International Biodeterioration & Biodegradation xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Enzymatic degradation of flax-fibers reinforced polylactide

Magdalena Stepczyńska*, Piotr Rytlewski

Department of Materials Engineering, Kazimierz Wielki University, ul. Chodkiewicza 30, 85-064 Bydgoszcz, Poland

ARTICLE INFO

Keywords:
Biocomposites
Polylactide
Flax fibers
Degradation
Enzymes

ABSTRACT

The present article described a study on the biodegradation rate of polylactide (PLA) with reinforcement of flax fibers. Specifically, enzymatic degradation of the flax-fibers reinforced PLA was addressed with particular attention paid to the processing conditions, the types of enzymes used for degradation, and time of treatments. Mass losses of the samples were measured as a function of time, and the degree of crystallinity was determined using a differential scanning calorimetry (DSC). The morphology of the samples was examined using scanning electron microscopy (SEM). Proteinase K was shown to exert the greatest effects on the degradation rate of all samples tested. For this enzyme, the mass loss of PLA samples without fillers was approximately 21%, but it was about 52% for PLA containing 30% (w/w) flax fiber. The lowest impact on the biodegradation rate of PLA was noted for protease and lipase enzymes tested in this study. Proteinase K also caused the greatest changes in the structure of all samples. Furthermore, samples treated with this enzyme also showed the highest degree of crystallinity as compared with non-treated ones.

1. Introduction

Constantly rising amount of plastic waste that loads natural environment, public awareness of threats associated with environmental pollution, legal regulations, and diminishing resources of crude oil have resulted in increasing interest in biodegradable materials. Due to their biocompatibility and biodegradability, polymers of that group are used in medicine and, now in a wider and wider range, in packaging industry, pharmacy, biotechnology, and horticulture (Ebnesajjad and Modjarrad, 2014). From among those materials, polylactide (PLA) properties are the subject of the most advanced scientific and technological studies. Production of PLA covers ca. 40% of all the biodegradable polymers being manufactured (Auras et al., 2010; Rasal et al., 2010). PLA has many advantages over conventional thermoplastic polymers manufactured from crude oil. It's characterized by good optical, mechanical and barrier properties. PLA is being produced from renewable resources, and it undergoes biodegradation within a dozen or so days under conditions of industrial composting, whereas biodegradation time for synthetic polymers, such as polyethylene (PE) or polystyrene (PS), is 500–1000 years (Błędzki and Fabrycy, 1992; Doi and Steinbuchel, 2002; Pluta, 2004).

Despite the many advantages brittleness, low impact strength and low thermal stability limit some applications of PLA. To improve its properties, various methods of modification are used (Rytlewski et al., 2011; Malinowski et al., 2011; Stepczyńska and Żenkiewicz, 2014). PLA's mechanical properties can be improved by reinforcing with fillers which are potentially susceptible to degradation (Gu et al., 1993a, 1996). The most widespread organic fillers are starch, sawdust, and natural fibers, mainly hemp, flax, wood and kenaf (Mantia and Moreale, 2011; Afiq and Azura, 2013; Bayerl et al., 2014; Rytlewski et al., 2014; Väisänena et al., 2016).

The extent and rate of the degradation of the studied samples (and other aliphatic polyesters) depend on the kind of extracellular enzymes produced by microorganisms. The enzymes, being macromolecular substances, cannot directly penetrate inside a polymer material but effectively cause decomposition of its external surface layer. Fragments of the material are absorbed by the microorganisms and undergo further degradation due to metabolic processes. It is accepted that aliphatic polyesters undergo the enzymatic degradation mainly by hydrolysis in which proteases, esterases, lipases, and cutinases are involved. Due to the mechanism of their action, these enzymes are considered as serine hydrolases, containing an active site in the form of a catalytic triad (Ser-His-Asp). Proteases (mainly serine ones) and esterases are the enzymes that are involved in the degradation of PLA (Tokiwa et al., 1993; Nowak et al., 2008; Qi et al., 2017).

The course and rate of degradation of the polymeric materials are influenced by physicochemical properties of the latter, including molecular weight, chain structure, crystallinity, and activity and durability of the applied enzyme in the case of the enzymatic degradation. Conditions under which the degradation occurs (e.g., pH and temperature) should also be considered as they affect properties of both the polymer and the enzyme itself. As a result of the degradation of a

* Corresponding author.
E-mail address: m.stepczynska@ukw.edu.pl (M. Stepczyńska).

http://dx.doi.org/10.1016/j.ibiod.2017.11.001
Received 29 January 2017; Received in revised form 17 October 2017; Accepted 3 November 2017

polymeric material, low molecular weight additives used in the processing procedure (e.g., stabilizers or activators) may penetrate into the reaction medium. These compounds may influence adsorption and activity of the enzyme and, thereby, kinetics of the enzymatic reactions (Azevedo and Reis, 2004; Lee et al., 2014; Barton-Pudlik et al., 2017).

Chemical or physical modifications of the biodegradable material can result in improving certain physicochemical and mechanical properties of these polymers, but at the same time they affect the rate of enzymatic degradation. The type and degree of modification determines whether the enzyme used for biodegradation is capable of degrading the modified material. Increasing the rate of degradation may be achieved by physical modification, for example mixing the components susceptible to biodegradation (including the fillers of plant origin) or by changing the physicochemical properties of biodegradable materials (Gu et al., 1993a,b; Petinakis et al., 2010; Hidayat and Tachibana, 2012; Catto et al., 2016). Sometimes degraded material can adversely impact the surrounding ecosystem. Biological factors, for example aquatic environment, may accelerate the deterioration of composite material (Mendez-Sanchez et al., 2003, 2004). Limiting the degree of decomposition is achieved by modifying the surface to make it more hydrophobic, or by selecting the chemical composition of the polymer in such a way as to increase the degree of crystallinity or molecular weight (Nowak et al., 2008; Żenkiewicz et al., 2012).

Knowledge of biochemical processes of PLA degradation is crucial for understanding biogeochemical cycles and developing environmental biotechnology. Biochemical processes of PLA degradation mainly include chemical hydrolysis and biodegradation in the natural soil. The ester bonds of PLA dissociate into carboxylic acid and alcohol by chemical hydrolysis due to hydrion, but the progress of complete hydrolysis requires much of energy and time. Therefore, analysis of biochemical processes of PLA biodegradation is a key issue for proposing new high efficient methods of PLA biodegradation (Qi et al., 2017).

At least two categories of enzymes are actively involved in biological degradation of polymers: extracellular and intracellular depolymerases. During degradation, enzymes from microorganisms first excrete extracellular depolymerase of PLA. The rapid production of extracellular depolymerase need usually to be stimulated by some inducers like amino acids, elastin or gelatin (Jarerat et al., 2004; Thanasak et al., 2015). Most of the inducers have L-alanine units, which is similar to L-lactic acid units of PLA in the stereochemical position of chiral carbon. Next, the depolymerase attack intramolecular ester links of PLA, which result in production of oligomers, dimers and monomers. Afterwards the low molecular weight compounds enter in microbial membranes and decompose into carbon dioxide, water or methane by intercellular enzymes (Gu, 2003; Tokiwa and Jarerat, 2004; Mueller, 2006; Musioł et al., 2016).

The aim of the paper was to present the influence of flax fibers on the biodegradation rate of PLA. Specifically, enzymatic degradation of flax-fibers reinforced PLA was addressed with particular attention paid to the processing conditions, the types of enzymes used for degradation, and time of treatments. Determination of the effect of flax fibers and enzymes on the biodegradation rate of PLA are the issues being in demand because they may be considered as a basis for development of novel methods for management of post-consumer waste. They contribute to quest for enzymes exhibiting high affinity to biocomposites and causing noticeable degradation of them in a relatively short time. Thus far, comparative studies of the influence the types of enzyme on the biodegradation rate of flax-fibers reinforced PLA have not been the subject of scientific publications.

2. Materials and methods

2.1. Materials

PLA type 2002D (D-repeat units: 3.5%, L-repeat units: 96.5%) of the melt flow rate of 4.2 g/10 min. (2.16 kg, 190 °C) and density of 1.24 g/cm^3 was obtained from Cargill Down LLC (USA),. The reinforcing flax fibres (Belgium, fibre length 5 mm) in the amount of 30% (w/w) were melt-compounded with the PLA matrix.

For enzymatic degradation, the following enzymes and reagents were used, proteinase K (≥30 units/mg) from *Tritirachium album* (Blirt, Poland), protease (7–15 units/mg) from *Bacillus licheniformis* (Sigma-Aldrich, Denmark), esterase (≥10 units/mg) from *Bacillus subtilis* (Sigma-Aldrich, Germany), and lipase (15–25 units/mg) from Candida rugosa EC 3.1.1.3 (Sigma-Aldrich, Switzerland); buffer 0.1 M Tris HCl (Blirt, Poland) and sodium azide (NaN_3) (Sigma-Aldrich, Germany).

The samples are denoted as follows: (i) P, (ii) 30%P, (iii) PLA, (iv) 30%PLA, where P indicates non-degraded polylactide, 30%P – non-degraded polylactide containing 30% (w/w) of flax fiber, PLA – degraded polylactide, and 30%PLA - degraded polylactide containing 30% (w/w) flax fiber.

2.2. Samples preparation

The PLA granulate with flax fibers was produced using a double-screw extruder type BTSK 20/40D (Buhler, Germany). The characteristic features of the extruder were as follows: the screw diameter of 20 mm, L/D ratio of 40. The screws were of a special shape, which limited cutting of the flax fibers into shorter fragments. Extrusion was carried out with no degassing, which required very well dried PLA and fibers. Thus, the PLA and the fibers were dried for twenty hours at 75 °C and 60 °C, respectively.

The temperatures of the cylinder heating zones and of the extruder head were 180, 182, 184, 186, and 185 °C, respectively. The rotational speed of the extruder screw was 150 min^{-1}.

The composites containing fibers were extruded by feeding the PLA using a volumetric feeder to the feeding zone of the extruder. The maximum feeding rate of the fibers was reduced by two parameters – (a) the torque, which reached 95 percent of the nominal value (32Nm) and (b) the pressure of the material in the head, which reached 10 MPa. The yield of the extrusion process was about 3 kg/h.

Subsequently, in order to produce samples in the form of thin films, granules were placed in a special compressing holder, attached to the dynamic mechanical analyzer (DMA). The samples were compressed at 160 °C under the pressure of 15 N, then cut into strips of films weighing from about 15 to 17 mg.

2.3. Enzymatic degradation testing

The samples were placed into the test tubes containing reaction mixtures of 2 mg of the respective enzyme, 10 ml of 0.1 M *Tris*-HCl buffer and 2 mg of sodium azide. Afterwards, the test tubes with investigated samples were placed in an incubator, where enzymatic degradation took place at the constant temperature of 37 °C.

The first measurements of mass loss were carried out after 24 h, then after five and seven days, and subsequently at weekly intervals for two to eight weeks of incubation. After a specified time, the samples were withdrawn from the reaction mixture, washed in distilled water, and dried in a moisture analyzer MAX 60/NH (Radwag, Poland) until constant weight was reached.

The mass loss was calculated using the formula:

$$\Delta m = \frac{(m_s - m_f)}{m_s} \cdot 100\% \quad (1)$$

in which m_s is the initial mass [mg] of a sample and m_f is the mass [mg] of the sample after the specified period of incubation.

2.4. The morphology testing

Images reflecting changes in the geometric structure of the surface