In Vitro assessment of the enzymatic degradation of several PVA/starch materials

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Plastic blends on poly(vinyl alcohol) (PVA) and natural polymer are ones of the most popular biodegradable plastics with many applications in packaging. In the present paper, the susceptibility of PVA/starch materials to enzymatic degradation by amylolytic enzymes (α-amylase) was investigated by incubating the materials with buffer enzymatic solutions at 27°C for 168 hours. Two polymeric blends of PVA with starch were studied. SEM micrographs show modification of polymer surface after enzymatic hydrolysis. The material degradation was characterized by Fourrier transform infrared-attenuated total reflectance (FTIR-ATR). FTIR-ATR spectra confirmed a decrease on the band corresponding to glycosidic linkage (-C-O-C-) of starch after incubation of the materials with α-amylase. The results showed that starch polymeric blends are susceptible to enzymatic degradation, as detected by 30% weight loss after enzyme incubation.

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1. Introduction

Polymer materials are widely used in daily life, in food industry, biomedical field and agriculture. The depolymerization of polymers by enzymes is of great interest for biodegradable plastics, a group of materials which has been developed as an answer of increasing problems in plastics waste management.

Poly(vinyl alcohol) is a biodegradable, biocompatible and non-toxic polymer with various applications. Blends of poly(vinyl alcohol with starch are prepared to achieve the desired performance for different applications [1, 2]. In such blends, the starch particles act as a promoter for plastic matrix biodegradation increasing the biodegradative process.

Microbial degradation of polymeric materials based on PVA and starch takes place with an important decrease of the physical – mechanical characteristics of the samples, as well as significant weight losses [3, 4, 5]. The biodegradation rate of the PVA/starch blends containing 20 and 40% of starch reaches 25.66 and 36.66%, respectively [6].

Within this frame of interest, a study of the enzymatic degradation of composites of poly(vinyl alcohol) with starch was performed in batch system with bacterial α-amylase. The blends were incubated with a buffer solution, containing enzymes at different concentrations, at 27 °C for 168 hours. The degradation was observed by SEM. The FTIR analysis showed some changes in the structures. The spectra confirmed a decrease on the band corresponding to glycosidic linkage (-C-O-C-) of starch after incubation of the materials with α-amylase.

2. Experimental part

2.1 Material and methods

Enzyme. α-amylase from bacteria supplied by Fluka AG; enzymatic activity was 10 IU α-amylase/g polymer

Preparation of blends films. Blend films were obtained by baking a mixture of PVA and starch and mixing on a Brabender Plastograph, followed by calendering and extrusion as films. The composition of PVA-starch blends (wt/wt): P1 – 50%, PVA; 15%, starch; 35%, glycerol; P2 – 40%, PVA; 15%, starch; 45%, glycerol. The films were cut into pieces of 2 cm × 2 cm and sterilized at UV light for 10 minutes. Each film was then aseptically transferred and individually placed into Erlenmayer flasks. Table 1 presents samples notation.

Table 1. Polymeric samples used in hydrolytic experiments.

Duration of hydrolysis (hours)	P1 sample (duplicates)	P2 sample (duplicates)
24	P1a/P1a1	P2a/P2a1
48	P1b/P1b1	P2b/P2b1
72	P1c/P1c1	P2c/P2c1
96	P1d/P1d1	P2d/P2d1
168	P1e/P1e1	P2e/P2e1
	P1M - control	P2M - control

Characterization of films biodegradation

Optical microscopy. The observations were done with optic microscope Olympus BX 51 (20x photos).

Scanning electronic microscopy (SEM). The observation of the film surfaces and fracture were performed using a scanning electron microscope FEI-QUANTA 200. Micrographs of the samples were taken at different magnifications to identify holes and other changes on the surface during the degradation process.

Characterizations by Fourier transform infrared spectroscopy. FT-IR spectra, in the range 400-4000 cm-1 using a Fourier transform infrared spectrometer (Tensor 37 from Bruker) were measured in ATR mode using Golden Gate unit.

Weight loss measurements: Determination of residual polymer. The polymer strips were preweighed and each film was then aseptically transferred and individually placed into sterile medium. At the end of experiments, the samples were removed and rinsed with distilled water to remove the enzyme, and dried in oven for 24 h at 50 C before they were weighed. The weight loss was calculated by the following equation:

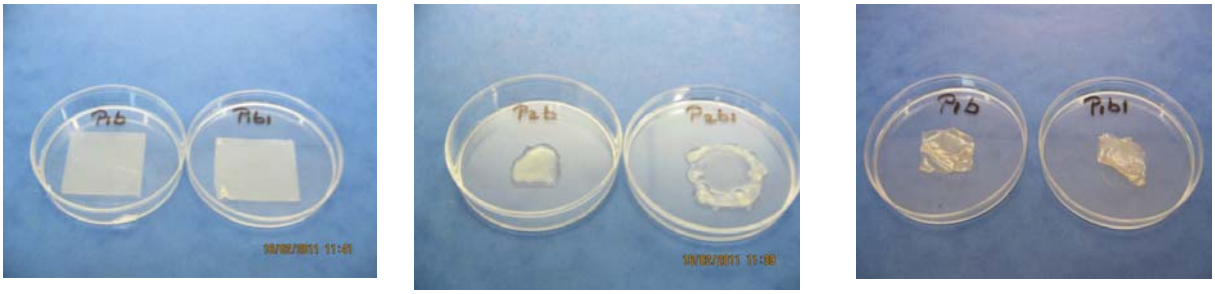
$$W_{loss}\ \% = (W_I - W_F)/\ W_I\ x\ 100 \qquad (1)$$

where W_F is the dry weight of the specimen after enzymatic treatment and W_I is the initial dry weight of the specimen. The percentage weight loss of the polymer due to enzymatic hydrolysis was corrected with controls weight loss.

3. Results and discussions

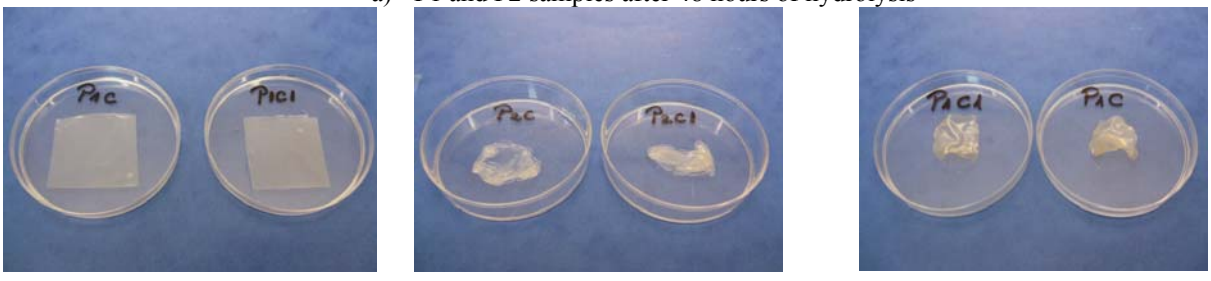
Enzymatic hydrolysis test is of interest to evaluate the indicator biodegradability of materials in a short period of time. With the addition of the biodegradable starch, the PVA blends became much easier to degrade. Starch a natural polymer is a mixture of amylose and amylopectin. α-amylase is the main enzyme involved in the hydrolysis of the 1,4-α-D-glucosidic linkage existing in starch [7]. Starch hydrolysis is proved by FTIR analysis and weight loss determination.

Figure 1 presents the polymeric samples after enzymatic degradation. As it can be seen, the samples P2 containing 40% PVA had a different behavior in connection with water solution as compared with P1. The contact with aqueous enzyme solution destroys samples P2 and, especially after drying, these ones were fragmented and dispersed. Thereby the weighing results were not taken into account.



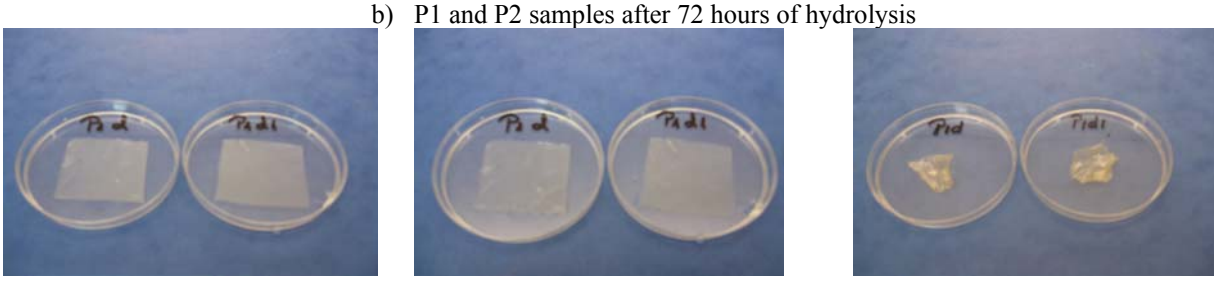
P1 wet samples P2 wet samples P1 dried sample

a) P1 and P2 samples after 48 hours of hydrolysis



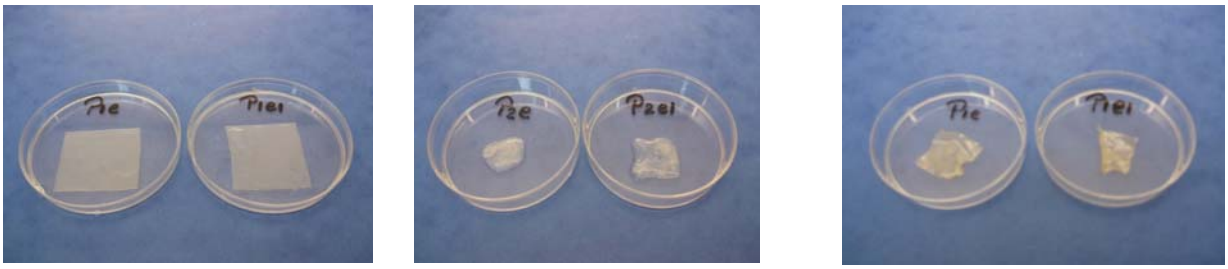
P1 wet samples P2 wet samples P1 dried sample

b) P1 and P2 samples after 72 hours of hydrolysis



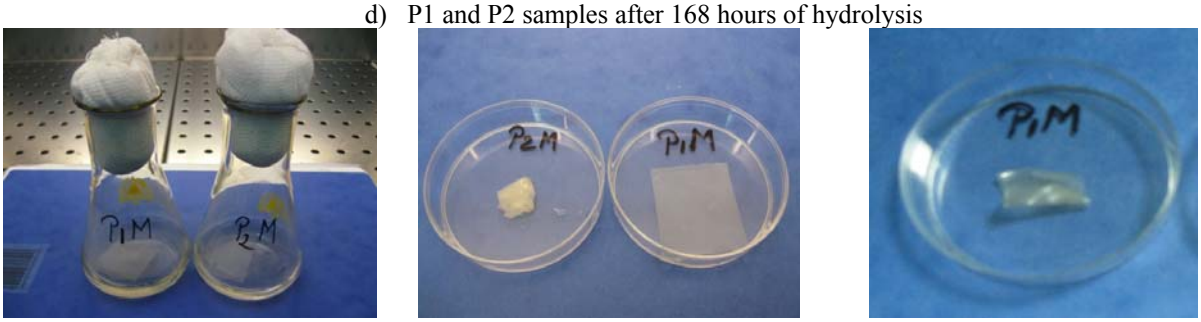
P1 wet samples P2 wet samples P1 dried sample

c) P1 and P2 samples after 96 hours of hydrolysis



P1 wet samples P2 wet samples P1 dried sample

d) P1 and P2 samples after 168 hours of hydrolysis

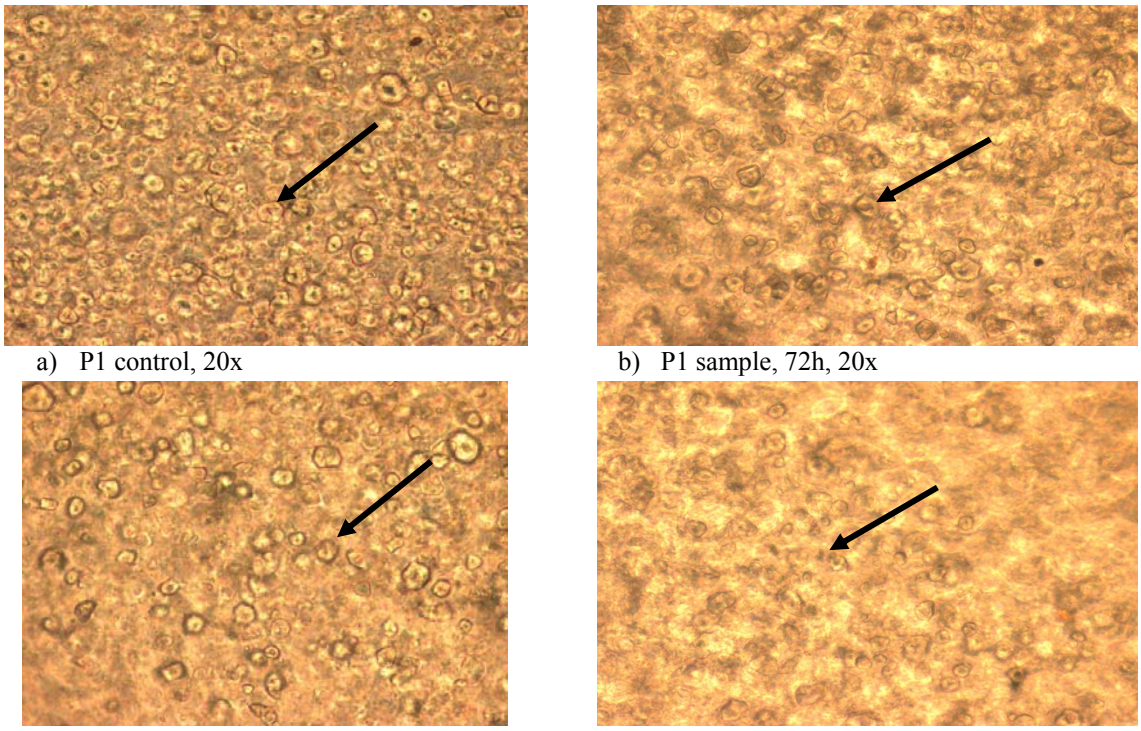


Sample in contact with enzyme Wet samples Dried sample

e) P1M and P2M (controls) after 168 hours

Fig. 1. Polymeric samples after enzymatic hydrolysis with bacterial α-amylase at 27° C.

In Fig. 2. it can be seen the modification of polymer aspect after enzymatic contact visualized with optical microscopic images. It is a partial dissolution of starch granules after 168 hours of incubation with enzyme (Fig. 2 c) as compared with initial sample (Fig. 2.a).

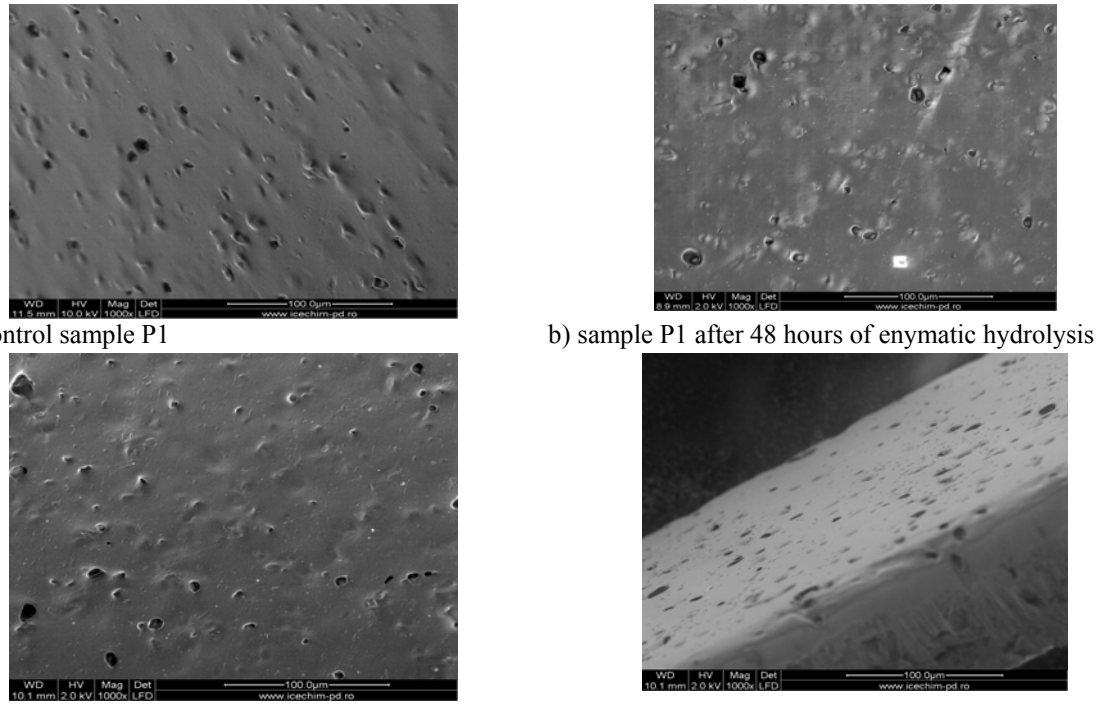


a) P1 control, 20x b) P1 sample, 72h, 20x

c) P1 sample after 96 hours of enzymatic hydrolysis, 20x d) P1 sample after 168 hours of enzymatic hydrolysis, 20x

Fig. 2. Optical microscopy applied to polymeric samples after enzymatic hydrolysis with bacterial α-amylase (black arrow - (starch granules).

Several SEM micrographs given in Fig. 4. show modification of polymer surface after enzymatic hydrolysis.



a) control sample P1 b) sample P1 after 48 hours of enymatic hydrolysis

c) surface of sample P1 after 96 hours of enzymatic hydrolysis d) sample P1 after 168 hours of enymatic hydrolysis

Fig. 4. SEM micrographs of the (a) control sample, (b, c and d) blends samples collected at different hydrolysis times (magnification 1000x).

Starch hydrolysis brings into polymer matrix some voids and polymer surface becomes less smooth. Several times, these irregularities stimulate the degradation process [8]. After enzymatic degradation, it can be seen some accents of polymer surface roughness, increasing the number of voids on the surface and in some cases increase the existing ones.

The enzymatic hydrolysis of the starch weakens the interaction between starch and PVA and thereby results in higher weight loss by the PVA/starch film (Fig. 5). The weight loss of samples exposed to enzymatic hydrolysis could be considered an assessment of degradation level. The ratio of the weight loss of enzymatic hydrolysis of the PVA blend reached almost 30%. This behavior is a consequence of the hydrolysis and solubility of starch in aqueous system. The polymeric material shows a weight-loss of 28.75% after being incubated with α-amylase for 168 hours. The degradation occurred rapidly in the initial 48 hours reaching 20% weight loss, followed by a slow degradation until the end of the experiment.

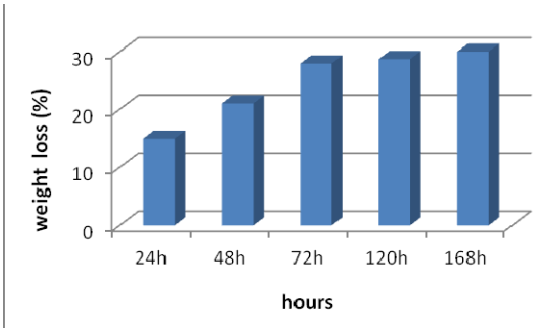


Fig. 5. Weight loss of polymeric samples after enzymatic hydrolysis.

FTIR spectra of films after enzymatic process along with individual components plotted in Fig. 6 reveal no significant differences in the general shapes. The similarity of FTIR spectra between treated samples and PVA is evident, suggesting that enzyme has hydrolyzed the starch, but not the PVA. It was an expected result because PVA is main component representing 50% or 40%, meanwhile starch only 15% in blends. The enzyme activity was addresses to starch component of blends and produces

modification only at its peaks. If we compare the spectra of control with treated samples, there is one major difference, the disappearance in treated samples of the "shoulder" existing at 999,45 cm^{-1} in starch.

The characteristic peaks of PVA are: 2939 cm^{-1} due to C–H group, 1424 cm^{-1} due to O-H group, 1740 cm^{-1} due to C=O, and 1088 cm^{-1} associated with –C-O specific to secundary alcohol. Hydrogen intermolecular bonds are present in the domain 3200 - 3400 cm^{-1} [9, 10].

In starch samples characteristics peaks are: 3290 cm^{-1} from water present in the system and hydroxyl groups of sugar units; at 2930 cm^{-1} as C–H stretch in glucose unit.

The O–H bending of absorbed water exhibits a peak at 1644 cm^{-1} and bands associated with C–O–C, C–O, C–O–H, C–C, C–H from 1150–800 cm^{-1} as mentioned by other reports [11]. Starch and PVA molecules are in general associated with inter- and intramolecular hydrogen bonding in the blends.

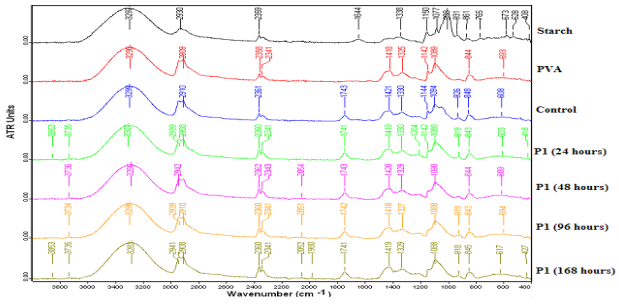


Fig. 6. ATR-FTIR spectra of the PVA/starch blends.

The similarity of treated samples with PVA is evident, suggesting that enzyme has hydrolyzed the starch, but not the PVA. The intensity of the peak at 1150-1040 cm^{-1} decreased, indicating the action of α-amylase in cleaving the glycosidic linkages of starch. After enzymatic degradation, the band at 1644 cm^{-1} from starch almost disappears indicating eventually changes in crystallinity. This phenomenon is attributed to the water absorbed in the amorphous regions of starch [12]

5. Conclusions

Several starch/PVA/glycerol polymer blends were prepared by a solution casting technique. The blends were examined for biodegradation in contact with α-amylase. The samples P1 were more stable in contact with aqueous enzyme solution. Samples P2 with 40% PVA were fragmented and dispersed after drying.

The optical microscopic images showed modifications of polymer aspect after enzymatic. It is a partial dissolution of starch granules after 168 hours of incubation with enzyme as compared with initial sample. SEM micrographs show modification of polymer surface induced by starch hydrolysis that brings into polymer matrix some voids. The weight loss of samples exposed to enzymatic hydrolysis could be considered an assessment of degradation level. The ratio of the weight loss reached 28.75%. The intensity of the peak at 1150-1040 cm^{-1} decreased, indicating the action of α-amylase in cleaving the glycosidic linkages of starch.

Acknowledgments

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