Enzymatic Degradation of LDPE / Corn Starch Blends Treated with [EMIM][Cl] Ionic Liquid

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The susceptibility of starch-based biomaterials to enzymatic degradation by α-amylase and peroxidase enzymes was investigated. A polymeric blend of corn starch with polyethylene, designated by SLDPE, was studied. The degradation was evidenced by gravimetry, scanning electron microscopy (SEM), FTIR spectroscopy, and X-ray diffraction. Analysis performed showed that starch blend is susceptible to enzymatic degradation, significantly in the presence of ionic liquid, as evidenced by increased weight loss and reducing sugars in solution. SEM analysis evidenced the presence of fractures and pores at the materials surface as a result of starch degradation by α-amylase. FTIR spectra confirmed a decrease on the band corresponding to glycosidic linkage (-C-O-C-) of starch.

Keywords: *corn starch, LDPE/starch blends, degradability, enzymes, ionic liquid*

Polymer blending is a convenient method to develop novel biocomposites with tailored properties [1-9]. Starch (S) is a (1→4)-linked polysaccharide composed of amylose, a linear α-D-(1→4)-glucan, and the extensively α-D-(1-4)-glucan branched by α-D-(1→ 6)-linkages, amylopectin. Susceptibility to biodegradation of the starch containing blends has been extensively studied [10-12]. Starch-based polymers have been studied as potential materials for biomedical applications [13-15]. The main enzymes involved in starch hydrolysis are α-amylases, β-amylases, glucoamylases, and α-glucosidases [16]. α-Amylase catalyses the hydrolysis of α-1,4-glycosidic linkages of starch to maltose and dextrins [17]. The enzymatic hydrolysis of the blends apparently involves surface starch granules [18].

Ionic liquids are solvents composed of ionized species and may be ideal solvents for biocatalytic reactions [19-21]. There were reported results on the activity of α-amylase in 1-butyl-3-methylimidazolium chloride [22].

The biodegradation of film made with LDPE-containing 6% starch by the fungus *Phanerochaete chrysosporium* has been attempted, but virtually no degradation of polyethylene was observed [23]. Peroxidases are very attractive biocatalysts for selective oxidative transformations. The thermal stability of horseradish peroxidase is improved in the presence of 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]) [24].

The present paper investigates the degradation behaviour of LDPE films containing starch (designed as SLDPE) treated with ionic liquid 1-ethyl-3-methylimidazolium chloride ([EMIM][Cl]) in the presence of enzymes (α-amylase and peroxidase). The modifications induced by the enzymatic degradation were evidenced by determination of the weight loss, sugars released during biodegradation, as well as by FTIR spectroscopy, scanning electron microscopy (SEM) and X-ray diffraction.

Experimental part

Materials

Low density polyethylene (MP 125°C, ρ=0.96 g/cm^3) was provided by SNP PETROM, Arpechim Pitesti, Romania. Corn starch was provided by S.C.Antibiotice SA Iasi, Romania. 1-ethyl-3-methylimidazolium chloride [EMIM][Cl] (>95% purity) was purchased from Fluka. α-Amylase (source *Bacillus licheniformis*)-Sigma and horseradish peroxidase-Merck were used for degradation tests.

LDPE-starch film manufacturing

A Brabender LabStation (Germany) with a mixer (30/50 EHT) was used for the melt blending of LDPE with starch. Starch/LDPE film containing 7.5% by weight (wt %) of starch and 100 μm in thickness was subjected to enzymatic degradation tests.

Enzymatic degradation tests

In vitro enzymatic degradation tests were carried out at 25°C, *p*H=6.9, using 6 IU enzyme/g material, up to 52 h, in the presence of [EMIM][Cl] using a ratio 1:25 (w/w). The weight loss for dry samples was monitored at pre-established time intervals, being calculated by equation:

$$\% \ degradation = (m_0 - m_d) / m_0 \ x \ 100$$

where: m_0 and m_d are the dry mass of the un-degraded and degraded samples at moment "t". Four replicates were performed for each experiment.

Reducing sugars

The assay method involved determination of the reducing groups released from starch in the presence of 3, 5-dinitrosalicylic acid [25], using a Carl Zeiss M42UV-spectrophotometer, maltose being used as standard. One unit releases from soluble starch one micromole of reducing groups per minute at 25°C and *p*H 6.9.

FTIR spectroscopy

FTIR spectroscopy was performed using a Bruker Vertex 70 equipment, at spectral resolution of 4 cm^{-1} and the scanning range from 400 to 4000 cm^{-1}.

X-ray diffraction

The X-ray diffraction patterns were registered on a Bruker AD8 ADVANCE X-ray diffractometer, with a conventional copper target X-ray tube set to 60 kV and 50 mA. The X-ray source was Cu Kα radiation. Data were collected for diffraction angle 2θ ranging from 5 to 60°.

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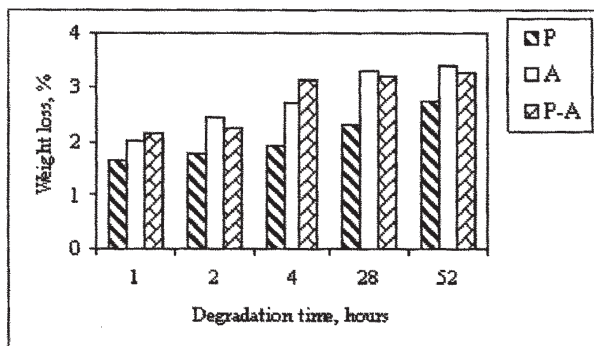

Fig.1. Weight loss for SLDPE blend treated with [EMIM][Cl] after incubation in enzyme solutions: P (peroxidase), A (α-amylase), P-A (enzymes mixture)

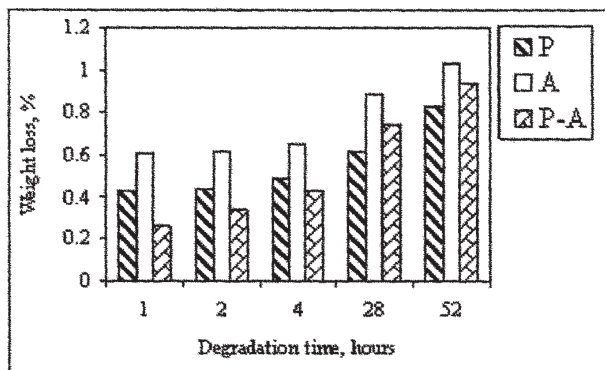

Fig.2. Weight loss for SLDPE blend after incubation in enzyme solutions without [EMIM][Cl]: P (peroxidase), A (α-amylase), P-A (enzymes mixture)

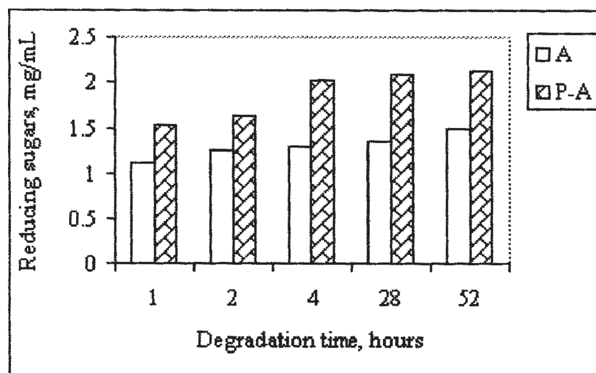

Fig.3. Enzyme activity on SLDPE blends treated with [EMIM][Cl] measured as concentration of reducing sugars released into the solution: A (α-amylase); P-A (enzymes mixture)

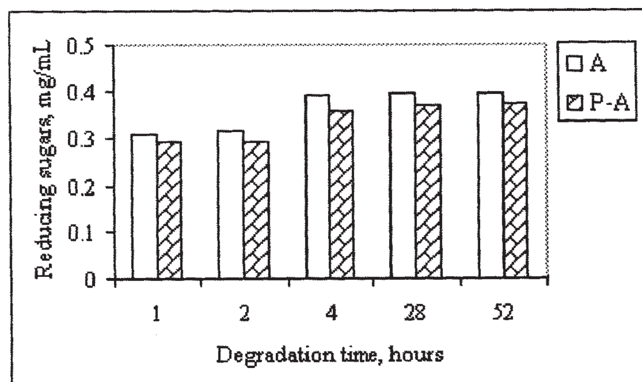

Fig. 4. Enzyme activity on SLDPE blends without [EMIM][Cl] measured as the concentration of reducing sugars released into the solution: A (α-amylase); P-A (enzymes mixture)

Scanning electron microscopy (SEM)

The surface properties of degraded blend samples were visually investigated using a QUANTA 200 instrument with an EDAX analysis system, a versatile high performance low-vacuum scanning electron microscope with a tungsten electron source, with three imaging modes (high vacuum, low vacuum and ESEM). The resolution used for surfaces investigation was 3 nm.

Results and discussion

Weight loss determination

The removal of part of the starch from the blend is observed mainly in the first hour as a result of enzymatic hydrolysis (figs. 1-2). The influence of enzymes upon weight change increased in the following order: peroxidase< enzymes mixture < α-amylase.

It is noticed that enzymes contribute, in different percentages, to the material degradation and the incubation with α-amylase leads to a higher weight loss. When these two enzymes were combined, the weight loss of SLDPE slightly increased, which may indicate a synergy effect.

Release of reducing sugars

Enzymatic hydrolysis of insoluble polymers is known to be affected by the mode of interaction between the enzymes and the polymeric chains [26]. The analysis of the reaction media after enzymatic hydrolysis (fig. 3) gives information about the mode of action of the enzymes toward different substrates. The content of sugars released during enzymatic degradation, expressed as maltose, is shown in figures 3 and 4.

From figures 3-4, it seems that enzymatic degradation is more efficient in the presence of [EMIM][Cl] in terms of sugars released during degradation. The high rate of releasing reducing groups from starch is evidenced in the first hour of enzymatic treatment.

FTIR spectroscopy

FTIR spectrum of corn starch (fig. 5) shows a strong and broad absorption peak at 3400 cm^{-1} assigned to the stretching vibration of –OH. The band at 2920 cm^{-1} is attributed to C-H stretching.

The characteristic peak occurred at 1652 cm^{-1} being related to water included in starch [27]. The absorption bands between 1000 and 1200 cm^{-1} are characteristic of the C-O bond stretching [28].

In figure 6, the spectra obtained for SLDPE films, before and after treatment with α-amylase without [EMIM][Cl] show differences due to the formation of new groups. The changes in the absorption bands at 1715-1740 cm^{-1} and 1620-1680 cm^{-1} corresponding to the carbonyl (C=O) and vinyl groups (CH_2=CH-), respectively, are due to the degradation process of the films. The proeminent bands of LDPE at 1467 and 1373 cm^{-1} arising from the -CH_2- group appear almost without modification. No differences are observed in the spectra for SLDPE films after enzymatic treatment with peroxidase and enzymes mixture.

Figure 7 presents the FTIR spectra obtained for SLDPE films subjected to enzymatic degradation in ionic liquid. After degradation with α-amylase, the intensity of the peak at 1150-1040 cm^{-1} decreased, indicating the cleavage of the glycosidic linkages from starch.

SEM investigation

The surface of the SLDPE films was investigated using SEM. It may be noted that the LDPE/starch interface does not present homogeneity due to the opposite nature of the components (LDPE hydrophobic versus starch hydrophilic).

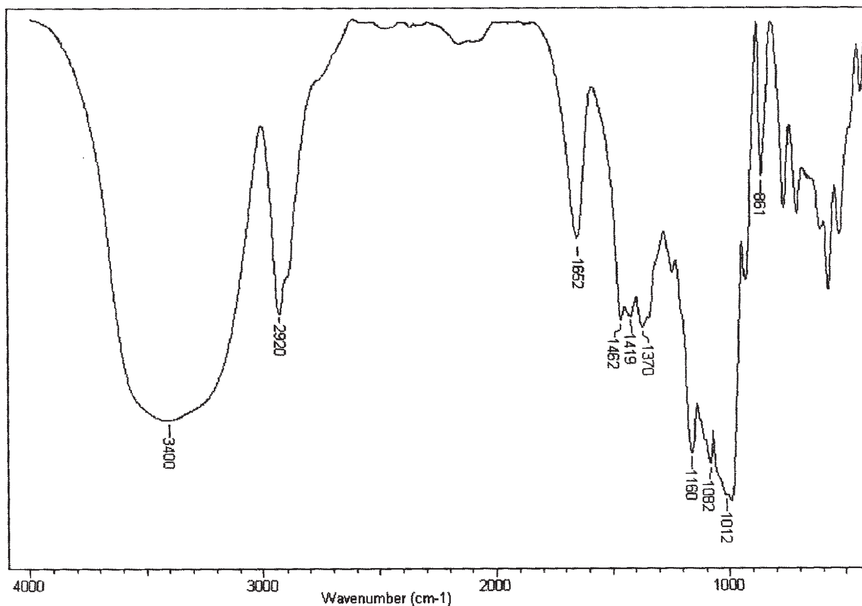

Fig. 5. FTIR spectrum obtained for corn starch

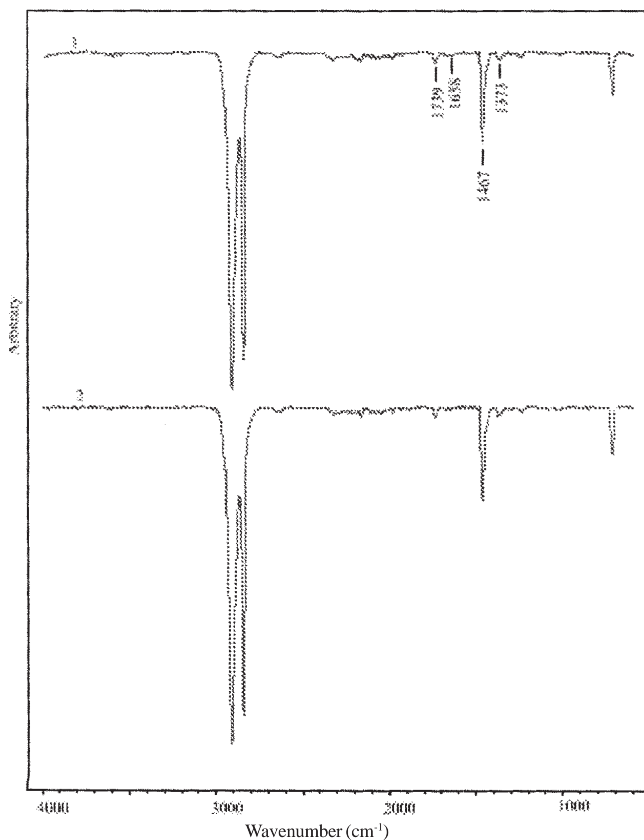

Fig. 6. FTIR spectra of SLDPE blend without [EMIM][Cl] before (1) and after 4h treatment with α-amylase (2)

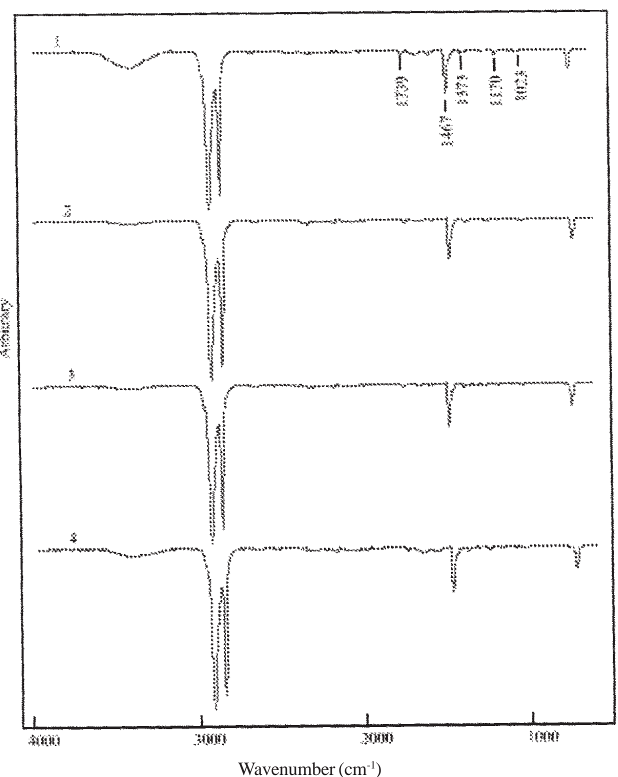

Fig.7. FTIR spectra of LDPE (1) and SLDPE treated with [EMIM][Cl] before (2) and after 4h treatment with enzymes (3: A; 4: P; 5: A-P)

Consequently, starch incorporation causes many imperfections into the polymeric matrix. The initial films showe a specific structure in the SEM micrographs (fig. 8 A), a slightly rough surface being evidenced. Figure 8 B-G show the surface of the SLDPE film subjected to the enzymatic degradation.

This became much less rougher, and is penetrated by a reduced number of craters and pinholes, which can be also explained by the presence of some imperfections. SEM images illustrate the increase in porosity of the SLDPE films as a result of enzymatic degradation.

The amorphous component of starch is the first to be consumed in the blend, since cavities are observed as a result of the starch consumption. Starch also seems to be consumed when the time of blend exposure to the enzymes is increased.

X-ray diffraction

The crystalline structure of the blend control sample and enzymatic treated SLDPE blend was studied by X-ray diffraction (fig. 9). The diffractogram of the native corn starch (fig. 9A) evidenced the A-style crystallinity.

The diffraction of SLDPE subjected to enzymatic treatments without [EMIM][Cl] is characterized by a strong magnitude peak at 19.6° (2θ) attributed to starch [4]. For SLDPE blend treated with α-amylase in ionic liquid, the diffraction peak is shifted to 18.5° (2θ) and its width decreases revealing the increasing of samples crystallinity.

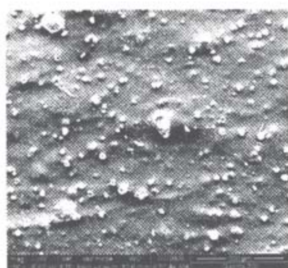

Fig. 8A

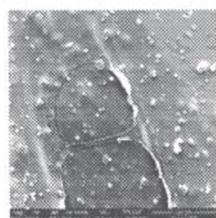

Fig. 8B

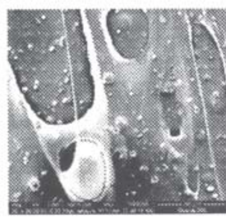

Fig. 8C

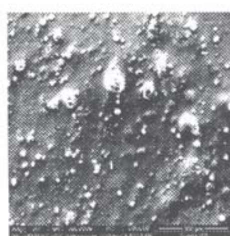

Fig. 8D

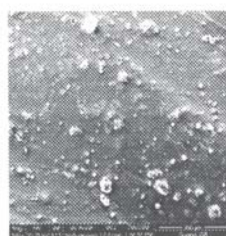

Fig. 8E

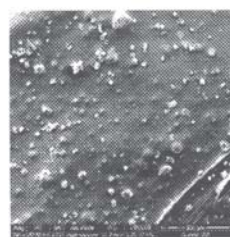

Fig. 8F

Fig. 8G

Fig. 8. SEM images revealing the changes in the surface morphology of SLDPE blend treated with [EMIM][Cl] in enzymes: control (A), peroxidase for 4 h (B) and 52 h (C), α-amylase for 4 h (D) and 52 h (E), enzymes mixture for 4 h (F) and 52 h (G)

Conclusions

The enzymatic degradation of SLDPE blend treated with ionic liquid [EMIM][Cl] was investigated by weight loss, FTIR spectroscopy, SEM and X-ray diffraction methods.

The α-amylase is the key enzyme involved in the starch degradation, contributing to major changes of materials. The enzymatic degradation ability in the presence of ionic liquid is: α-amylase>enzymes mixture>peroxidase.

SEM analysis detected the presence of fractures and pores at the materials surface as a result of starch degradation which seems to be consumed when the time of blend exposure to the enzymes is increased.

X-ray diffraction data evidenced a slightly increase in degree of crystallinity for samples during enzymatic treatment, mainly in the presence of enzymes mixture.

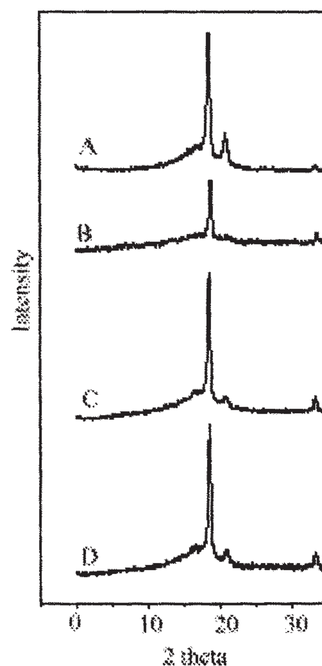

Fig. 9. X-ray diffraction scans for SLDE blend treated with [EMIM][Cl] in enzymes: (A) control, (B) peroxidase, (C) α-amylase, (D) enzymes mixture

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