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Surface modification of poly(ethylene terephthalate) (PET) fibers by a cutinase from *Fusarium oxysporum*

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ABSTRACT

Synthetic polyester fabrics occupy a great part of the textile industry production satisfying variable ordinary needs. Nonetheless, their high hydrophobicity constitutes an important weakness that impedes process manufacture, as well as permeability and evaporation of sweat when used in clothing industry. The enzymatic treatment of these materials is a modern and eco-friendly procedure that aims at the increase of the hydrophilicity through superficial modification. In this study, the enzymatic surface hydrolysis of poly(ethylene terephthalate) (PET) fabric is succeeded using a recombinant cutinase from *Fusarium oxysporum*. The effect of various parameters is studied for the enzymatic modification of PET, such as temperature, pH, enzyme loading and reaction time. The optimal parameters are found to be 40 °C, pH 8, and 1.92 mg enzyme loading per gram of fabric. The controlled enzymatic hydrolysis of PET textile is further confirmed and characterized using various spectroscopic and analytical methods, including Fourier Transform Infrared (FT-IR) in the Attenuated Total Reflectance mode (ATR) and X-ray photoelectron spectroscopy (XPS). Tensile test and dyeability analyses were also employed achieving a *K*/*S* increase up to 150%, confirming the successful surface modification without degrading the quality of the starting material.

1. Introduction

In modern times, technology and science have met advances that have encouraged fundamental changes and improvements in several industrial sectors, such as textile industry. Synthetic fabrics prove to be useful in many applications because of their advantages as far as their physical properties are concerned, presenting strength and elasticity [1]. In addition, synthetic textiles present less wrinkles, fast drying properties and can be produced massively in a short period of time with lower cost as opposed to natural textiles. Their main drawback is the low hydrophilicity that impedes the process of finishing and dyeing, as well as the permeability and evaporation of sweat [2–5] and stain resistance [6]. Towards this direction, enzymatic treatment consists an innovative biotechnological and eco-friendly approach to increase fabrics' hydrophilicity and improve their properties, as opposed to chemical treatment, which has higher manufacturing cost and energy consumption and leads to strength loss and degradation of fabric bulk properties [7–12]. Enzymes are macromolecular compounds that present selectivity and cause superficial modification of the fibers, as they cannot diffuse further into the fibers' bulk part because of their size [13–15]. Modification of poly(ethylene terephthalate) (PET) fibers aims at the surface hydrolysis of the ester bonds releasing carboxyl- and hydroxyl- end groups [12,16] and as a result terephthalic ions in the reaction supernatant. According to literature, enzymes suitable for polyester modification are esterases, lipases and cutinases [16–18].

Cutinases are serine esterases that belong to the α/β-hydrolase fold family. Their role in nature is the hydrolysis of ester bonds in cutin, a wax biopolyester found in plant cuticle of aerial surfaces of plants [19]. Furthermore, cutinases have the ability to hydrolyze

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the ester bonds of synthetic polyesters and this ability makes them suitable for many industrial applications such as the surface modification of PET [17,20].

In literature, commonly used hydrolases for PET surface modification are cutinases from *Fusarium solani* sp. *pisi* [7–9,21]. However, it has previously been reported that a cutinase from *Fusarium oxysporum* proved to be more efficient as far as PET fabric modification is concerned, presenting higher activity in comparison to a cutinase from *F. solani* sp. *pisi*. [22]. In addition, cutinase from *F. oxysporum* is considered as highly organic solvent-tolerant, indicating its potential in biotechnological applications including the essential area of Biocatalysis [20].

In the present work, the ability of a *F. oxysporum* cutinase (*Fo*Cut5a) for the surface hydrolysis of PET fibers was investigated. The potential of this enzyme in PET modification was proved by its capability to hydrolyze two PET model substrates, bis(benzoyloxyethyl) terephthalate (3PET) and commercial bis(2-hydroxyethyl) terephthalate (BHET) [23]. Various parameters were studied aiming at the efficient surface modification of PET textile, such as the effect of temperature, pH, incubation time and enzyme loading. The extent of the enzymatic treatment was monitored by the quantification of terephthalic acid (TPA) and its derivatives (TPA equivalents) released in reaction supernatant. In addition, the modification of the polymeric material was further justified through the application of different analytical techniques, including FTIR-ATR, SEM, XPS, DSC, TGA, as well as through dyeability tests.

2. Material and methods

2.1. Materials, chemicals and enzymes

Commercial PET woven fabric with tricot knit was kindly supplied by Colora S.A (Thessaloniki, Greece) with density 126 columns in^{-1} and 58 rows in^{-1}, weight 52.90 g m^{-2} and thickness 42 μm. The polyester fabric was washed with detergent Felosan NFG from CHT Bezema (Tübingen–Germany) for the removal of paraffins used during weaving. Recombinant cutinase from *F. oxysporum* was heterologously expressed in *Escherichia coli* BL21 (DE3) and purified by immobilized metal affinity chromatography, as described previously [23]. TPA and BHET were supplied by Merck (Whitehouse Station, New Jersey) and Sigma (St. Louis, MO), respectively. Trifluoroacetic acid and acetonitrile were of HPLC grade (Sigma, St. Louis, MO). The reactive dyes used were Novacron Deep Cherry S–D, Novacron Yellow S-3R (Everberg, Belgium) and Jakazol Black 133% (Huntsman, Gujarat, India). All other chemicals were of analytical grade.

2.2. Enzymatic treatment of polyester fabric

Polyester fabrics were cut into pieces of 0.5 g and were incubated in glass vessels with bath ratio of textile mass over buffer mass 1:50 under stirring (170 rpm) for 24 h. For the enzymatic treatment, different parameters were studied including the effect of temperature (25–50 °C), pH (phosphate buffer 100 mM pH range 5.8–8.0, glycine-NaOH buffer 100 mM pH range 9.0–10.0), incubation time (0–24 h) and enzyme loading (0.096–3.840 mg g^{-1} of fabric). After enzymatic treatment, the fabrics were washed with 2 g L^{-1} Na_2CO_3 at 60 °C for 1 h and afterwards double-washed with deionized water for 1 h, as previously described [13]. All experiments were performed in duplicate.

2.3. Monitoring of enzymatic modification

For the determination of the polyester hydrolysis degree, TPA or its derivatives released in samples' supernatants were quantified spectrophotometrically at 241 nm in a BOECO S-20 Spectrophotometer (Hamburg, Germany). Possible hydrolysis products were TPA, BHET and mono(2-hydroxyethyl) terephthalate (MHET). A calibration curve of TPA was used for the calculation of the total amount of products released since all three products have the same molar absorption due to their carbonyl groups [8]. Samples of 0.6 mL of the supernatant were taken and the enzyme was precipitated with the addition of 1:1 methanol prior to centrifugation. Subsequently, in 1 mL of the supernatant sulfuric acid was added (18 mM) in order to convert TPA and any of its derivative ions into their neutral counterparts. Control samples without the addition of the enzyme or without the addition of fabric were measured as well.

The reaction supernatants from the hydrolysis at optimal conditions, were concentrated and analyzed by HPLC, using a SHIMADZU LC-20AD pump equipped with a Jasco UV-975 detector recording at 241 nm. The reversed phase column Eurospher-100 C18 from KNAUER (Berlin) was maintained at room temperature. A linear gradient method, involving 1% trifluoroacetic acid solution and acetonitrile as eluents, at a flow rate of 0.8 mL/min, was applied, as previously described [8].

2.4. Dyeing and color measurements

Polyester fabrics were dyed in 390 cm^3 glass tubes in a laboratory scale dyeing Ahiba Texomat machine (Datacolor, Lawrenceville, NJ, USA). According to dyeing procedure, 55 g L^{-1} of Na_2SO_4 and 2% w/w of each dye were dissolved and introduced in the glass tubes where fabrics were also introduced. The dye bath was heated up to 60 °C and after 20 min, 5 g L^{-1} Na_2CO_3 was added. Finally, 0.4 g L^{-1} of NaOH were incorporated 30 min before the end of the dyeing procedure. For the fabric neutralization to pH 6 a small amount of HCOOH was added after removing the fabrics from each dye bath, and then, a wash procedure followed at 95 °C with soap (Cibapon R) for 1–2 h, in order to remove the non-reacted dye.

Color changes (ΔE) were evaluated with a color tristimulus colorimeter (Data Color International, Spectraflash SF450) (Lawrenceville, NJ, USA). The spectrophotometer was calibrated according to the manufacturer's instructions, using the supplied black and white calibration standards. The color alterations were calculated using the CIE L*a*b* System, established by the "Commission Internationale de l'Eclairage-CIE" according to ASTM D 2244-68. For each sample, four repeated measurements were taken to determine the color coordinates L, a, b. "L" indicates the brightness, "a" describes the red-green content and "b" the yellow-blue content. Color change can be calculated using Eq. (1):

$$\Delta E = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2} \quad (1)$$

Studies have concluded that the $\Delta E = 1.0$ is a color difference that can be perceived by 50% [24] by the human eye, while $\Delta E = 2.0$ is a color difference that can be seen at 100% [25].

In order to determine the dye absorption, the color strength (K/S) of the dyed samples was measured at the wavelength of minimum reflection by using Eq. (2):

$$\frac{K}{S} = \frac{(1-R)^2}{2R} \quad (2)$$

where, R refers to the reflection value of the sample.

Practically, the ΔE value is used when it is desired to compare two shades. The color strength, i.e. the ratio K/S, is the parameter that is used to evaluate the dyeing process and dyeing capacity.