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Short contribution

Monitoring Biotransformations in Polyamide Fibres

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The enzymatic hydrolysis of polyamide fibres yields amino and carboxylic groups. These groups can be found in solution treatments as polyamide monomers and soluble oligomers. The amino groups can also be found at the surface of the fibres as end group chains. In this paper we report two methods to quantify the formation of these groups as a result of the enzymatic action. Soluble amino groups can be quantified with 2,4,6-trinitrobenzenesulfonic acid (TNBS), which yields a coloured complex which can be determined spectrophotometrically. The amino groups on the fibre surface can be quantified by reaction with a wool reactive dye and determination of colour intensities after a dyeing procedure below the glass transition temperature of polyamide.

Keywords: Esterase; Cutinase; Polyamide 6.6; Hydrolysis; Amines; Synthetic fibres

INTRODUCTION

Polyamide fibres are obtained by the condensation of adipic acid and hexamethylenediamine. These fibres are hydrophobic and have poor wettability in an aqueous medium. Alkaline hydrolysis is an effective way to improve fibre wettability, but the action of concentrated solutions of NaOH or KOH is hard to control and extensive damage can result. (Silva, 2002) The use of hydrolytic enzymes can lead only to superficial hydrolysis of polyamide fibres. This is due to the fact that enzymes are relatively large molecules and do not penetrate into the tight hydrogen bonded structure of polyamide. These superficial changes would improve the fibre hydrophilicity and chemical reactivity towards other agents for new finishing effects of polyamide, without causing significant damage.

Previously we have shown that cutinase can be used to modify the surface of polyamide fibres by hydrolysis of the amide linkages with the formation of amino and carboxylic groups. (Silva *et al.*, 2005) Enzyme action produces some superficial cuts along the polymer, corresponding to breakage of the amide linkages. From the breakage of these linkages two hydrolysis products might result, adipic acid and hexamethylenediamine. Some of the groups formed stay on the surface of the fibres but some compounds can be found in the liquid phase after treatment.

The aim of this work was to describe new methodologies to measure the extent of enzymatic hydrolysis and therefore to quantify functional groups formed after enzymatic treatment. The measurement of amino groups in the treatment liquor is a direct method to measure enzymatic hydrolysis. For this purpose the TNBS (2,4,6-trinitrobenzenesulfonic acid) method was used, which is based on reaction with primary amino groups (Morçöl *et al.*, 1997).

Some groups formed during enzymatic processes stay on the surface of the fibre and their presence can be detected by dyeing with a wool reactive dye, specific for the primary amino groups. Standard reactive dyeing fixes the chromophoric structures to hydroxyl groups of the fibres. In the case of polyamide, the free amino groups at the surface of the fibres can be detected by the specific reaction with the α-bromoacrylamido dye reactive group. (Lewis, 1992)

MATERIALS AND METHODS

Materials

Commercial polyamide (100% PA 6.6) woven fabric taffetá, obtained from Rhodia, with 30/28 yarns cm^{-1}

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and 63 $g\,m^{-2}$, was used in all experiments. Cutinase (GCI 2002/1410) was generously supplied by Genencor International. The reactive dye used, Lanasol Red 5B–CI Reactive Red 66 (C.I.: 17555), was generously supplied by CIBA SC and 2, 4, 6-trinitrobenzenesulfonic acid was obtained from SIGMA. All other reagents used were laboratory grade reagents.

Enzymatic Hydrolysis

To remove some impurities from the fabrics, before enzymatic treatment all polyamide fabric samples were washed with 2 gL^{-1} of a non ionic agent, Lutensol AT 25 (10 gL^{-1}) and with 2 gL^{-1} of Na_2CO_3, both at 50°C.

Two sets of experiments were performed. In the first set, 0.2 g of polyamide fabric, cut in small pieces, was incubated in a glass vessel with different concentrations of cutinase (1.58×10^3 UL^{-1} to 20.10×10^3 UL^{-1}) at 30°C, using 50mM borate buffer pH 8.5, for 24 hours with a bath ratio of 1:50. Aliquots were taken after 6 and 24 hours for each enzyme concentration assayed. All experiments were performed using duplicate samples. (Fig. 1)

For the other set of experiments, 1 g of polyamide fabric (10×14 cm) was incubated with 12.98×10^3 UL^{-1} of cutinase at 30°C, using 50 mM borate buffer pH 8.5, for 70 hours. A bath ratio of 1:200 was used. The enzymatic treatment was performed in sealed, stainless steel dye pots of 250 cm^3 in a laboratory scale machine (vertical agitation simulating European washing machines-Rotawash). Samples of fabric were taken after 4 and 70 hours for posterior dyeing. All experiments were performed using duplicate samples. (Fig. 2)

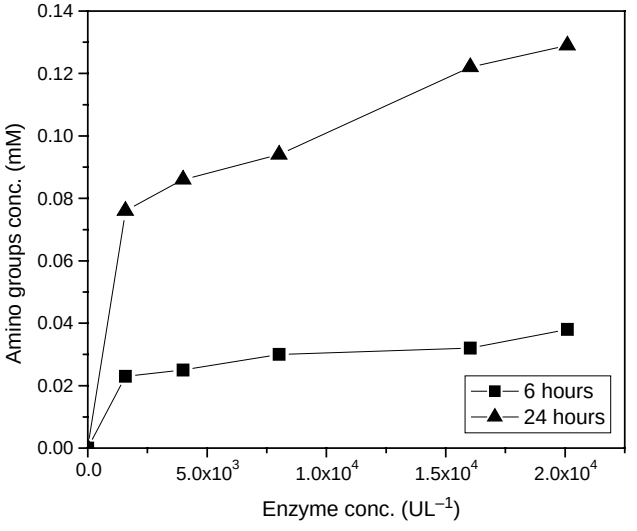

FIGURE 1 Production of amino groups in the treatment bath of polyamide samples.

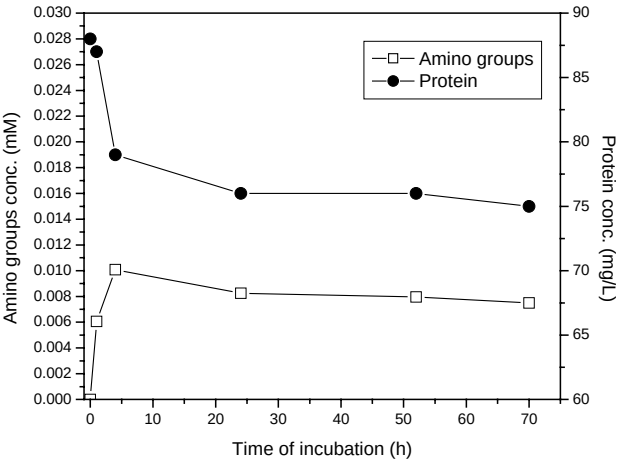

FIGURE 2 Variation of protein and amino group concentration vs. time of incubation in the bath solution of polyamide samples treated with 12.98×10^3 UL^{-1} of enzyme in a Rotawash machine.

Determination of Amino Groups in the Bath Solution

To follow the formation of amino groups and quantify the groups present in the treatment liquid, the TNBS method was adapted from a methodology described by others. (Morçöl *et al.,* 1997) This method is based on the reaction of the primary amino groups with the sodium salt of trinitrobenzenesulfonic acid (TNBS). The product of this reaction is a complex between the amino groups and TNBS, which can be measured read spectrophotometrically at 420 nm. The first step of the procedure was the precipitation of protein present in the treatment solutions, which was done with trichloroacetic acid (TCA) (110 mM) giving total elimination of the protein content. (Lowry *et al.,* 1951)

Afterwards the assays were done by mixing 4 mL of the supernatant with 100 μL of 30 mM aqueous TNBS and incubating for 30 minutes at room temperature. The blank for the assay consisted of 4 ml of borate buffer. The absorbance was read against the blank at 420 nm in a Helios Alfa spectrophotometer. All measurements were performed using at least triplicate samples.

Determination of the Protein Concentration at Different Times of Incubation

To study the relationship between the adsorbed protein and the time of hydrolysis, the quantity of protein present in the treatment liquid was measured.

The determination of protein concentration was done following the Bradford method (Bradford *et al.,* 1976).

All measurements were performed using at least triplicate samples.

Determination of the Amino End Groups on the Fibre Surface by Reactive Dyeing

The amino end groups on the surface of polyamide fabrics were analyzed by dyeing the treated samples with a wool reactive dye (Lanasol Red 5B). Standard reactive dyeing fixes the chromophoric structures to hydroxyl groups of the fibres. In the case of polyamide, the free amino groups at the surface of the fibres are detected by the specific reaction with the α-bromoacrylamido dye reactive group. (Lewis, 1992)

The fabric samples of polyamide were dyed with 2% of reactive dye at 50, 60 and 70°C, in the presence of NaCl (50 gL^{-1}), an auxiliary product, in order to promote dyeing at neutral pH (7). The dyeing was performed in sealed, stainless steel dye pots of 120 cm^3 capacity in a laboratory scale dyeing machine (Ahiba) at 30 rpm with a gradient of 2° min^{-1}. A bath ratio of 1:50 was used and the dyeing time was 90 minutes. After dyeing, the samples were washed with a Lutensol solution (2%) at 50°C and then with distilled water at 50°C, followed by washing with tap water. The color measurements were carried out using a spectrophotometer (illuminant D_{65} at 570 nm) coupled to a PC. All measurements were performed using at least triplicate samples

RESULTS AND DISCUSSION

The cutinase treatment of polyamide yields primary amino groups resulting from the cleavage of the amide bonds. These groups can be found in the liquid bath or as amino end groups on the fibre surface. The amount of soluble amino groups produced after 6 and 24 hours of enzymatic treatment is shown in Fig. 1. These results confirm that very low levels of soluble amines are produced after enzymatic hydrolysis of the polyamide and also confirms our previous findings that 1 U of cutinase measured towards pNPP (p-nitrophenyl palmitate) yield only 2.30×10^{-3} U of activity over the polyamide fibre. (Silva *et al.,* 2005) This is mainly due to the heterogeneity of the reaction, where the enzyme can only access the amide groups on the fibre surface. The results (Fig. 1) show that saturation levels for very high dosages of enzyme in the range of 2×10^4 UL^{-1} can be observed, confirming the superficial treatment mode. Two sets of experiments were performed. In the first set (Fig. 1) the treatment of polyamide fibres was done in a shaker bath and therefore very low quantities of soluble amino groups would be expected. The results obtained confirm previous work done by others with cotton fibres. The enzymatic treatment of cotton fibres is also restrained to the fibre surface and a synergistic action was found with high levels of mechanical agitation and enzyme activities (Cavaco-Paulo, 1998). In the second set of experiments (Fig. 2), high levels of mechanical agitation were provided in a Rotawash machine (vertical agitation simulating European washing machines) and a saturation enzyme dosage of 12.98×10^3 UL^{-1} was used. The results obtained are shown in Fig. 2. Despite the high levels of mechanical agitation, a plateau in amine production was found after 4 hours of treatment with a maximum of approximately 0.01 mM. Protein adsorption levels were also monitored, revealing a maximum adsorption level of 15% after 4 hours of treatment (Fig. 2). It is evident that, after 4 hours of incubation, lower amounts of protein are adsorbed and virtually no more amino groups are formed. The results obtained seem to show that there is a correlation between maximum adsorption levels and amino group formation.

The percentage of K/S increases in samples treated with cutinase in comparison with the controls (Fig. 3). The reactive dye reacts with the primary amino groups present on the fibre surface and the colour intensities are proportional to the amount of those free groups formed. Fig. 3 seems to show the formation of amino end groups only after 4 hours of treatment, while after 70 hours no more end groups were formed, due to solubilization of the fibre monomers (hexamethylenediamine). This feature is extremely important with respect to future applications of this enzyme in the textile industry, where the time of incubation is a crucial factor in textile treatments.

The best dyeing results were obtained at 50°C (4 hours of treatment), below the glass transition temperature. Dye fixation occurs only at the surface of the fibre, where it is believed that cutinase can act.

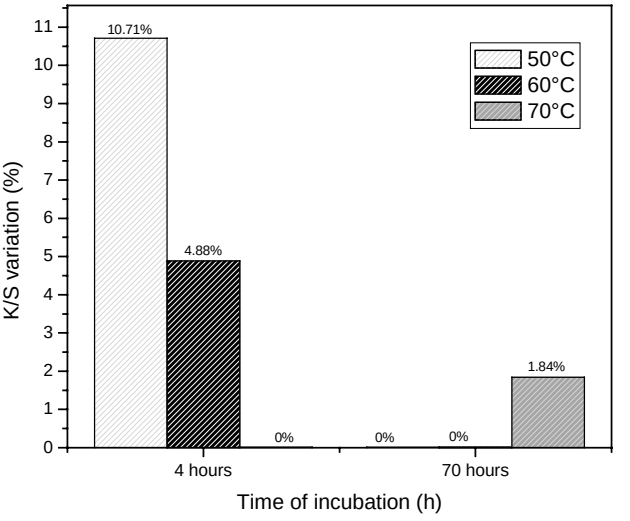

FIGURE 3 Increase (%) of K/S (λ = 570 nm) of samples treated with 12.98×10^3 UL^{-1} of cutinase and dyed with a reactive dye (Lanasol Red 5B), corrected for controls.

Above the glass transition temperature, around 57°C, the polyamide structure is more open and the amino groups, formed by enzymatic action, might be hidden as suggested by the dyeing results at 70°C.

CONCLUSIONS

We have shown that enzymatic hydrolysis of polyamide fabrics can be followed by determination of amino groups in the solution and on the fibre surface. Using the TNBS method we have detected amino groups in the treatment liquid of the polyamide fabrics. When analyzing the dyeing results, it is evident that after enzymatic hydrolysis, some groups resulting from the cleavage of the amide bonds, are left at the surface of the fibre. These groups react with the reactive dye, as revealed by the increase in coloration levels. Both methods are direct ways to demonstrate enzymatic hydrolysis and useful tools to ascertain what really happens when fibres are treated with enzymes.

Acknowledgements

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