ISSN : 0974 - 7451 Volume 7 Issue 11

Trade Science Inc.

Environmental Science
An Indian Journal
Sustainability Engineering and Green Chemistry

ESAIJ, 7(11), 2012 [425-429]

Enzymatic degradation of nanocomposites poly (ε-Caprolactone) and starch containing sodium montmorillonite clay by amyloglucosidase and α-amylase

Mohammad Alikarami[1], Zahra Abbasi[2]*

[1]Department of Chemistry, Ilam Branch, Islamic Azad University, Ilam, (IRAN)

[2]Department of Chemistry, Eyvan-e-Gharb Branch, Islamic Azad University, Eyvan, (IRAN)

E-mail : zahra.abbasi886@gmail.com

ABSTRACT

The aims of the study were to investigate the effect of poly (ε-caprolactone) (PCL) and Sodium Montmorillonite (MMT-Na) within the thermoplastic starch (TPS) blends on the rate and extent of starch enzymatic hydrolysis using enzymes α-amylase and amyloglucosidase. The results of this study have revealed that blends with a MMT-Na content at 6 wt% exhibited a significantly reduced rate and extent of starch hydrolysis. The results suggest that this may have been attributed to interactions between starch and MMT-Na that further prevented enzymatic attack on the remaining starch phases within the blend. The total solids that remained after 3000 min were 52 wt.% (TPS: PCL); 56.3 wt.% (TPS: PCL: 2% MMT-Na); 61.7 wt.% (TPS: PCL: 4% MMT-Na); 65.4 wt.% (TPS: PCL: 6% MMT-Na). Enzymatic degradation behaviour of TPS: PCL: MMT-Na was based on the determinations of Water resistance, Weight loss and the Reducing sugars.

© 2012 Trade Science Inc. - INDIA

KEYWORDS

Nanocomposites;
Polymer composites;
Biodegradable polymers;
Water resistance;
Reducing sugars.

INTRODUCTION

Biodegradable polymers have been extensively investigated since the 1970s in order to protect the environment from non-biodegradable plastic wastes[1,2]. Among such compounds, starch has received much attention in its use as biodegradable packaging materials because it is readily available at a low cost and has very fast biodegradability[3–6]. Apart from favourable physico-chemical and mechanical properties, a biodegradable polymer to be used in medical applications needs to be biocompatible in a specific environment and its degradation products should not be cytotoxic[7]. The use of synthetic degradable polymers as biomaterials implies they are biocompatible by themselves and the use of particular additives and/or processing technologies should not interfere with the biocompatible behaviour[8]. Among biodegradable polymers, poly (ε-caprolactone) (PCL), a synthetic aliphatic polyester, has been widely used in medical, packaging and agricultural applications because of its excellent mechanical properties, including its flexibility. The major disadvantage of PCL is its price, which limits its wider use as a substitute for conventional polymers. Polymeric blends,

i.e., mixtures of two or more polymers that may or may not be biodegradable, are commonly used in the plastic industry[9]. In particular, blends of PCL and natural materials, such as starch and cellulose derivatives[10] have been extensively studied because of their lower cost compared to other materials[11]. Amylose is linear and its composition is around 25% in the starch. Amylopectin is branched and has a higher molar mass than amylose; it is found to be around 75% in the starch composition. The linear portion of amylopectin forms double helical structures stabilized by hydrogen bonds between the hydroxyl groups and forms the crystalline region of starch granules. The amorphous region is composed of amylose and amylopectin chains[12]. Starch is currently used in the development of thermoplastic materials. The addition of starch to synthetic polymers enhances the microbiological degradation of the blend. Starch can be processed as a thermoplastic and also can be incorporated as a filler in traditional plastics or associated with plasticizers. Enzymatic degradation, using α-amylase and amyloglucosidase, is one of a number of possible methods that can be employed to hydrolyse starch[12, 13]. Both fractions are readily hydrolysed at the acetal link by enzymes. The α-1,4-linkage in both components of starch is attacked by amylase; the α-1,6- linkage in amylopectin is attacked by glucosidases[12]. α-amylase are endoamylases catalysing the hydrolysis of internal α-1, 4- glycosidic linkages in the starch in a random manner. The microbial α- amylase for industrial purposes are derived mainly from *Bacillus licheniformis*, *Bacillus amyloliquefaciens* and *Aspergillus oryzae*. PCL was chosen because starch/ PCL blends have demonstrated excellent compatibility. Gan et al. (1999) reported that PCL was easily degraded by lipases from microorganisms, especially Pseudomonas[14]. Similarly, Marten et al. (2005) also studied the effect of enzymes on polyesters[15]. Li et al. (2003) described a system to study the biodegradation of PCL and poly (L-lactide) blends using a Pseudomonas Lipase[16]. The addition of poly (L-lactide) to PCL markedly reduced the degradation of the former polymer. In this system, the presence of cracks and an elevated lipase concentration favoured enzymatic degradation. Sivalingam et al. (2003) studied the enzymatic biodegradation of PCL by two enzymes, novozyme 435 and lipolase, and found that there was less degradation with the former enzyme than with lipolase[17]. Braganca (2003) reported that hydrolysis was the principal mechanism for the biodegradation of Cellulose acetate (CA), with the first step as depolymerization and being in contact with extracellular microbial enzymes; the resulting oligomers were then easily phagocytosed by the cells, followed by mineralization[18]. The current paper studies the α-amylase and amyloglucosidase actions on starch/ PCL composite film containing MMT-Na at temperature 37 °C. The modifications induced by the enzymatic treatment were evidenced by determination of weight loss, water absorption capacity, sugars released during biodegradation, as well as by UV spectroscopy and Total sugars were estimated by dinitrosalicylic acid (DNS) method.

EXPERIMENTAL

Materials

Starch (ST) was provided by Merck company, and PCL (type P-767) was supplied in pellet form by Dow Quý´mica S.A. (Cubata˜ o, SP, Brazil). The melt flow at 80 °C was 1.970.3 g/10 min (ASTM D-1238), with a density of 1,145 kg/m^3 and a average molecular weight (Mw) of 50,000. The water used was distilled and deionized water. α-Amylase (source from *Bacillus Subtilis*) and amyloglucosidase (sourced from *Aspergillus niger*) The clay used in this study was based on naturally occurring sodium montmorillonite clay (Cloisite Na+ – supplied by Southern Clay Products) – Na-MMT and Reagent DNS was used for determination sugars released during degradation.

Film preparation

The nanocomposite of PCL with TPS containing sodium montmorillonite clay were prepared by casting. The nanocomposite have been prepared from 50 wt% PCL–50 wt% starch containing small amounts of plasticizers, stabilizers and destructuring agents (stabilizers or destructuring agents such as sodium montmorillonite clay and plasticizer such as glycerol). The solutions were prepared by dissolving the material in 10% (w/v) acetone, with stirring at 60± 5 °C for 6 h. The mixtures were then poured into culture dishes and the solvent was allowed to evaporate in an atmosphere saturated with acetone.

Enzymatic degradation test

Each sample was placed in a vial filled with 20 ml

of 0.05 M phosphate buffer, pH 6.9, containing 1.0 mg of amyloglucosidase and 1.0 mg of α-amylase, and then incubated in a thermostatted oven at 37 °C. The buffer/ enzyme system was changed for every 24 h during the evaluation period in order to maintain the original level of enzymatic activity. For every 48 h, the samples were removed from the incubation medium, washed with distilled water, wiped dry, weighed, and examined by light microscopy before being returned to the incubation medium. The controls consisted of samples incubated in buffer without enzyme. The dried samples were cut into 4 cm× 4 cm square specimens, weighted, and immersed in the conical flasks. The flasks were placed in a shaking incubator (Fanavaran Sahand Azar Co. 1SH 554D, Iran) with a rate of 180 rpm for 50 h at 37 °C. After 1, 2, 3, 5, 7, 9, 12, 18, 24, 29, 36, 40, 45 and 50 h, the samples were removed and rinsed with distilled water to remove the enzymes, dried and weighed, respectively. The degree of enzymatic degradation (DED) was calculated as:

$$DED\ \% = (W_0 - W_1) / W_0 \times 100$$

where W_0 represents the initial weight of a specimen and W_1 is the weight of a specimen after degradation.

Water absorption test

Pieces of the films were placed in a freeze dryer (Pishtaz Engineering Co, FD-4, Iran) and dried for least 24 h. then samples were weighed for the dry weight, and then placed in a bath in distilled water at room temperature. After 1, 2, 3, 5, 7, 9, 12, 18, 24, 29, 36, 40, 45, 50 h, the samples were removed from distilled water and weighed. The water absorption capability (WAC) was calculated with the equation below:

$$WAC\ \% = (W_{wet} - W_{dry}) / W_{dry} \times 100$$

Where W_{wet} represents the weight of the wet specimen and W_{dry} represents the weight of the dry specimen.

Detection of reducing sugars

The reducing sugars in the degradation solutions were quantified by the dinitrosalicylic acid method: 1 ml of reagent DNS was added to 1 ml of the sample to be analysed. using 1 mg/ ml glucose stock solution as a standard. At the same time, the blank was prepared using 1 ml of control sample. The mixture was heated at 90-100 °C for 10 min. After cooling to room temperature, 5ml of distilled water was added, and the absorbance at 540 nm was measured. The respective carbohydrate concentration was obtained by comparison with a standard curve.

Scanning electronic microscopy (SEM)

The morphology of the surface of the films, before and after biodegradation, was investigated using a scanning electronic microscope of XL30 type (Netherland). The films were covered with pure metalic Ag. The laying down of Ag was carried out using evaporation of the metal under a high vacuum, to give a thickness of around 100 A°.

RESULTS AND DISCUSSION

Degradability of polymers is a critical functionality for their application. Currently, no official standard method was established in determining biodegradability of polymers. The enzyme method[19] the microbiological method[20] and the soil burial method[21] have been used by different researchers. Moreover, the biodegradability was also recorded by diverse indexes even in the same method[6]. The current paper studies the α-amylase and amyloglucosidase actions on starch/ PCL composite film containing MMT-Na at temperature 37 °C. Bajpai and Shrivastava[22] who studied the biodegradation of carboxymethyl-cellulose/ starch blends, found that, at small amounts of starch in the blend, a high percent of weight loss occurred while, at high starch contents, the weight loss was lower. This variation was explained in the first case, by the increase of the number of starch molecules contacting the α-amylase, so that the amount of degraded starch was higher. At high starch contents, the material becomes much more compact, which hinders the α-amylase diffusion in the polymer film.

Weight loss and water uptake

The water absorption capacity and the degradability are the most important properties for biodegradable materials. The water absorption capacities of the TPS: PCL: MMT-Na blend film were found to have significant difference. The increase of nanoparticale leads to the decrease of both weight loss and Water uptake Figuer 1 and Figure 2 clearly show that degradation is much more pronounced when the WAC % is high. A comparison between the variation of the DED % and WAC % with respect to MMT-Na clearly show that

degradation is much more pronounced when the water sorption is high. The total solids that remained after 3000 min were 52 wt.% (TPS: PCL); 56.3 wt.% (TPS: PCL: 2% MMT-Na); 61.7 wt.% (TPS: PCL: 4% MMT-Na); 65.4 wt.% (TPS: PCL: 6% MMT-Na). TPS: PCL exhibited both a high water sorption and the most significant weight loss.

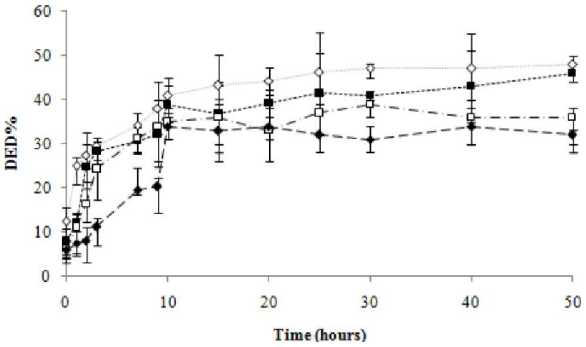

Figure 1 : Enzymatic degradability of the TPS: PCL (◊), TPS: PCL: 2% MMT-Na (■),TPS: PCL: 4% MMT-Na ( ),TPS: PCL: 6% MMT-Na (◆)

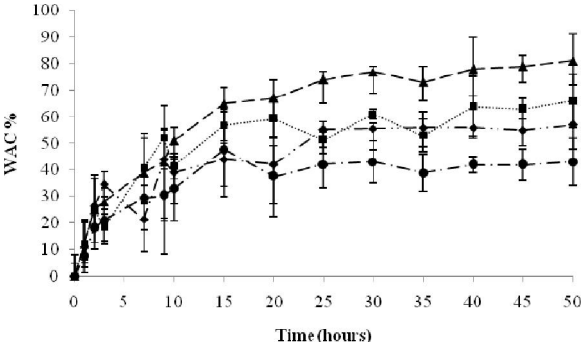

Figure 2 : Water absorption capability (WAC) of the TPS: PCL (▲),TPS: PCL: 2% MMT-Na (■),TPS: PCL: 4% MMT-Na (♦),TPS: PCL: 6% MMT-Na (●)

Rate and extent of glucose production

The rate and extent hydrolysis by the actions of α-amylase and amyloglucosidase was measured using the DNS method glucose assay of four blends of varying MMT-Na. The production of glucose was used as a measure of starch hydrolysis. Figure 3. Shows the extent of glucose over a 50 h hydrolysis time for each substrate. Figure 4. illustratess the initial rate of glucose production by each substrate up to a hydrolysis time 10 h. The rate of glucose production was calculated; refer to TABLE 1. by assuming a linear relationship between the concentration of glucose and time for the first 10 h of hydrolysis. The rates of glucose production from each composite substrates, were most rapid for the substrate without MMT-Na and decreased with the addition of MMT-Na, for TPS: PCL blend (374 µg/ml.h), 289 µg/ml.h (TPS: PCL: 2% MMT-Na), 267 µg/ml.h (TPS: PCL: 4% MMT-Na), 219 µg/ml.h (TPS: PCL: 6% MMT-Na). The rate of starch hydrolysis was most rapid for the substrate Starch/PCL and decreased with the addition of MMT-Na. The amount of reducing sugars in the degradation solutions, reduced by dinitrosalicylic acid, increased since the beginning until the end of the assay the relative amount of reducing sugars in the degradation solutions in similar assays without enzymes was about 100 times lower. One of the routes of biodegradation is by hydrolysis, and the enzymatic hydrolysis of starch is

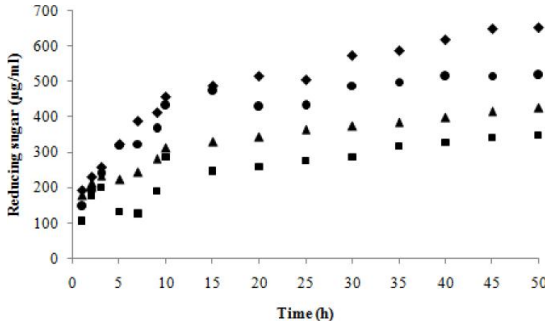

Figure 3 : Concentration of glucose produced for nanocomposite films in the 50 h of enzymatic degradation due to the action of <alpha>-amylase and amyloglucosidase. TPS: PCL (♦),TPS: PCL: 2% MMT-Na (●),TPS: PCL: 4% MMT-Na (▲),TPS: PCL: 6% MMT-Na (■)

TABLE 1 : A summary of the rates of glucose production due to the action 1.0 mg of amyloglucosidase and 1.0 mg of -amylase from each substrates

substrate	Rate (µg/ ml. h)	R^2
TPS: PCL	374	0.99
TPS: PCL: 2% MMT-Na	289	0.97
TPS: PCL: 4% MMT-Na	267	0.98
TPS: PCL: 6% MMT-Na	219	0.99

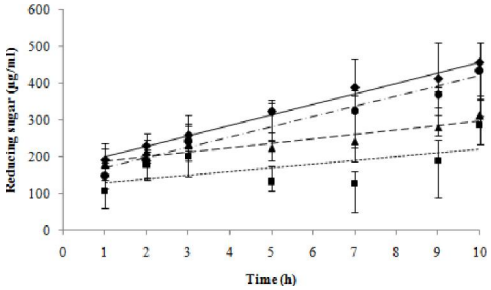

Figure 4 : Concentration of glucose produced for nanocomposite films in the first 10 h of enzymatic degradation due to the action of <alpha>-amylase and amyloglucosidase. TPS: PCL (♦),TPS: PCL: 2% MMT-Na (●),TPS: PCL: 4% MMT-Na (▲),TPS: PCL: 6% MMT-Na (■)

accompanied by the release of glucose. Figure 4 shows the release of glucose (μg/ml) during exposure to α-amylase and amyloglucosidase. The amount of free glucose increased with time for the blends showed a peak release of glucose at 10 h, followed by a decline. Apparently, the MMT-Na has a stabilizing effect against the enzymatic attack, even after increasing the content of insoluble fraction.

Scanning electronic microscopy (SEM)

Several scanning electronic microscopy images of nanocomposites are given in Figure 5. One may observe that the films are considerably destroyed, although during degradation a much more stable fibrillar fraction is revealed.

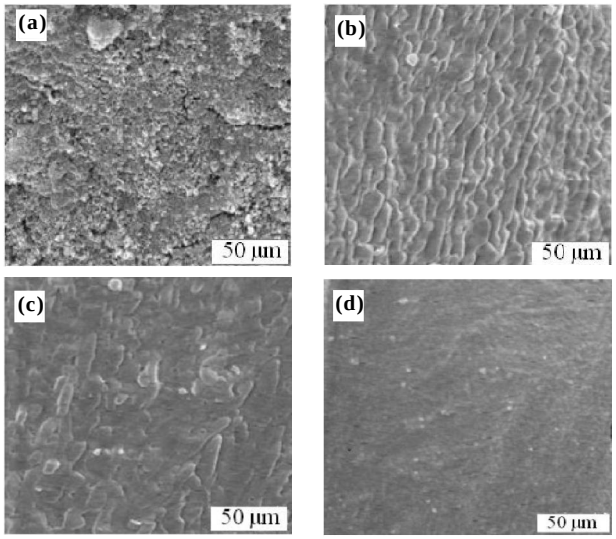

Figure 5 : Scanning electron micrographs of degradable films in 50 h of enzymatic degradation due to the action of <alpha>-amylase and amyloglucosidase: (a) TPS: PCL degraded; (b) TPS: PCL with 2 wt% MMT-Na degraded; (c) TPS: PCL with 4 wt% MMT-Na degraded; (d) TPS: PCL with 6 wt% MMT-Na degraded

CONCLUSIONS

The present study shows the role of α-amylase and amyloglucosidase nanocomposites degradation. The MMT-Na content significantly impacted on the rate of starch solubilistion. The decrease of the degradation rate observed in the final stage can be explained to the lower degradability of the MMT- PCL domains that remain in the material. After 10-50 hour, the variation is almost negligible, nearly zero, as no saccharides and other compounds leached to the solution, as demonstrated before. The reduction of the degradation rate is also influenced by the water uptake ability of these polymers.

REFERENCES

[1] H.S.Yang, J.S.Yoon, M.N.Kim; Polymer Degradation and Stability, **87**, 131 **(2005)**.
[2] S.Kiatkamjornwong, P.Thakeow, M.Sonsuk; Polymer Degradation and Stability, **73**, 363 **(2001)**.
[3] Z.Abbasi; Journal of the Taiwan Institute of Chemical Engineers, **43**, 264–268 **(2012)**.
[4] Z.Abbasi, M.Alikarami, M.T.Taghizadeh, N.Saouri, F. Raoufi; Journal of Chemical Science and Technology, **1(3)**, 70-73 **(2012)**.
[5] M.Kim; Carbohydrate Polymers, **54**, 173 **(2003)**.
[6] M.N.Kim, A.R.Lee, J.S.Yoon; I.J.Chin.European Polymer Journal, **36**, 677 **(2000)**.
[7] J.Lunt; Polym.Degrad.Stab., **59**, 145 **(1998)**.
[8] M.T.Taghizadeh, Z.Abbasi, Z.Nasrolahzadeh; JTICE, **43**, 120 **(2012)**.
[9] U.S.shiaku, K.W.Pang, W.S.Lee, Z.A.M.Ishak; European Polymer Journal, **38**, 393 **(2002)**.
[10] C.Bastioli, A.Cerutti, I.Guanella, G.C.Romano, M.Tosin; J.Environ.Polym.Degrad., **3**, 81 **(1995)**.
[11] C.Bastioli; Polym.Degrad.Stab., **59**, 263 **(1998)**.
[12] M.T.Taghizadeh, Z.Abbasi; J.Iran.Chem.Res., **4**, 77 **(2011)**.
[13] S.Helena; L.Rui; Reis.Acta BIOMATERIALIA, **5**, 3021 **(2009)**.
[14] Z.Gan, D.Yu, Z.Zhong, Q.Liang, X.Jing; Polymer., **40**, 2859 **(1999)**.
[15] E.Marten, R.J.Muler; Polym.Degradat.Stabil., **88**, 371 **(2005)**.
[16] S.Li, L.Liu, H.Garreau, M.Vert; Biomacromolecules, **4**, 372 **(2003)**.
[17] G.Sivalingam, S.Chattopadhyay, G.Madras; Polymer Degradation and Stability, **79**, 413 **(2003)**.
[18] F.C.Braganca; Universidade Sa˜o Francisco, Itatiba, **(2003)**.
[19] C.V.Benedict, W.J.Cook, P.Jarrett, J.A.Cameron, S.J.Huang, J.P.Bell; J.Appl.Polym.Sci., **28,** 327 **(1983)**.
[20] M.Huskic, I.Brnardic, M.Zigon, M.Ivankovic; J.Non-Cryst Solids, **354,** 3326 **(2008)**.
[21] Y.X.Xu, Y.X.Hanna; Carbohydr.Polym., **59** , 521 **(2005)**.
[22] A.K.Bajpai, J.Shrivastava; Polym.Int., **54**, 1524 **(2005)**.