA); the modified starch Penetrose 80, in the form of powder, with contents of 27% amylose and 73% amylopectin and average molar mass of 340.000 g.mol^{-1}, provided by Corn Products Brazil Industrial Ingredients (Jundiaí, SP) and glycerin (Cromine Fine Chemical). Low density polyethylene (LDPE) TX 7003, supplied by Braskem (density 0.922g / cm3 and flow rate 0.27g / 10min) was used as a negative control of the enzymatic hydrolysis, since it is known that it does not degrade under the conditions studied.

3. Methods

Preparation of the TPS

To obtain the thermoplastic starch (TPS) 2000 g of modified starch and 500 g of glycerine were added in a mechanical propeller mixer, which were mixed under stirring for 5 minutes, keeping the temperature at 70 °C. The mixtures were prepared using PLA and small contents (5 and 10% w/w) of TPS, yielding the compositions indicated in Table 1.

Table 1 - Quantity of each component in the blends.

	% (w/w)		
Samples	**PLA**	**TPS**	**LDPE**
100PLA	100	0	0
95PLA/5TPS	95	5	0
90PLA/10TPS	90	10	0
100TPS	0	100	0
100LDPE	0	0	100

Preparation of the TPS

Films of the studied samples were prepared, according to compositions described in Table 1, by thermopressing in hydraulic press MH, model 8MN, following the temperatures of 185 and 110 ºC and the pressures of 10 and 15 tons, for pure PLA and their mixtures, respectively [8].

Contact Angle (CA)

The determination of the water-sample contact angle was performed with the aid of a DCAT Tensiometer, with three-phase Air / Water / Sample stage, using the static contact angle method (θest) with drops of 4.00 μL, temporal monitoring of 300s, and deionized water.

Enzymatic Hydrolysis

To evaluate the behavior of enzymatic hydrolysis, two different enzymes, alpha-amylase and proteinase-K, were used, which present specifics to the different polymers studied, TPS and PLA, respectively [9 e 10].

As a control, a sample of each composition was used, with the same dimensions previously presented, which were immersed in buffer solution in the absence of the enzyme.

alpha-amylase

Four samples measuring 2.5 x 1 cm were prepared from each of the 5 compositions studied (Table 1). Their initial masses were determined and then each of these samples were transferred to a vial containing 5 mL of 0.05 M tris-HCl buffer pH 8.6 and 1.35 mg of Bacillus licheniformis alpha-amylase enzyme (Termamyl ® 2X-Novozymes®).

The flasks were placed in a thermostatic bath (Quimis, model: Q - 334-28, Diadema, SP, Brazil) and kept at a constant temperature of 60 ° C. The samples were removed after different degradation intervals, that is, after 0.24,72,120,168,240,336 and 480 hours, after which they were washed with distilled water, dried and weighed to determine the percentage of mass retained.

proteinase-K

Four samples measuring 2.5 x 1 cm were obtained from each of the 5 compositions studied (Table 2). Their initial masses were determined and then each of these samples was transferred to a vial containing 5 mL of 0.05 M tris-HCl buffer, pH 8.6, 1.0 mg of sodium azide and 1.0 mg of enzyme proteinase K from Tritirachium album (Ludwig Biotechnology Ltd.). As a control, a sample of each analyzed composition was used, with the same dimensions previously presented, which were immersed in buffer solution with sodium azide in the absence of the enzyme.

The flasks were placed in a thermostatic bath (Quimis, model: Q - 334-28, Diadema, SP, Brazil) and kept at a constant temperature of 30 ° C. The samples were removed after different degradation intervals, that is, after 0.24, 96, 192, 342, 456 and 600 hours, after which they were washed with distilled water, dried and weighed to determine the percentage of mass retained

Scanning Electron Microscopy (SEM)

The surface of the samples was analyzed by scanning electron microscopy (SEM), before and after the enzymatic hydrolysis. For this, the samples were cryo-fractured in liquid nitrogen and then covered with gold with the aid of the SCANCOAT equipment of the company EDWARDS, model PIRANI 501, at a pressure of $3x10^{-1}$mbar, 1,5Kv, 42,5mA, for 35 seconds. Finally, images with magnifications of 100µ, 50µm and 10µm were obtained with the aid of a JEOL - JCM 600 microscope, operating with a 5Kv beam.

4. Results and Discussions

Figure 1 (a) and (b) shows the percentage of retained mass of the samples submitted to the enzymatic hydrolysis assay in the presence of α-amylase and proteinase-k enzymes, respectively.

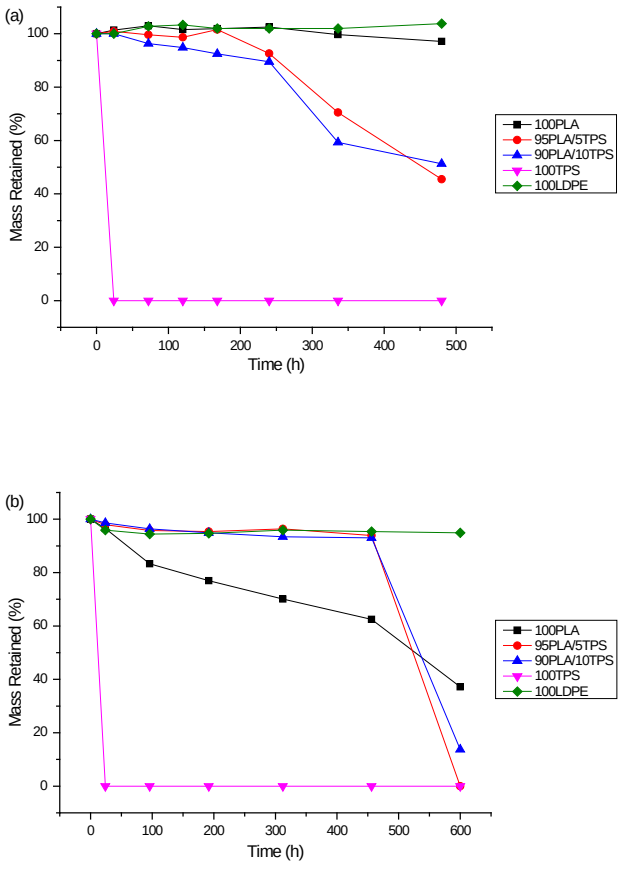

Figure 1: mass retained after enzymatic hydrolysis promoted by α-amylase (a) and proteinase-K (b) enzymes.

It is possible to observe in figure 1 (a) that the samples 100PLA and 100LDPE presented, during the first hours, a small mass gain, not very significant. The increase in mass values can be explained by the absorption of water by the polymer.

After approximately 24 hours it can be seen that the 100TPS sample was completely degraded. This result corroborates with that reported by Rosa et al. [7], who prove experimentally that TPS begins to lose mass after 6 hours of degradation in the presence of the α-amylase enzyme and is totally degraded after 24 hours.

The pure PLA (100PLA) and the physical mixtures of PLA / TPS (samples 95PLA / 5TPS and 90PLA / 10TPS) showed continuous reduction of mass, whereas LDPE (100LDPE) showed small variations in the intermediate intervals. This result can be justified by the fact that LLDP contains in its structure only non-C-C and C-H bonds, which limits its chemical reactivity and does not provide centers for nucleophilic attacks. This, combined with the low water diffusion, reduces the degradability of the material.

In the assay performed with the proteinase-K enzyme (Figure 1 (b)), it was not possible to observe the mass gain observed in the hydrolysis with the α-amylase enzyme (Figure 1 (a)). The 100TPS sample showed the same behavior in the presence of the two enzymes, ie it was completely degraded after 24 hours of assay. This behavior was expected for the hydrolysis in the presence of the α-amylase enzyme, since it has specificity for the degradation of the starch. However, the 100TPS sample reproduced the same behavior in the presence of the proteinase-k enzyme, which can be justified by the hydrophilicity and consequent solubility of the starch which, in the presence of the aqueous medium and with increasing temperature, is solubilized.

It is important to observe, analyzing the two images, that the PLA / TPS blends presented lower mass retention than the pure PLA samples. PLA is characterized as a somewhat hydrophobic material, that is, its affinity with water is not the greatest possible, as is the case of TPS, for example. In addition, its semicrystalline structure, which features intense molecular interaction force, shows resistance to enzymatic activity. Despite this, it is possible to note that there was degradation of this material, especially in the proteinase-K assay, although lower than that presented by the blends. This is due to the presence of TPS in the composition of samples 95PLA / 5TPS and 90PLA / 10TPS, which increases the hydrophilic profile of the mixture, facilitating the diffusion of the water and, consequently, its degradation.

Figure 2 shows the scanning electron photomicrographs (SEM) of the materials after hydrolysis in the presence of α-amylase and proteinase-k enzymes.

The PLA film (100PLA) presents, in its initial stage, a smooth and homogeneous matrix. A large difference is observed in the surfaces of the sample after the enzymatic hydrolysis. While the matrix remains homogeneous after α-

amylase assay, pores and cavities resulting from loss of mass are observed when the samples are hydrolyzed with proteinase-K. The process leads to the removal of material from the surface, changing the appearance of the polymer matrix.

The 95PLA / 5TPS sample shows a rough but homogeneous matrix in the initial stage. As with the 100PLA sample, more water-eroded regions are seen in the hydrolysis assay with the proteinase-K enzyme.

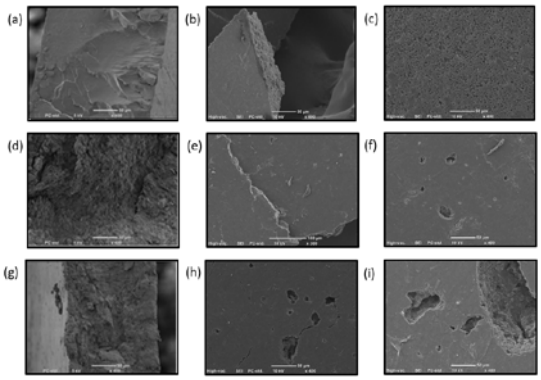

Figure 2: Electronic photomicrographs (SEM). Sample 100PLA: (a) initial stage; (b) after α-amylase hydrolysis; (c) after hydrolysis with proteinase-K. Sample 95PLA / 5TPS: (d) initial stage; (e) after hydrolysis with α-amylase; (f) after hydrolysis with proteinase-K. Sample 90PLA / 10TPS: (g) initial stage; (h) after α-amylase hydrolysis; (i) after hydrolysis with proteinase-K.

The 90PLA / 10TPS sample also presented rough matrix in the initial stage, but shows some heterogenous phases in the middle of this matrix. This is in contrast to that shown by the 100PLA and 95PLA / 5TPS samples, since there was a superficial modification after hydrolysis with the α-amylase, in addition, there are larger cavities in the results after hydrolysis with the proteinase-K enzyme when compared to the samples 100PLA and 95PLA / 5TPS.

By analyzing the photomicrographs of the samples, the highest affinity of the samples with the proteinase-K enzyme was verified, since its enzymatic activity was more efficient, providing better results.

Figure 3 shows the contact angle (CA) values of the samples during the enzymatic hydrolysis assays.

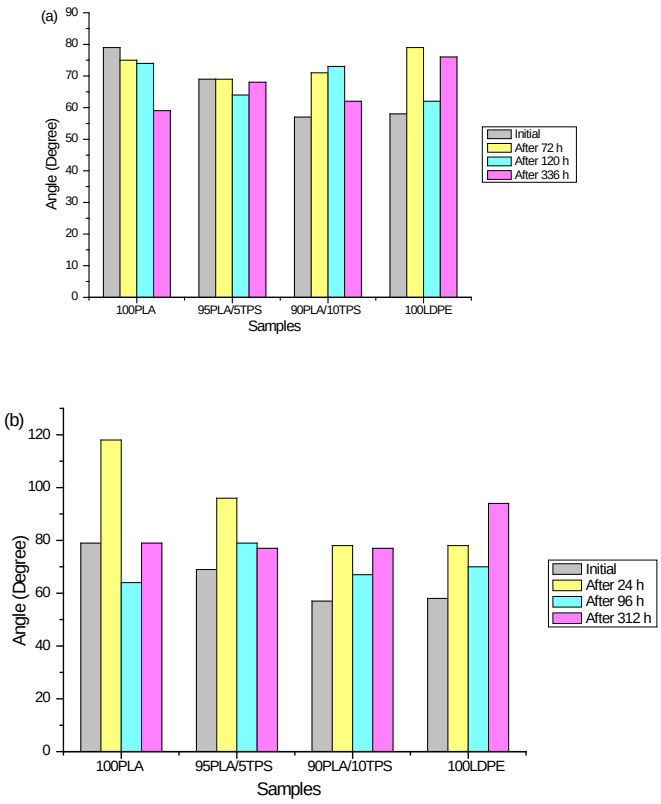

Figure 3: Contact Angle (CA). initial stage and after enzymatic hydrolysis with (a) α-amylase hydrolysis and (b) with proteinase-K.

In both tests, it is noticed that the samples initially have contact angles lower than 90 °. In the alpha amylase assay, in the first 72 hours, only the pure PLA (100PLA) showed a reduction in the angle value. In the other samples, either there was an increase in value, or permanence of the initial value. From 120 hours, some samples show a considerable decrease of the angle value, with the exception of sample 100LDPE, which showed an increase of almost 30 °. At the end of the test, all other samples presented lower value than the initial one, indicating that the degradation contributed to increase the hydrophilic profile of the surfaces. According to Göpferich (1997) [11], the degradation of polymers causes a superficial erosion of the polymers due to the loss of mass. There is, therefore, a greater penetration of the water, due to the erosion, that facilitates the breakdown of the structures of the matrices. In the 24-hour proteinase-K assay, all samples had higher values than the initial ones, being greater than 90 °, only those of the 100PLA and 95PLA / 5TPS samples. These numbers decreased when they reached 96 hours of assay. At the end of the test, it is observed that the contact angles are larger than the initial ones.

4. Conclusions

The results allowed to conclude that TPS increases the roughness of the studied surfaces, and higher levels potentiate this structural alteration, favoring the enzymatic hydrolysis of the systems. It can also be concluded that studied samples presented greater affinity with the proteinase-K enzyme.

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