CHEMICAL ENGINEERING TRANSACTIONS, Volume 14, 2008
Edited by G. Marino, E. Bardone, A. Viglia
 ISBN 978-88-95608-02-0

Water contact angle and FTIR study of the surface modification of PET by lipolytic enzyme

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The goal of this study was to investigate the interaction of a lypolytic enzyme with poly-(ethylene terephthalate) (PET). Chemical, physical and structural changes induced on PET substrates with different degree of crystallinity were systematically investigated by water contact angle measurements and FTIR-ATR analyses. Treatment of PET with a commercial cutinase resulted in increased hydrophylicity and improved wettability of the PET materials. Water contact angle measurements indicate that cutinase displayed higher hydrolytic activity towards less crystalline PET. FTIR-ATR allowed to elucidate some chemical and structural features of the enzymatically-modified PET surface and provided some insight into the mechanism of the interaction of the lypolytic enzyme with PET.

1. Introduction

Polyethylene terephtalate, PET, is a polymer of major industrial importance because of its unique physico-mechanical and chemical properties. It is widely used in different commercial applications such as textile fibers and plastics. The material shows excellent strenght properties, a high hydrophobicity and resistance to chemicals. However, to improved dyeability with water-soluble dyes, an increased hydrophilicity of the surface is necessary. High temperature alkali treatment with sodium hydroxide is a conventional way of rendering PET fibres hydrophilic, but also the bulk properties of fibres are affected.

Recent studies have demonstrated that enzymes appear to be a safer and ecologically acceptable alternative to conventional chemical techniques for improvement of synthetic fibres properties in an environmentally friendly process (Alisch et al., 2004; Fischer-Colbrie G. et al., 2004; Gübitz and Cavaco Paulo, 2003; Heumann et al., 2006; Vertommen at al., 2005; Yoon et al. 2002). A highly specific and controlled enzymatic modification of synthetic fibres generates great benefits in terms of quality and performance. Enzymatic systems active on synthetic polymers are esterases, lipases and cutinases. In particular, the hydrolysis of PET by a fungal cutinase from *Fusarium solani* f. sp. *pisi*, wild-type (Alisch et al., 2006; Vertommen at al., 2005; Nimchua et al., 2006) or genetically modified near the active site (Araújo et al., 2007), and by a bacterial thermostable hydrolase from *Thermobifida fusca* has been demonstrated (Muller et al., 2005). Contrary to lipases, whose activity is greatly enhanced in the

presence of a lipid-water interface, cutinases do not display, or display little interfacial activation, being active on both soluble and emulsified substrates.
For this study, a commercial cutinase has been chosen. The activity of this enzyme increases the number of hydroxyl and carboxyl free groups on the surface, improving hydrophilicity and faciliting dyeing. The effects are clearly visible, but the parameters that regulate such activity are still poorly understood. In fact, recent studies have underlined that chemical and physical properties of PET, in particular the degree of crystallinity, affect the capability of the enzyme to hydrolize the ester bonds. The goal of this study was to investigate the interaction of the enzyme with PET, in relation to chemical and physical features of the sample.

2. Materials and Methods

2.1 Materials

2.1.1 Enzymes and chemicals

TEXAZYM EM, a commercial enzyme formulation, was obtained from inoTEX Ltd, Dvur Kralove, Czech Republic.

2.1.2 Polyester substrates

Two different poly(ethylene terephthalate) (PET) membrane substrates were used: crystalline/oriented PET, obtained from Fait Plast S.p.A. (Italy) and amorphous PET, obtained from University of Twente (The Netherlands), abbreviated as M-PET-A and M-PET-B, respectively.
The M-PET-A thickness is 70 µm, while that of M-PET-B is 200 µm. The degree of crystallinity of M-PET-A and M-PET-B is about 35% and 10%, respectively, as determined by single DSC run (Sichina, 2000).

2.2 Methods

2.2.1 Pre-Treatment of the commercial enzyme preparation

The commercial enzyme preparation TEXAZYM EM was diluted 1:1 with water and dialyzed in a cellulose tube (molecular cut-off: 12000 Da, purchased from Sigma-Aldrich, product no. D9652-100FT) against distilled water for 2 days at room temperature. After dialysis, the aqueous enzymatic solution was centrifuged at 10000 rpm for 25 minutes, freeze-dried and stored in a refrigerator prior to use. The aim was to remove the low molecular weight additives (wetting and softening agents) present in the commercial enzyme formulation in order to avoid any undesired interference during the reactions

2.2.2 Activity assay

Esterase activity (EC 3.1.1.1) was measured using 4-nitrophenyl butyrate as a substrate (Sigma-Aldrich assay). The increase of the absorbance at 405 nm was measured at room temperature using Schimadzu UV 1601 spectrophotometer. The increase of the absorbance at 405 nm indicates an increase of 4-nitrophenolate due to hydrolysis of the substrate. The activity was calculated in units, where 1 unit is the amount of enzyme required to hydrolyze 1 µmol of substrate per minute under the given assay conditions (pH 7.5 at 25°C).

2.2.3 Enzyme treatment of PET membranes

The samples were cut into pieces of 10 x 1.5 cm and washed with 1% SDS solution for 30 minutes, at 50/60°C and then thoroughly rinsed with distilled water. The reactions were done in phosphate buffer (50 mM, pH 8.0). Reaction conditions are shown in

Table 1. Control samples without enzyme were prepared as well. After incubation, the samples were washed with 1% SDS solution for 30 minutes at 50/60°C and then with distilled water until foam was removed. Samples were dried at room temperature overnight.

Table 1: Texazyme Em reaction conditions

Temperature	40°C ± 1°C
Time	120 min
Material/Liquor ratio mg/ml	1:85
Enzyme/Substrate ratio	20 U/mg
Mixing (Agitation)	40 rpm/min ± 2 in Linitest Heraeus

2.2.4 Contact angle measurements

Water contact angles were measured at room temperature using a FTA188 Contact Angle and Surface Tension Analyzer (First Ten Ångstroms). Each value reported is the average of left and right contact angle with respective standard deviation. Samples were fixed to the support with sticky tape and measurements were made each 5 mm. The aim was to reduce the superficial variability of measurements. Therefore, a profile of the superficial properties was obtained for each sample. Measurements were made on the same sample before and after enzyme treatment.

2.2.5 Infrared Spectroscopy (FTIR-ATR)

Measurements were performed on a NEXUS Thermo Nicolet FTIR spectrometer employing an Attenuated Total Reflection (ATR) accessory. All spectra were obtained with a Ge crystal cell (maximum depth 0.8 μm). Spectra were normalized to the 1410 cm^{-1} peak. Each spectrum reported is the average of three spectra.

3. Results and Discussion

To evaluate the superficial properties of the samples after enzyme treatment, water contact angle measurements were performed by sampling progressively the surface of each sample before and after treatment. The average water contact angle values of untreated membranes were 74.1°±1.3° and 75.1°±1.4° for the crystalline and amorphous sample, respectively. They decreased to 67.6°±4.1° (crystalline) and 58.3°±3.1° (amorphous) after cutinase treatment, as shown in Figure 1.

These results indicate that cutinase displayed higher hydrolytic activity towards amorphous PET, confirming the data reported in a previous work about a higher amount of soluble hydrolysis products after cutinase treatment of a less crystalline sample (Vertommen at al., 2005).

FTIR-ATR measurements of PET membranes were performed on both crystalline and amorphous samples before and after enzyme treatment. Here we report the data obtained on the amorphous membrane M-PET-B, where spectral changes were more evident. Typical FTIR-ATR spectra before and after cutinase treatment are shown in Figure 2. Several spectral changes attributable to enzymatic modification are observable in the 1800-800 cm^{-1} wavenumber range.

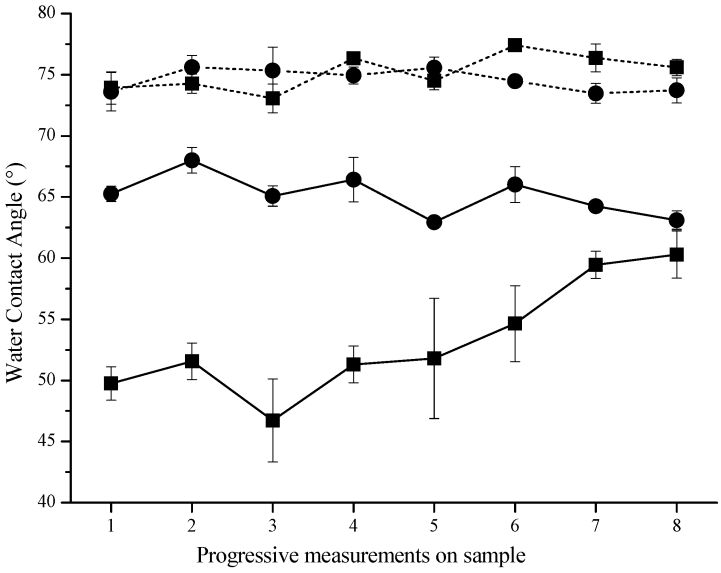

Figure 1: Average water contact angle profiles of M-PET-A (•) and M-PET-B (■) membranes. Dotted line: untreated samples; solid line: samples treated with the enzyme.

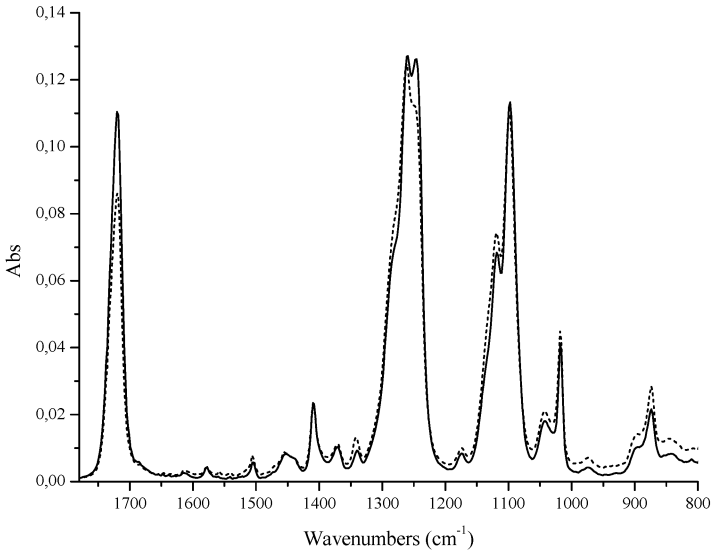

Figure 2: FTIR-ATR spectra of M-PET-B membrane untreated (solid line) and after enzyme treatment (dotted line).

One of the most important aspects of PET structure concerns the existence of *trans* and *gauche* rotational conformers for the ethylene glycol moiety. Both types of conformers are present in the amorphous phase, but only the *trans* conformer is present in the crystalline phase (Cole et al., 1994; Walls, 1991; Ward, 1977). Because of the sensitivity of molecular vibrations to bond strengths and configurations, IR spectroscopy is a sensitive method for determining conformers and potential changes induced to them from enzyme activity.

With reference to the remarkable spectral changes induced on the amorphous M-PET-B membrane by the enzymatic reaction, the absorbance of the carbonyl stretching band at 1725 cm^{-1} decreased significantly after enzyme treatment, and the strong bands near 1260 and 1100 cm^{-1}, attributed mainly to C-O stretching, also changed in shape and relative intensity of band components after reaction. These changes confirm that the ester bonds of PET were the main target of the enzyme activity.

Additionally, the peak at 1340 cm^{-1}, which arises from CH_2 wagging of *trans* conformers of the ethylene glycol unit, became much stronger than the peak at 1370 cm^{-1}, which corresponds to *gauche* conformers. It can be inferred from this result that upon enzyme treatment the *trans* conformers were enriched either by selective hydrolysis and removal of the *gauche* conformers or by *gauche* → *trans* conversion. Therefore, it is confirmed that cutinase displays significantly higher hydrolytic activity towards amorphous regions of PET. The mechanism of enzyme-PET interaction is still under investigation through quantitative analysis of the above discussed spectral changes.

4. Conclusions

In this study, the hydrolytic activity of a lypolytic enzyme, cutinase, toward PET materials was confirmed. In particular, water contact angle measurements and FTIR-ATR analysis indicated that cutinase displays higher activity toward the amorphous phase of PET.

FTIR-ATR spectra allowed to highlight some chemical and structural changes induced by the enzyme on the outermost layers of the PET substrates. These changes are worth of being further investigated in order to obtain more information on the mechanism driving the enzyme-PET interactions.

Detailed and fundamental knowledge in this field may not only contribute to the development of bio-treatment processes for polyester substrates, but also address the genetic engineering of lypolytic enzymes with enhanced hydrolysing activity with respect to the native ones.

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