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Short contribution

Monitoring Biotransformations in Polyesters

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This paper describes two methods to monitor esterase hydrolysis at the surface of polyester fibres (PET-Polyethylene terephthalate). The hydroxyl groups were determined on the fibre surface by alkaline reaction with a reactive dye (CI Reactive Black 5) and colour intensity was determined using a reflectance spectrophotometer. The terephthalic acid solution formed was also quantified after reaction with peroxide by fluorimetric determination of the resulting hydroxyterephthalic acid. Detailed descriptions of those methods are given in this paper.

Keywords: Esterase; Cutinase; Polyester; Hydrolysis; Terephthalic acid

INTRODUCTION

Polyethylene terephthalate (PET) can be hydrolysed under strong alkaline conditions yielding a massive weight-loss and a highly hydrophilic fibre. The alkaline hydrolysis is an "all-or-nothing" mechanism providing more than superficial changes. Since enzymes are large molecules, diffusion inside the fibres will not happen, and only superficial formation of hydroxyl and carboxylic groups is achieved. Since this reaction leads to a more hydrophilic fibre, the applications of esterases to PET and other polyesters has received much attention recently (Huskic and Zigon, 2003), (Marten *et al*., 2003), (Walter *et al*., 1995). Most of the claims of the enzyme action are due to new properties induced in PET fibres, like better hydrophility, but in some cases no direct evidence has been shown (Hsieh *et al*., 1998).

Some reports indicate that the formation of terephthalic acid could be monitored at 240 nm (Yoon *et al*., 2002). This method has been investigated in the current study, but showed large errors due to the protein present in the enzyme liquors. Terephthalate and its esters have characteristic absorbance peaks between 240–244 nm (UV) but in this case the protein also adsorbs strongly in this region.

Enzyme action will leave cleaved ester bonds, i.e., hydroxyl and carboxylic acids at the fibre surface and also terephthalic acid and ethylene glycol in solution. The terephthalic acid yields hydroxy-terephthalic after reaction with peroxide under boiling conditions. As the hydroxy-terephthalic acid has a sharp emission peak at 425 nm using an excitation at 315 nm it can be determined by fluorescence. This method has been adapted from quantification of radical formation by ultrasound using a similar procedure. In alkaline aqueous solutions, terephthalic acid produces terephthalate anions, which react with hydroxy radicals ($HO^{\bullet}$) from hydrogen peroxide to produce highly fluorescent hydroxy-terephtalate ions (HTA) (Mason *et al*., 1994).

The hydroxyl groups in the fibres can be determined by specific reaction with a vinylsulphonic dye at alkaline pH and further determination of colour levels with a reflectance spectrophotometer. This method has been adapted from reactive dyeing of cotton using vinylsulphonic dyes, where the fixation is done on the hydroxyl groups of the glycosidic units of cotton cellulose (Scheme 1).

If hydrolysis occurs in polyester, the number of –OH end groups will increase and therefore more –OH groups will react with a reactive dye. In alkaline media the reactive dye reacts with the OH end groups on polyester fibres. In this paper we describe two new methods to follow the formation of terephthalic acid in solution and hydroxyl end groups on the fabric surface resulting from the enzymatic hydrolysis of PET.

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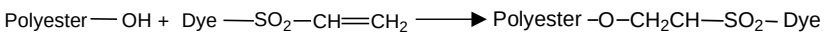

SCHEME 1 Reaction between polyester − OH end groups and the reactive dye.

EXPERIMENTAL

Materials

Polyester fabric taffeta with 62 g/m^2 and 22 yarns/cm (warp) and 30 yarns/cm (weft) was obtained from Rhodia. Cutinase (GCI 2002/1410) was supplied by Genencor International. Terephthalic acid and Hydrogen Peroxide 35% were purchased from Sigma Chemical Company. The dye used was C.I. Reactive Black 5, from the chemical class di-azo, with a reactive system vinylsulphonyl purchased from Ciba SC. All the other chemicals used were laboratory grade reagents.

Enzyme Activity

Activity of the cutinase preparation was assayed towards p-nitrophenylpalmitate (pNPP) as described by Quyen, *et al*. (1999).

Enzymatic Treatment

Polyester fabric was pre-treated by washing with 1 gL^{-1} of Calgon T at 70°C for 60 min and dried in an oven at 40°C for 24 h. The enzymatic treatment was performed in sealed, stainless steel dye pots of 250 cm^3 in a laboratory scale dyeing machine (Rotawash), using a cutinase concentration of 89 U, in 0.1 M phosphate buffer, pH 8.0 at 30°C (Carvalho *et al*., 1999). The samples were incubated for 168 hours with agitation. For each incubating time (9, 24, 120 and 168 h) a sample of the enzymatic solution was taken for chemical analysis.

After enzymatic treatment, all samples were washed first with tap water, then with a 2 g/l sodium carbonate solution for 30 min at 70°C (to remove the remaining protein) and finally with distilled water at 70°C for 1h (2 times).

Terephthalic Acid Determination by Fluorescence Monitoring

After reaction of terephthalic acid solution with 35% hydrogen peroxide at 90°C a sample was scanned between 300 and 600 nm using a luminescence spectrometer with an excitation wavelength of 315 nm. The presence of HTA was detected by the emission at 425 nm. For the assay, 1 ml of solution was added to 2 ml of hydrogen peroxide and heated at 90°C for 30 min. After cooling to room temperature, samples were measured by fluorescence and the intensity of the peak at 425 nm, due to the presence of HTA ions, was used for the determination of TPA concentration.

A calibration curve was determined using standard solutions with different concentrations of TPA (0.006, 0.03, 0.06 and 0.12 mM) dissolved in a 0,05 M NaOH solution. The correlation obtained was Intensity (425 nm) $= 3.13349 + 585.17224 \times$ Conc. (mM) with $R^2 = 0.9993$ and was linear between 0.006 and 0.12 mM TPA.

The influence of NaOH on the fluorimetry method was also studied, and no change in intensity was detected when NaOH was added to the TPA solutions.

Protein in the enzymatic solution was measured at 595 nm following the Bradford method, using bovine serum albumin (BSA) as standard (Bradford, 1976). All measurements were performed using duplicate samples.

Influence of Protein

Terephthalic acid solutions of 0.12 mM and 0.60 mM were prepared in 0.05 M NaOH by heating at 100°C until completely dissolved. The lower concentration had to be used for the fluorimetric method, due to the fact that 0.60 mM gave an intensity at 425 nm which was out of range. The influence of protein on the fluorimetric and UV methods was measured preparing solutions with and without 0.1 gL^{-1} BSA and comparing the resulting values. All measurements were performed using duplicate samples.

Dyeing

Polyester fabrics were dyed in sealed, stainless steel dye pots of 120 cm^3 capacity in a laboratory scale dyeing machine (Ahiba). The dyeing was performed with owf 2%, bath ratio of 1:100, at pH = 11 using 20 mg/ml sodium carbonate and 60 mg/ml sodium sulphate at 60°C for 90 min with agitation of 30 rpm. After the dyeing process, a reductive wash was done with 2 gL^{-1} of sodium hydrosulphite for 30 min and samples were then dried in an oven at 40°C for 24 h. Samples were all dyed in the same bath at the same time to detect the colour difference among them. The dyeing was performed at 60°C, below the glass transition temperature (T_g) of polyester fibre, which is approximately 69°C.

Colour measurement

Colorimetric data for CI Reactive Black 5 was determined using a Spectraflash 600 plus

spectrophotometer interfaced to a PC, using an illuminant D_{65} with a wavelength range between 360 and 700 nm. Each fabric sample was folded once in order to get two thicknesses and an average of five readings was taken each time. The K/S values were taken at 590 nm.

RESULTS

Terephthalic Acid Determination by Fluorescence and Protein Adsorption

Figure 1 shows a typical curve of TPA formation with cutinase hydrolysis and protein adsorption. The maximum TPA formation and protein adsorption was found after approximately 24 h incubation. The protein adsorption level in this assay was around 40–45%. The concentration of hydroxyterephthalate ions (HTA) increased, indicating that the concentration of terephthalic acid increased with the time of incubation for the samples treated with 89 U of cutinase.

The results obtained in the presence of additional protein in the TPA solutions show that this is a better method for detecting the formation of TPA than measuring at 240 nm (UV) because the protein has a much lower influence. In the fluorescence method the additional protein interfered by less than 4–5% compared to approximately 70% for the UV-240 nm method. This means that 70% of the absorbance at 240 nm (UV) was due to the presence of the 0.1 gL-1 of BSA in the TPA solution.

Colour Differences (K/S)

K/S values for the samples treated with enzyme were higher than the control for all samples and the difference between the sample and the control increased up to the maximum incubation time of 168h (Fig. 2). The increase in the K/S values is related to the presence of more $-$OH end groups after enzymatic hydrolysis of the polymer. This increase was between 0.15 and 0.60, which indicates a significant increase of dye reacting with the fibre, reflecting the increase in $-$OH end groups at the surface of the fibre. The dyeing results were better at temperatures below the glass transition temperature of the fibre (T_g), probably because the hydroxyl end groups formed at the fibre surface can be hidden when the dyeing procedure is performed above T_g. We have some evidence for this (not shown here) from samples dyed at 80°C with reactive dye after enzymatic treatment with cutinase.

DISCUSSION

The formation of terephthalic acid has been detected in solutions after cutinase treatment of polyester, using the fluorescence method at 425 nm, confirming that enzymatic hydrolysis of polyester occurs with cutinase. Terephthalic acid formed by enzymatic hydrolysis increased with incubation time and the intensity of the sharp peak at 425 nm increased over 24 hours of incubation. With the method involving measurement at 240 nm (UV), the influence of protein on the assay was very high (approximately 70% of the absorbance value was due to the protein), which is likely to result in significant errors. The new method is more effective and shows a much lower error. The amount of protein present in the enzymatic treatment solution decreased during the incubation, mainly due to its adsorption on the polyester fibres.

After dyeing with a reactive dye for cellulosic fibres, the K/S values were shown to increase with time of incubation with cutinase, due to the increase in the number of $-$OH end groups after enzymatic hydrolysis. This confirms that significant enzymatic hydrolysis occurred even over short incubation

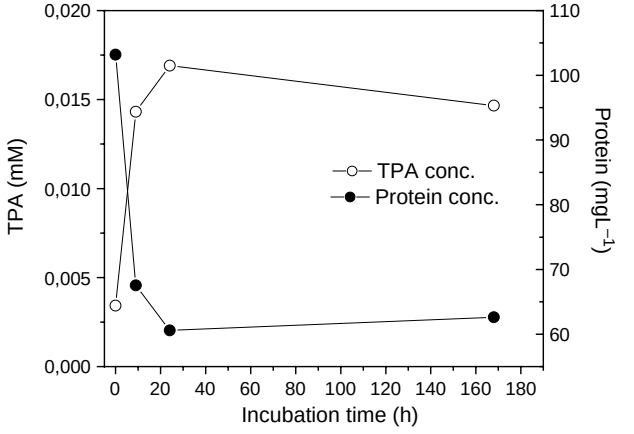

FIGURE 1 Terephthalic acid (mM) and protein (mg L^{-1}) concentrations vs. incubation time (h) for enzymatic treatment solutions of polyester incubated with 89 U of cutinase. The values are average differences between the enzymatic treatment and a control without enzyme.

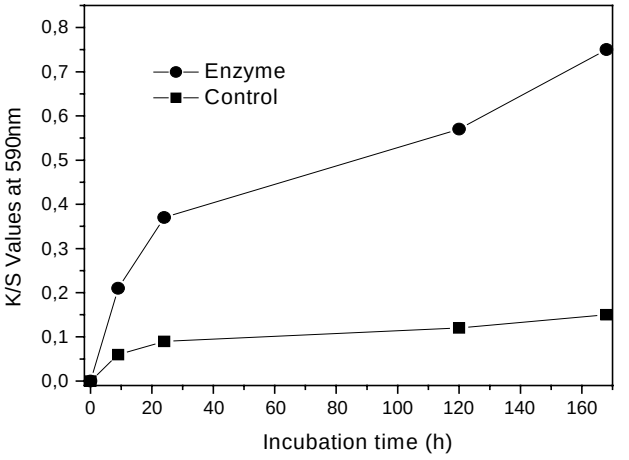

FIGURE 2 Colour levels (measured at 590 nm) of samples dyed with Reactive Black 5 vs. enzyme incubation time.

times (24 h), with the K/S value increasing from 0.09 to 0.37. However, the highest increase (from 0.15 to 0.75) was obtained after a long period of incubation which indicates that the hydrolysis continues to occur over a long time.

The methods described in this paper are novel and directly assay enzymatic esterase action on polyester fibres. The fluorimetric determination for soluble terephthalic acid yields a reliable method to assay enzyme action on the fibre. The dyeing of polyester, using cotton reactive dyes specific for hydroxyl groups, gives a direct measure of the ends groups remaining at the fibre surface after enzyme action.

Acknowledgements

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