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Influence of mechanical agitation on cutinases and protease activity towards polyamide substrates

Carla Silva [a], Rita Araújo [b], Margarida Casal [b], Georg M. Gübitz [c], Artur Cavaco-Paulo [a,*]

[a] *University of Minho, Textile Engineering Department, 4800-058 Guimarães, Portugal*
[b] *University of Minho, Biology Department, 4710-057 Braga, Portugal*
[c] *Graz University of Technology, Department of Environmental Biotechnology, 8010 Graz, Austria*

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Abstract

Two polyamide 6,6 substrates with different constructions, namely a model substrate and a fabric, were hydrolyzed using native cutinase and L182A cutinase mutant (from *Fusarium solani pisi*) and a protease (subtilisin from *Bacillus* sp.). The catalytic efficiency of these enzymes, measured in terms of hydrolysis products release, was measured for both substrates and the protease released five times more amines to the bath treatment. The L182A cutinase mutant showed higher activity when compared with the native enzyme.

All enzymes have shown activity additive effects with higher levels of mechanical agitation for polyamide fabrics. The results achieved are of paramount importance on the design of a process of enzymatic functionalization of polyamide.

Keywords: Polyamide; Cutinase; Protease; Hydrolysis

1. Introduction

Polyamide 6.6 (nylon 6.6) is an aliphatic semi-crystalline polymer made up of adipic acid and hexamethylenediamine. The amide groups –(–CO–NH–)– provide hydrogen bonding among polyamide chains, giving high strength properties at high temperatures, toughness at low temperatures, as well as stiffness, wear and abrasion resistance, low friction coefficient and good chemical resistance. Nylons are therefore one of the strongest synthetic fibers commonly used, with an extensive range of applications such as clothing, apparel, carpets, tyre reinforcement, parachutes and many other applications [1,2]. Despite of all the excellent properties exhibited, nylon fibers present low hydrophylicity and low reactivity with the most usual finishing and colouring agents [3,4]. Coating finishing effects are difficult to obtain when hydrophobic polyamide fabrics are used. Recent studies clearly indicate that the modification of synthetic polymers with enzymes is an effective and environmentally friendly alternative to chemical methods using alkaline products [5]. New processes using cutinases have been developed for the surface modification of polyamide fibers and quite satisfactory results were obtained [3–6].

Cutinase from *Fusarium solani pisi* is a α/β hydrolase that degrades cutin, the cuticular polymer of higher plants, which is an insoluble hydrophobic polyester composed of hydroxyl and epoxy fatty acids [7–9]. This enzyme has a catalytic mechanism similar to that presented by serine proteases. It is characterized by the triad Ser, His, Asp residues and by an oxyanion binding site that stabilizes the transition state via hydrogen bonds with two main chain amide groups [7–12].

Serine proteases such as subtilisin have a structural homology however they do not recognize the same substrates [13].

Being cutinase an esterase, it seems improbable that it will hydrolyze polyamide substrates. Although, polyamide has a structure quite similar to the cutin, that is an aliphatic polyester. This similarity and the diversified substrate recognition of cutinase makes it able to modify the polyamide surface, showing however a slow enzymatic kinetics [3].

The high crystallinity of the polyamide structure and the low affinity of the enzyme to the non-natural substrate are the main factors responsible for that. A more detailed study is needed in a way to know which process variables are the most significant in determining the efficiency of enzymatic hydrolysis. These vari-

* Corresponding author. Tel.: +351 253 510271; fax: +351 253 510293.
E-mail address: artur@det.uminho.pt (A. Cavaco-Paulo).

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ables may include different genetic modifications as well as the more usual operating conditions such as enzyme concentration, liquor ratio, treatment time, temperature, pH and mechanical agitation [14].

Site-directed mutagenesis is a way of developing the cutinase in order to obtain a higher specific activity to insoluble substrates like polyamide fiber. The modeling studies are based on the substitution of the specific amino acid residues close to the active site of cutinase, resulting in a modified enzyme with different properties and bigger binding sites. A previous work was performed in order to increase cutinase activity towards polyamide substrates [15]. A site-directed mutagenesis of cutinase was performed and five genetically modified enzymes were obtained by changing specific amino acids residues around the active site by alanine (L81A, N84A, L182A, V184A and L189A). The L182A mutant form was the most efficient in the catalysis of the amide linkages [15].

The process of enzyme adsorption is also of main importance to the enzymatic hydrolysis of polyamide. Different studies reveal that the adsorption of proteins follows different steps and that the mechanical agitation plays an important role in all of them [16,17]. Earlier investigation with cellulases proved that higher mechanical agitations increased greatly the enzyme performance at the fibers surface, although it can lead to an increase of the weight loss [14–19].

Different levels of mechanical agitation might lead to different levels of protein adsorption and enzymatic hydrolysis. The surface properties have an enormous effect on the mechanism, rate and degree of adsorption. The hydrophilicity of the surface has generally been regarded as a very important factor: the hydrophobicity of the surface increases the adsorption degree [20]. Cutinases, as well as proteases, have hydrophobic amino acids exposed on the surface which can increase the binding to the hydrophobic surface of polyamide fibers.

The purpose of the present work is to provide new insights about the influence of mechanical agitation on cutinase and protease activities towards polyamide substrates. The interaction between the activity of the genetically modified cutinases, the activity of a protease and the mechanical agitation were studied.

2. Materials and methods

2.1. Enzymes and reagents

Commercial polyamide (PA 6.6) woven fabric, a plain woven structure with 63 g^{-2}, was supplied by Rhodia (Switzerland). The polyamide model substrate was synthesized as described [21]. The genetic modification of cutinase was performed as previously described [15]. The protease, subtilisin from *Bacillus* sp. (E.C. 3.4.21.62), was a commercial enzyme purchased from Sigma (St. Louis, USA). The reactive dye used, Lanasol Red 5B (C.I. Reactive Red 66—17555), was generously supplied by CIBA (Switzerland). All other reagents used were laboratory grade reagents.

2.2. Quantification of protein concentration

Total protein concentration was determined by the Bradford methodology using bovine serum albumin (BSA) as standard [22]. For each sample three determinations were made.

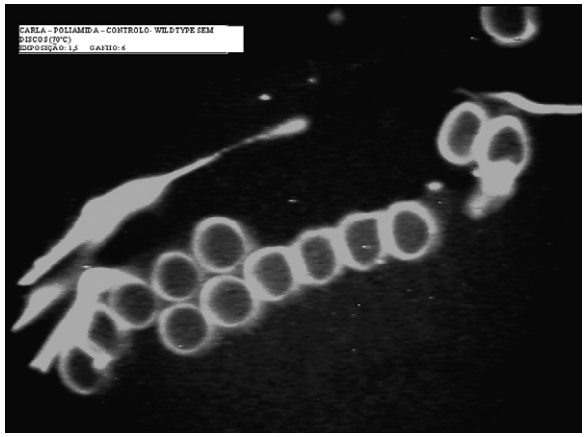

Fig. 1. Microscopic image of the polyamide stained samples (the reactive dye is linked only at the surface of the fabric).

2.3. Determination of protein adsorption

The protein adsorption was obtained measuring the protein content in the incubation solution before and after the enzymatic treatment of polyamide samples. The difference between the values obtained for these two periods was considered the protein adsorbed by the substrate.

2.4. Determination of the amino groups in the liquor treatment

To quantify the amino groups released during enzymatic hydrolysis, the trinitrobenzenesulfonic acid (TNBS) method was adapted from a methodology already described [3].

2.5. Determination of the amino end groups at the fiber surface by reactive staining

The amino groups at the surface of the polyamide fabric resulting from the enzymatic hydrolysis, were detected by staining polyamide with a wool reactive dye, specifically designed to react with the primary amino groups. The reaction occurred only at the surface of the fabric as can be depicted from Fig. 1 and the free amino groups were detected by the specific reaction with the α-bromoacrylamide dye reactive group [23].

All stainings were carried out in a 150 cm^3 capacity sealed stainless steel dyepots, housed in a dyeing machine (AHIBA Spectradye, from Datacolor). Stainings of 4% o.w.f. (weight of fabric) were obtained using a liquor ratio of 1:100 at different temperatures (50, 60 and 70 °C) for 90 min with a temperature gradient of 4 °C min^{-1}. After staining, the samples were washed with 2 g L^{-1} of a Lutensol AT 25 solution and then rinsed in running cold tap water for 10 min and air dried. Two independent staining experiments were performed and the results represent the mean of these experiments.

Colour differences of the stained fabrics were measured by using a reflectance-measuring apparatus, Spectraflash 600 Plus, from Datacolor International according to the CIELab colour difference concept, at standard illuminant D65. The colour strength was evaluated as *K/S* at maximum absorption wavelength (570 nm) and the results were summarized by the overall *K/S* differences [24].

2.6. Determination of the wettability of the polyamide treated fabric samples

In order to obtain the degree of wettability (hydrophilicity) of the untreated and treated polyamide fabrics, a water drop test was applied according AATCC standard method [25]. The wetting time was determined by placing a drop of distilled water on the stretched fabric sample (5 cm × 5 cm) from a burette held 1 cm from the fabric. The time of disappearance of the water-mirror on the surface (in other words the time for the water drop to lose its reflective power)

was measured as the wetting time. This procedure was applied to both untreated and treated fabrics.

2.7. Enzymatic hydrolysis of polyamide model substrate

2.7.1. Cutinases

In the first part of the work, a native and a mutated cutinase (L182A) were used to incubate the polyamide model substrate. Two sets of experiments were performed where 0.01 g of a polyamide model substrate was incubated in two different solutions. The first solution contained 10 mL of phosphate buffer (pH 7.5) and 3.5 U (μmol of *p*-nitrophenol-*p*-NP per min)/mL of native cutinase and the second solution contained the same amount of buffer and 4.7 U (μmol of *p*-NP per min) mL of cutinase mutant (L182A). Both experiments were performed at 35 °C for 8 h under continuous shaking (using an AHIBA Spectradye, from Datacolor, with vertical agitation). At different periods of incubation, the total protein content in the solution was determined as described in Section 2.2. After 8 h of incubation, a protein precipitation step was performed (the solution was mixed with perchloric acid in a ratio of 1:2; the mixture was centrifuged and the supernatant was neutralized with 2 M of KOH for posterior analysis) and the primary amino groups resulting from enzymatic hydrolysis were quantified by the TNBS method [3].

2.7.2. Protease

On this set of experiments, 10 mL of Tris–HCl buffer (pH 7.6) containing 1.8 U (μmol of Tyrosine per min)/mL of protease were incubated with 0.01 g of model substrate under the same conditions already described for cutinases. At different periods of incubation, the total protein content in solution was determined as described in Section 2.2. After 8 h of incubation, a protein precipitation step was performed (as previously described) and the primary amino groups resulting from enzymatic hydrolysis were quantified by the TNBS method [3].

2.8. Enzymatic hydrolysis of polyamide fabric pre-treatment

All samples of polyamide fabric used on this work were subjected to a previous washing with 2 g L^{-1} of a non-ionic agent, Lutensol AT 25 (10 g L^{-1}) and water for 1 h, followed by washing with a 2 g L^{-1} of Na_2CO_3 solution for 1 h, both at 50 °C.

2.8.1. Cutinase—vertical agitation

In 500 mL stainless steel pots of a laboratory Rotawash MKII machine, from SDL International Ltd., rotating at 20 rpm, 1 g of pre-treated polyamide fabric was incubated with 8 U (μmol of *p*-NP per min)/mL and 28 U (μmol of *p*-NP per min) mL of native cutinase in 300 mL of phosphate buffer (0.1 M NaOH, 0.1 M KH_2PO_4, pH 7.8) at 37 °C for 4 h under continuous vertical agitation. A higher level of mechanical agitation was achieved by adding five stainless steel discs (each disc with average weight of 19.1 g, 32 mm × 3 mm) into the reaction mixture. The L182A cutinase mutation was also tested, where 15 U (μmol of *p*-NP per min)/mL were incubated using the same conditions of the native one, as already described.

The experiments were performed in the presence of the discs as well as in their absence. For protein and amino groups quantification, aliquots were taken from the liquor treatment at 0.5, 1, 2, 3 and 4 h. After 4 h of incubation, the fabrics were removed from the liquor and rinsed in sodium carbonate solution (2 g L^{-1}) for 2 h to stop the enzymatic reaction and remove the protein adsorbed, followed by washing with 2 g L^{-1} of Lutensol AT25 solution for 1 h. After that, the samples were rinsed in running cold tap water for 5 min and allowed to dry at open air. Two independent experiments were done for each treatment, and the results represent the mean of these experiments.

2.8.2. Cutinase—orbital agitation

In 300 mL of phosphate buffer (0.1 M NaOH, 0.1 M KH_2PO_4, pH 7.8), 1 g of pre-treated polyamide fabric was incubated with 8 U (μmol of *p*-NP per min)/mL of native cutinase at 37 °C for 48 h under continuous orbital agitation. The low level of mechanical agitation was achieved using an Erlenmeyer held in a shaking water bath operating at 90 strokes min^{-1}. For protein and amino groups determination, aliquots were taken from the liquor treatment at 4, 6, 24, 36 and 48 h of incubation. After 48 h of incubation, the same procedure already described for the enzymatic treatment using vertical agitation was followed.

2.8.3. Protease—vertical agitation

In this set of experiments, 3 g of pre-treated polyamide fabric were incubated with 4 U (μmol of Tyrosine per min)/mL of subtilisin in 300 mL of Tris–HCl buffer (0.3 M Tris, 3 M HCl, pH 7.5) at 35 °C for 4 h under continuous vertical agitation. A higher level of mechanical agitation was achieved by adding five stainless steel discs (each disc with average weight of 19.1 g, 32 mm × 3 mm) to the reaction mixture contained in 500 mL stainless steel pots of a Rotawash MKII machine, rotating at 20 rpm. The experiments were performed both in the presence and absence of stainless steel discs. For protein and amino groups determination, aliquots were taken from the liquor treatment at 0.5, 1, 2, 3 and 4 h. After 4 h of incubation, the same procedure already described for the enzymatic treatment with cutinase using vertical agitation was followed.

2.9. Wide-angle X-ray diffraction (WAXD)

WAXD patterns of the PA 6.6 fabric were obtained for the samples treated with cutinases and protease, both in the presence and absence of stainless steel discs. The X-ray diffractometer used was the model PW1710, from Philips. The Cu Kα radiation source (λ = 0.154 nm) was operated at 40 kV and 30 mA. The WAXD spectra were continuously recorded in the diffraction angular range of 5–35° (2θ). The scan speed was 0.01° s^{-1}.

The WAXD data were analyzed by profile fitting of the obtained scans. The Pearson VII functions were applied and several simulations were performed in order to provide the best fit. At the end several parameter values were obtained such as pick intensities, pick positions, full width at half-maximum and others.

The crystallinity value (CV) of the different assayed samples was obtained using Eq. (1), available in literature where d_{100} and d_{010} are the interplanar distances related to the planes (1 0 0) and (0 1 0), respectively [26,27].

$$\mathrm{CV} = \frac{[d_{010}/d_{100}] - 1}{0.189} \times 100 \quad (1)$$

Eq. (1) can be simplified and expressed as Eq. (2), where the θ_{100} and θ_{010} are the angles related to the (1 0 0) and (0 1 0) interplanar distances (*d*); 546.7 is a constant related with polyamide crystallinity and 0.50 is the other constant obtained by calculating the reason between the crystalline area and the total area (crystalline and amorphous) of each sample.

$$\mathrm{CV} = \left(\frac{2\theta_{010}}{2\theta_{100}} - 1\right) \times 546.7 \times 0.5 \quad (2)$$

2.10. Fourier transformed infrared spectroscopy (FT-IR)

Infrared spectra were taken with a Bomem M Series Spectrophotometer. All the spectra reproduced were collected using an attenuated total reflectance accessory (ATR). Before collecting, the background scanning was performed using KBr powder. The fabric samples were placed in a large sample cup in top of KBr. At least 32 scans were obtained to achieve an adequate signal to noise ratio. The spectra were taken in the region of 800–4000 cm^{-1} with a resolution of 8 cm^{-1} at room temperature.

2.11. Scanning electronic microscopy (SEM)

The scanning electronic microscopy pictures were obtained in a scanning electronic microscope model LEICA S360 with a backscattered and secondary electron detector.

3. Results and discussion

3.1. Enzymatic activity of cutinase and protease towards polyamide model substrate

One of the objectives of this work was to prove that cutinase and protease were able to hydrolyze polyamide surface sub-

Table 1
Protein adsorption and enzyme activity (measured as amines formation) of native cutinase, L182A cutinase mutant and protease

Enzyme	Protein adsorption (%)	Amines (mM)
Native (4 U mL^{-1})	18	0.0728
L182A mutant (4.7 U mL^{-1})	53	0.1053
Protease (1.8 U mL^{-1})	54	0.3228

strates. Therefore, before the enzymatic treatment of the main substrate (polyamide fabric), a smaller substrate was studied. Our purpose was to prove that if these enzymes were able to work on the hydrolysis of small polyamide substrates, they could probably act also on bigger ones. For that reason a small amount of PA 6.6 model substrate was incubated with native, mutated cutinase (L182A) and protease and their activity was measured as the amino groups formation. The protein adsorbed during the enzymatic process was also quantified for all the enzymes assayed. Comparing the obtained data for both cutinases assayed, it can be observed (Table 1) that the protein adsorption, as well as the enzymatic activity, expressed as mM of amines in solution, is higher when L182A mutant was used. These values are explained based on the assumption that the site-directed mutagenesis, that consisted on the substitution of the Leucine amino acid by a smaller (Alanine) amino acid, close to the active site, resulted in a more "open" enzyme structure. This allows for a better "accommodation" of the bigger polyamide substrate into the active site. The protein adsorption values for protease were quite similar to those obtained for the L182A mutant but the enzyme activity was higher. This result can be attributed to the specificity of this enzyme to hydrolyze amide bonds.

The data obtained for PA model substrate show relative ability to hydrolyze small substrates of polyamide. A posterior study was performed to confirm these results and the ability of these enzymes to modify the surface of a bigger substrate (PA fabric).

3.2. Enzymatic activity of cutinase and protease towards polyamide fabric

3.2.1. Cutinase

The ability of cutinase to modify polyamide substrates with higher DP and MW was determined as well as the interaction of mechanical agitation with enzymatic activity.

Different levels of mechanical agitation were applied on the described experiments in order to measure its influence on enzymes activity.

The activity of native cutinase, measured as amino groups released in the liquor bath treatment, increased when higher mechanical agitation was used (stainless steel discs addition) (Fig. 2). The findings seem to suggest that mechanical agitation influences greatly the enzyme hydrolysis on the polyamide fabric. Comparing the experiments performed on the Rotawash machine (vertical agitation), it seems clear that the increase of the native cutinase activity was due to the incorporation of the stainless steel discs on the treatment pots. This process variable leads to an increase of the fiber–metal friction, as well as an increase of the beating effects during enzymatic incubation.

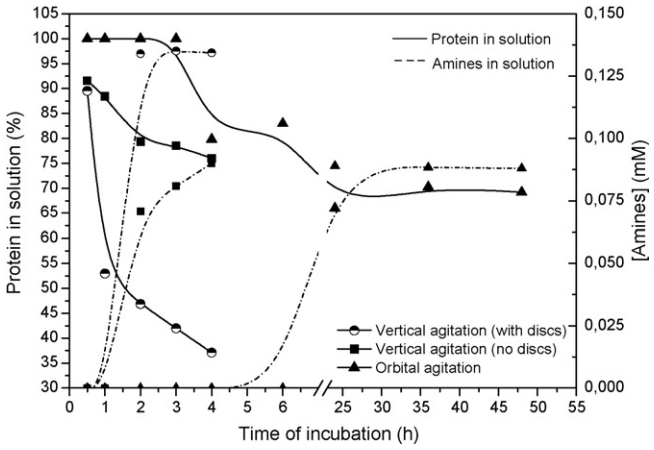

Fig. 2. Native cutinase (8 U mL^{-1}) activity (measured as amines released to the bath treatment) vs. protein adsorption.

The higher mechanical agitation used increased the action of cutinases. The combined action of the enzyme and the mechanical agitation lead to a more pronounced effect compared with the enzyme action itself. The additive effects of the enzyme and the mechanical agitation created more superficial cuts along the polymer, corresponding to the breakage of the amide linkages. Mechanical action raised these broken ends, creating micro fibrils and consequently more sites for possible enzyme attack. This phenomenon was accomplished by the mechanical abrasion of the fabrics' surface where the amino end groups formed by enzymatic action were released to the liquor bath treatment and could be spectrophotometrically quantified (Fig. 2). In order to measure the hydrolysis extent, a fabric reactive staining was performed (Fig. 3). In the absence of the stainless steel discs was obtained an increase of the staining values corresponding to an increase of the amino groups at the surface of the treated fabrics since they were not released to the bath treatment. The *K/S* values decreased when temperatures above T_g were applied. Above 57 °C the polymer structure is more exposed and the dye does not link only at the surface of the fabric (Fig. 1) but is also able to penetrate into the interior of the fibers. It is important to notice that, to measure the hydrolysis extent at the surface of the treated fabric, the reactive staining should be per-

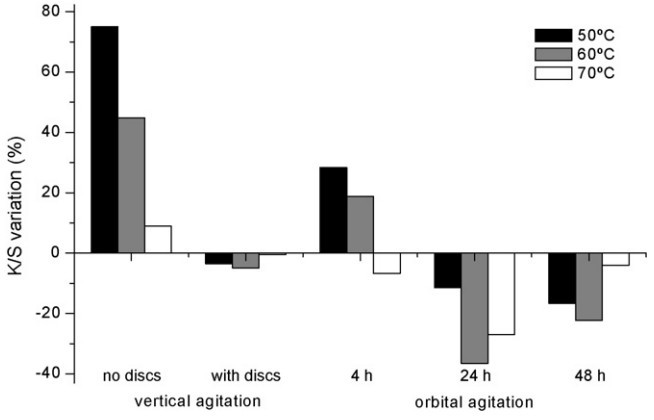

Fig. 3. *K/S* variation, proportional to the amino groups at the surface of the treated fabric (4% of reactive dye; liquor ratio 1:100; 90 min).

formed below glass transition temperature. Regarding the other values obtained, it can be seen that mechanical agitation preferentially removed microfibrillar material with a high content of end groups which cannot be detected by reactive staining. High levels of mechanical agitation are aggressive and cause fabric fibrillation. The formed fibrils (pills) represent a more exposed specific surface area of enzyme attack and will present a more pronounced colour intensity compared with the other part of the treated fabric. However, these results were not considered because the spectrophotometrically measure is technically difficult to obtain. The fibrils resulted from strong beating effects are too small and dispersed on the fabric sample surface.

In the same set of experiments described a shaker bath was used with orbital agitation. In this apparatus the polyamide samples were incubated for a long period (48 h) at 90 strokes min^{-1}. A slow kinetic of enzymatic activity, measured in terms of amino groups formed, in the first 24 h of incubation was obtained. After this period the activity of cutinase, measured as amino groups formation, increased reaching the same level of the one obtained on the Rotawash machine (vertical agitation), without discs (Fig. 2).

The results of reactive staining obtained for vertical agitation, as well as the results obtained for orbital agitation show that the incorporation of discs on the system increased the release of the amines to the bath system (Fig. 3). The *K*/*S* values decreased when a higher level of mechanical agitation was applied.

Adsorption studies were also performed in order to measure the influence of mechanical agitation on the protein adsorption on the fibers. The results given in Fig. 2 show that protein adsorption increased when stainless steel discs were included in the system, reaching a high level of about 60% of adsorption. Orbital agitation provided values of protein adsorption in the order of 30%.

As obtained for polyamide model substrate (Table 1), on fabric the L182A cutinase mutant presented a considerably higher activity when compared with the native one (Fig. 4a and b). Protein adsorption values are similar for both enzymes and presented higher values when stainless steel discs were included on the enzymatic system.

3.2.2. Protease

Similarly to the results obtained for cutinases, the protease activity as well as the protein adsorption, increased when high levels of mechanical agitation were used. Mechanical abrasion has been indicated as to synergistically cooperate with protease activity. This is explained by the fact that mechanical agitation causes more fibrillation. In this situation, the loose fibrils (pills) formed represent an increased and more exposed specific surface area for enzyme attack. The synergistic action of the enzyme specificity and the mechanical agitation leads to a higher activity, measured as amino groups released, compared with the cutinases (Fig. 5b). The amino groups concentration, when protease was used, reached 1 mM, a value which is five times higher when compared with that obtained for cutinases (0.2 mM) (Fig. 4b). Spectral values obtained after reactive staining of treated fabric samples increased which can be correlated with an increase of the amino groups at the surface of the treated fabrics (Fig. 6).

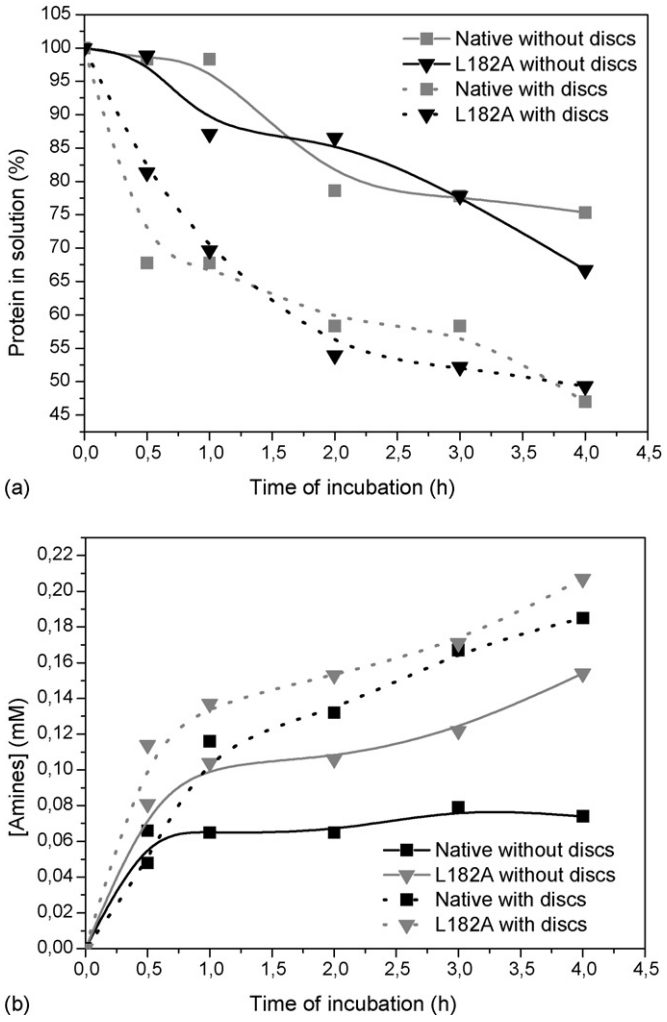

Fig. 4. (a) Protein content in the liquor treatment after 4 h of incubation with native (28 U mL^{-1}) and cutinase mutant (15 U mL^{-1}). (b) Amino groups concentration in the liquor treatment after 4 h of incubation with native (28 U mL^{-1}) and cutinase mutant (15 U mL^{-1}).

The increase of the staining temperature leads to a lower increase of the *K*/*S* values due to the fact that above glass transition temperature the polymer structure is more open and the dye penetrates in the interior of the fiber. Differences between samples are not so easily detected when analyzing the obtained data. When a higher level of mechanical action was used, the amino groups at the surface were partially removed and consequently the *K*/*S* values decreased. The values obtained when samples were dyed at 70 °C should be lower. This result can probably be an error. Protease hydrolysis was efficient on surface fabric modification.

3.3. Wettability

Polyamide fabric samples were tested in terms of water absorption after enzymatic incubation procedure. This result indicates that the amount of hydrophilic groups at the surface has increased. The samples without treatment presented an hydrophobic behaviour (>10 min of absorption). The enzymatic

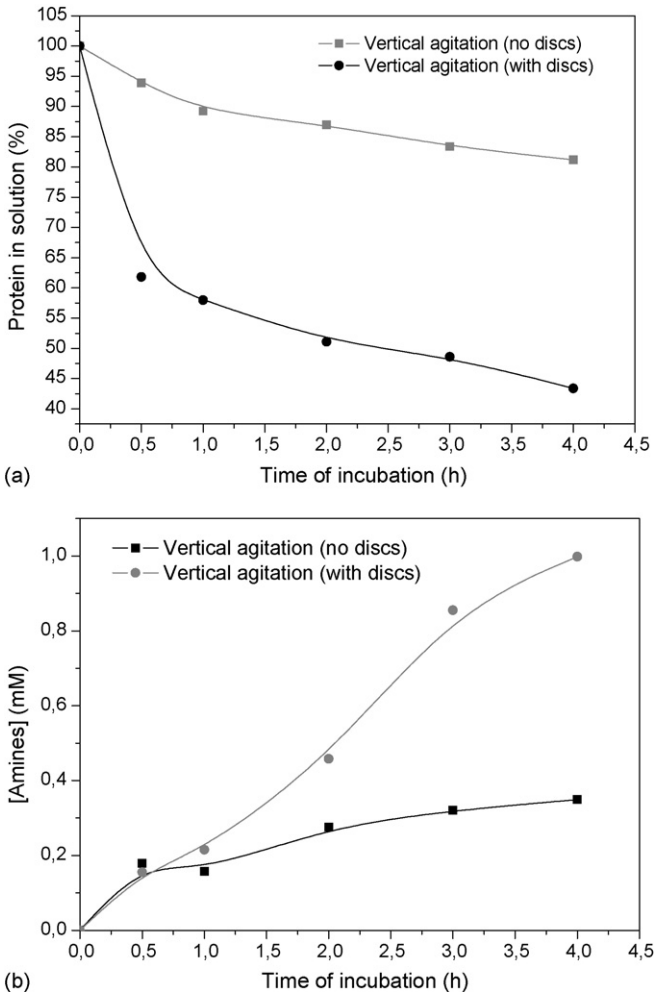

Fig. 5. (a) Protein adsorption after 4 h of incubation with protease (4 U mL^{-1}). (b) Amino groups concentration in the liquor treatment after 4 h of incubation with protease (4 U mL^{-1}).

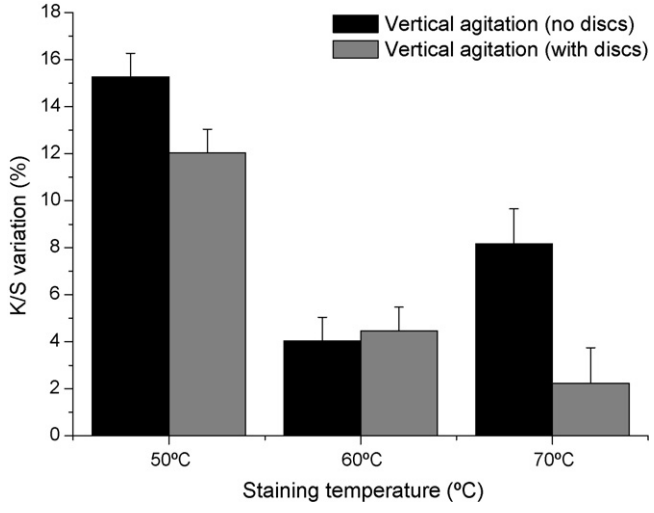

Fig. 6. *K/S* variation, proportional to the amino groups at the surface of the fabric samples treated with protease (4% of reactive dye; liquor ratio 1:100; 90 min).

Table 2
Time of water drop absorption of fabrics treated with different enzymes

Enzymes	Time of water drop absorption (min)
Control	>10
Native (28 U mL^{-1})	5.34 ± 0.20
L182A mutant (15 U mL^{-1})	4.67 ± 0.20
Protease (4 U mL^{-1})	5.00 ± 0.40

hydrolysis with cutinases and protease were able to modify the surface of polyamide fabrics with a consequent decrease of the time of water drop absorption to 5 min (Table 2). The surface of polyamide fabrics became more hydrophilic and probably easier to finish.

3.4. Crystallinity determination through WAXD

WAXD studies of treated polyamide samples show, as expected, two strong diffraction peaks, one located at $2\theta = 20.2^\circ$ and the other one at $2\theta = 23.4^\circ$ [27]. The crystallinity value (CV) of the control and the treated samples was calculated as defined in Eq. (2). As expected, no significant changes were observed. Enzymatic action occurs only at the surface of the fabric and the formation of hydrophilic groups by hydrolysis does not influence the intrinsic physical properties of polyamide polymer (Table 3).

3.5. Infrared studies

Infrared spectra, taken for PA 6.6 samples are shown in Fig. 7a and b, as an example. In this study the different spectra obtained for control and each treated sample were compared. All the spectra were collected using the region of 800–4000 cm^{-1}, although the band region used for comparison between samples was 1700–1650 cm^{-1} where it seams to have some relative intensity differences. This region corresponds to the amide bands, where a decrease of the peak intensity can be correlated with the breakage of some amide linkages at the surface of the fabric, as theoretically expected. The carbonyl amide stretching vibrations (1663 – Amide I band of secondary amide; 1558 – Amide II band: N–H bending and –N stretching) presented different intensities depending on the enzyme.

Regarding the spectra obtained for samples treated with the native, L182A mutant cutinase and protease without stainless

Table 3
Crystallographic results of polyamide samples treated with cutinases and protease

Sample	Crystallinity (%)
Control (no discs)	42.837
Native	42.964
L182A mutant	42.911
Protease	42.957
Control (with discs)	43.037
Native	43.419
L182A mutant	43.723
Protease	43.838

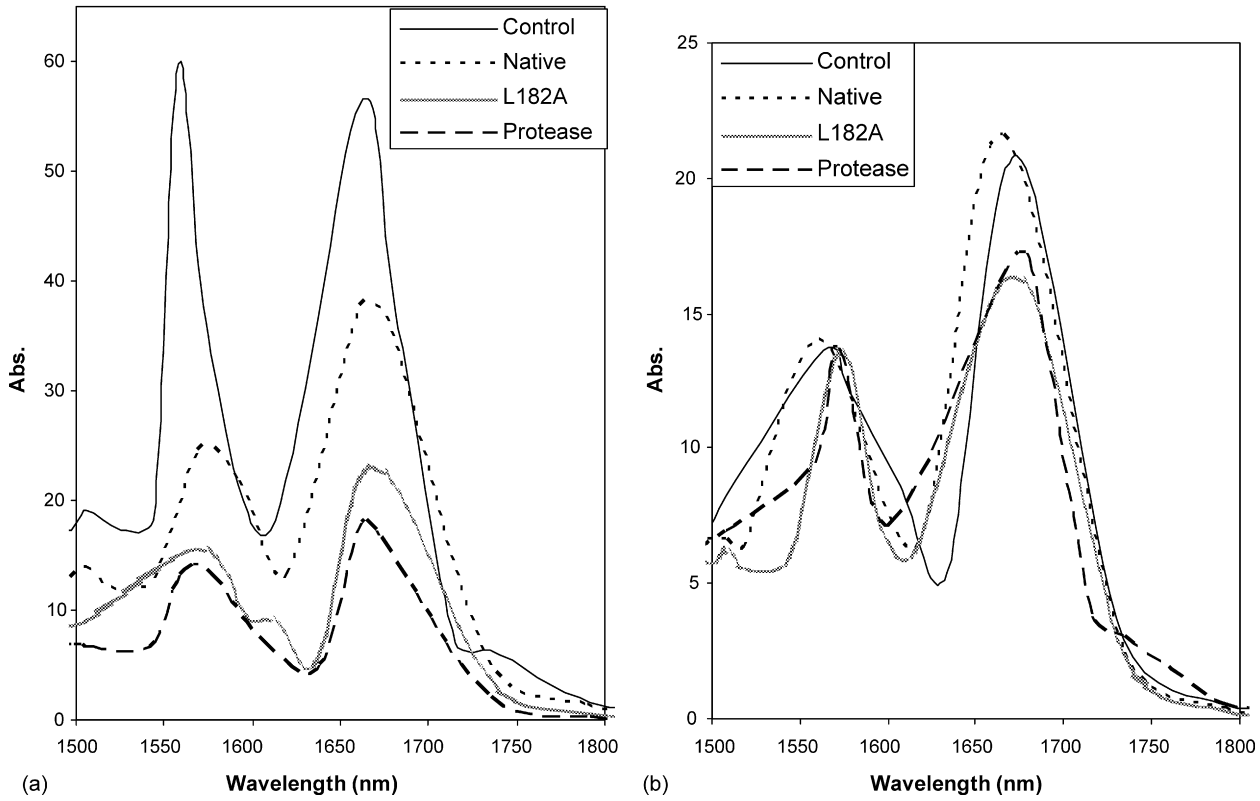

Fig. 7. (a) Infrared spectra of samples treated with Native, L182A cutinase mutant and protease in the absence of stainless steel discs (most significant band). (b) Infrared spectra of samples treated with Native, L182A cutinase mutant and protease in the presence of stainless steel discs (most significant band).

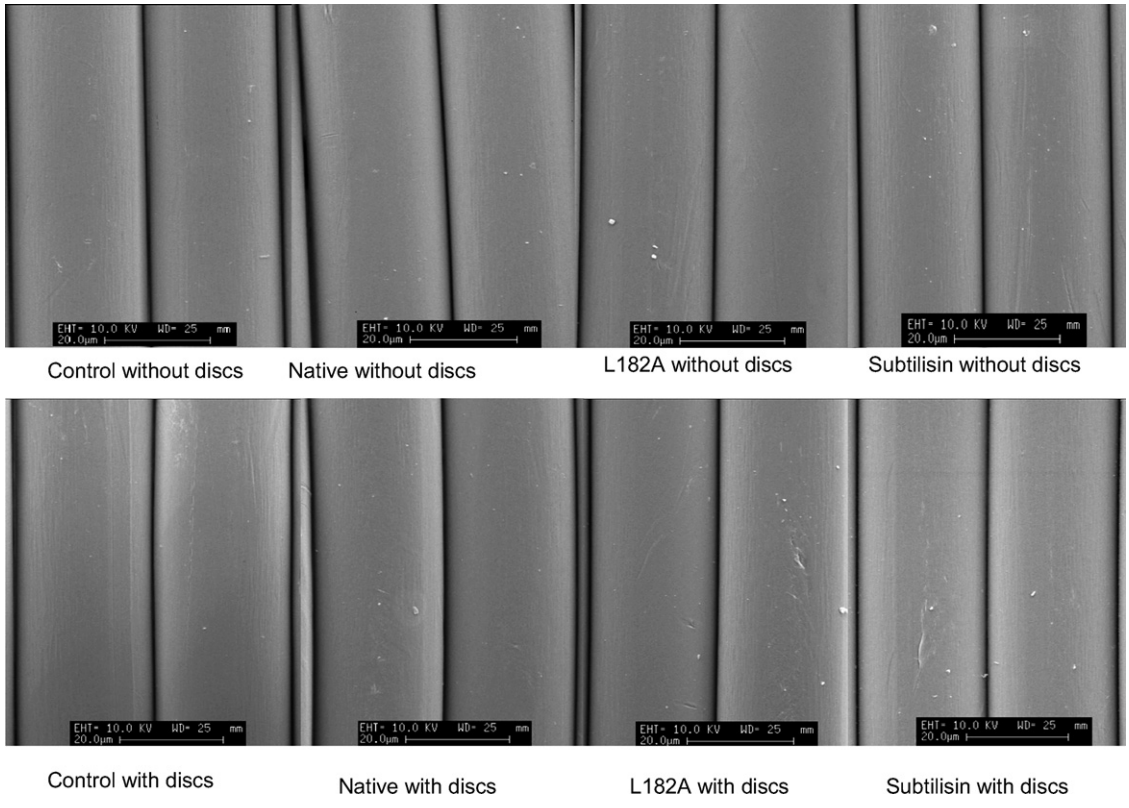

Fig. 8. Scanning electronic microscopy (SEM) of samples treated with native, L182A mutant and proteases in the absence and in the presence of stainless steel discs.

steel discs it seems clear that there is a decrease of the absorbance values relatively to the control. The breakage of the amide linkages of the polyamide polymer and the increase of the other groups at the surface of the fabric might influence the amide vibrations and consequently its absorbance intensity. The spectra obtained for the samples treated with all the enzymes in presence of stainless steel discs does not show significant changes on the absorbance intensity value.

These results can be correlated with the spectral results already described. Samples treated in the absence of discs presented higher *K*/*S* values than samples treated in their presence. The simultaneous action of enzyme and mechanical agitation lead to the surface abrasion of the superior layer of polyamide fabric (Fig. 8) and consequently the hydrophilic groups formed by enzymatic hydrolysis cannot be so easily detected by reactive staining.

4. Concluding remarks

This study provided new insights about the influence of mechanical agitation on cutinase and protease activities towards polyamide substrates. The cutinase mutant (L182A) showed more ability to modify the surface of polyamide substrates when compared with the native one. However, the higher catalytic efficiency was obtained for protease due to its enzymatic specificity. The results obtained support the idea that when higher levels of mechanical agitation were introduced on the system the level of surface modification increased. The simultaneous action of the enzymes and the stainless steel discs lead to an increase of the enzymatic conversion, although a careful balance between the enzyme activity and the mechanical agitation is required to achieve higher level of hydrolysis without excessive fabric strength and weight loss.

For a future industrial application of this process it is necessary to find this equilibrium. To produce the large amount of amino groups, short times of incubation must be used as well as vertical agitation. More studies have to be performed in order to predict and better control the polyamide finishing.

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