Enzymatic Hydrolysis of Nylon 6 Fiber Using Lipolytic Enzyme

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ABSTRACT: This study confirms the structural changes of nylon 6 fibers using lipase by measuring the dyeability, hydrophilicity, chemical changes, and fastness properties. For this purpose, nylon 6 fabrics were first treated separately with different concentrations of lipase enzyme. The dyeing process was then carried out on the treated fabrics with two disperse and acid dyes. A UV-vis spectrophotometer was used for determination of dyebath exhaustion. Acid and disperse dyes showed higher dyebath exhaustion on the enzyme treated samples compared to raw material. The intensity of major peaks in FTIR spectra of the lipase treated samples are in favor of chemical changes of the polypeptide functional groups in fabric. Tensile strength of treated fabrics was decreased due to enzyme treatment. The results of color measurements in the CIELAB system showed that the darkness of the samples increased with an increase in the enzyme percentage in the solution. The results of moisture regain showed that treatment of nylon fabrics with lipolytic enzymes caused to increase the moisture absorbency. The wash and light fastness properties of samples were measured according to ISO 105-CO5 and Daylight ISO 105-BO1 and discussed. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 3140–3147, 2010

Key words: nylon 6; lipase; hydrolysis; FTIR spectroscopy; moisture regain

INTRODUCTION

Nowadays, textile processing based on biotechnology has gained importance in view of stringent environmental and industrial safety conditions.[1–3] The best established application of biotechnology in textile industry is the use of enzymes. These vital parts of all living organisms are specific organic catalysts in the reaction catalyzed and substrates selectivity. Traditional chemical treatments are replaced by enzymatic ones because of their lower product quality, higher manufacturing cost, more waste, and added energy consumption. The main enzymes used in textile processing are amylases, cellulases, proteases, esterases, nitrilases, catalases, peroxidases, laccases, and pectin-degrading enzymes.[1,2,4,5]

Many studies have been carried out on the application of enzymes on natural fibers, including cotton surface modification to enhance the softness and appearance[5–8]; removing undesirable byproducts from the unscoured cotton[5,9]; desizing by enzymatic hydrolysis[3,5]; enzyme treatment of bleaching effluent[10,11]; softening woody fibers during retting[12,13]; shrink-proofing, softening, improving dyeability and pilling behavior of wool and cotton[14–31]; and silk degumming.[32,33]

Recently, enzymatic hydrolysis of synthetic fibers to improve some undesired properties such as hydrophobicity, low dyeability, and insufficient washability is considered to chemists.[34–38]

Many research articles have been published on enzymatic surface hydrolysis of polyester and on its dyeing behavior[36,39–41] but few have been reported on dyeing of hydrolyzed polyamides. Studies showed that enzymatic hydrolysis can be achieved on nylon oligomers.[42–46] Researchers claim that surface of PA66 and PA6 can be modified by oxidative enzymes without reducing the fiber diameter.[47] Furthermore, different types of enzymes can hydrolyze the surface of polyamide fibers.[38,48–50] It was indicated that the metal-free, tannic acid/enzyme after treatment of acid dyed PA 66 fibers is highly effective on improving its wash fastness.[51] Surface amino groups formed during the enzyme hydrolysis can also be determined based on dyeing with reactive dyes.[49]

Thus, to fulfill further studies on dyeing behavior of enzyme treated polyamide, the present research work focuses on the physical and chemical properties of acid and disperse dyed-lipase treated PA 6 fibers.

MATERIALS AND METHODS

Plain weave PA 6 fabric with warp and weft yarn count of 40/2 tex was used. Nonionic detergent (SDL

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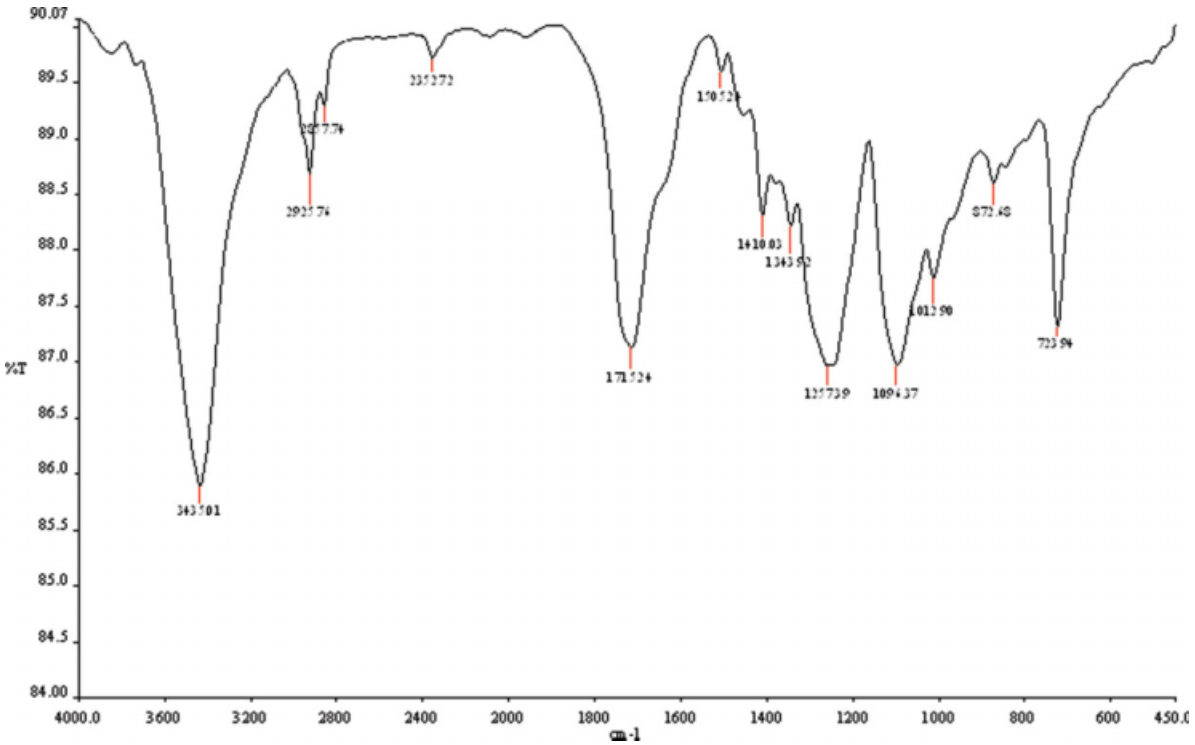

Figure 1 FTIR spectra of untreated nylon 6 fabric. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com].

Technologies, Stockpot, England) was used for scouring the PA 6 fabrics. The lipase enzyme used was Lipex 50T purchased from Novo Nordisk (Bagsward, Denmark). It was produced by fermentation of *Aspergillus terreus* microorganism. Acetic acid (85%) from Merck (Darmstadt, Germany) was applied for acid and disperse

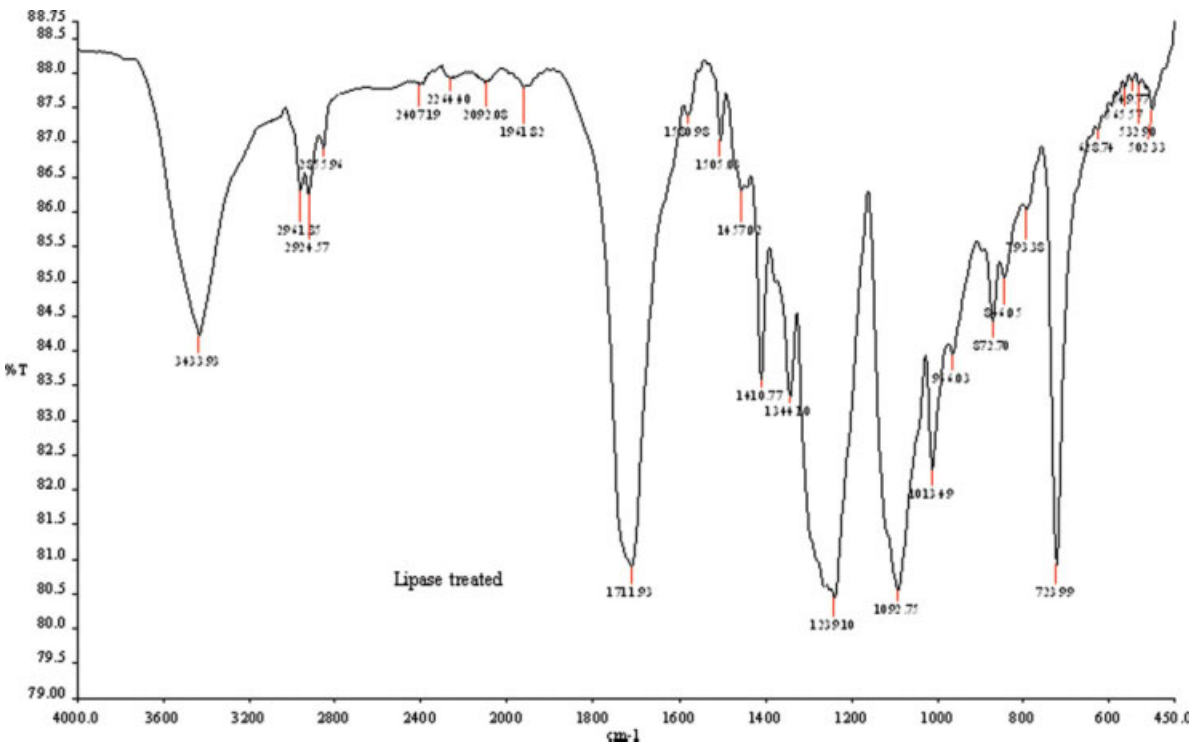

Figure 2 FTIR spectra of nylon 6 fabric treated with lipase enzyme 6% o.w.f. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com].

TABLE I
Exhaustion of Untreated and Enzyme Treated Acid and Disperse Dyed Nylon 6 Fabrics

Dyes used for dyeing (1%)	Enzyme (%)	Exhaustion (%)
Acid dye	0	65 (1.5)
	1	67 (1.7)
	2	68 (1.3)
	4	70 (0.8)
	6	73 (1.7)
Disperse dye	0	50 (1.2)
	1	51 (0.7)
	2	52 (0.8)
	4	55 (1.8)
	6	58 (1.1)

Standard deviations in parentheses.

dyeings. Dispersing agent was Ekalin F from Sandoz Company (Holzkirchen, Germany) for disperse dyeing of enzyme treated fabrics. The acid and disperse dyes used were Cyanine Blue R (C. I. Acid Blue 92) and Serene Red Brown RFS (C. I. Disperse Brown 1) from Serene Industries Ltd. (Mumbai, India) for dyeing of lipase treated PA 6 fabrics.

Fabric samples were scoured with 5% nonionic detergent. The L:G (liquor ratio) of the scouring bath was kept at 20:1 for 15 min at 60°C. The enzymatic treatments were carried out for 80 min at pH 6.5 and 30°C. Enzyme concentrations of 1%, 2%, 4%, and 6% o.w.f. were used. The liquor ratio was 20:1. Following the enzymatic treatment, the fabrics were maintained for 5 min at a temperature of 90°C and a pH lower than 4, to denature the enzyme. The fabrics were then well rinsed to eliminate any remaining enzyme. For acid dyeing of treated fabrics, solutions were prepared by adding the acid dye (1% o.w.f.) and acetic acid (4%) at liquor ratio of 40:1. The dyeing process was started at 40°C and the temperature was raised to 95°C over 20 min and then held at that temperature for 1 h. For disperse dyeing, solutions were prepared by adding the disperse dye (1% o.w.f), acetic acid (4%), and dispersing agent (1% o.w.f.) at liquor ratio of 40:1. The disperse dyeing process was the same as acid dyeing.

Some of the physical and chemical properties of treated PA 6 fabrics (Fourier-transform infrared spectroscopy, dyebath exhaustion, moisture regain, microscopic characterization, tensile strength test, reflectance measurement, and color fastness) were discussed. All measurements were repeated five times, together with calculating the standard deviation (the coefficient of variation was below 5% for all cases).

Fourier-transform infrared spectroscopy

The effect of the enzyme treatment on the chemical structure of PA 6 fabric was examined by the FTIR spectroscopy and KBr method [Bomem-MB100 Series (Hartmann and Broun)].

Determination of dyebath exhaustion

The effect of enzyme treatments on the percentage of dyebath exhaustion was calculated according to "eq. (1)":

$$\text{Exhaustion } \% = [(A_0 - A_d)/A_0] \times 100 \qquad (1)$$

where A_o and A_d are the absorbances (at λ_{max}) of the initial concentration of dye in the dyebath and the residual dye, respectively. The absorbances were measured using a Shimadzu UV-2101 PC UV-Vis spectrophotometer.

Determination of moisture regain

Moisture regain was calculated using "eq. (2)" according to ASTM method 2654-76:

$$\text{Moisture regain } \% = [(W_1 - W_2)/W_2] \times 100 \qquad (2)$$

Where W_1 is the weight (g) of the sample after saturation at standard humidity and W_2 is the weight (g) of the sample dried to constant weight.

Microscopic characterization

The surface of fabrics was investigated using a Scanning Electron Microscope (SEM XL30, Philips). The surface of samples was first coated with a thin layer of gold ($\sim$ 10 nm) by Physical Vapor Deposition method (PVD) using a sputter coater (SCDOOS, BAL-TEC).

Determination of tensile strength

The tensile strength of the enzyme treated and untreated PA 6 fabrics was evaluated using an Instron TE-500 from Farayab with gauge of 20 cm and a cross-head speed of 25 cm/min, after conditioning the specimen for 24 h and 65% relative humidity and 20°C (ASTM D2256).

Reflectance measurement

The reflectance of the dyed samples was recorded using a Gretagmacbeth COLOREYE 7000A spectrophotometer integrated with an IBM personal computer. CIELAB color coordinates (L^*, a^*, b^*, C^*, and h) were calculated from the reflectance data for 10°

TABLE II
Moisture Regain of Fabrics Before and After Treatment with Lipolytic Enzyme

Enzyme (%)	Moisture regain (%)
0	3.35 (0.07)
1	3.68 (0.08)
2	4.05 (0.05)
4	4.43 (0.05)
6	4.58 (0.07)

Standard deviations in parentheses.

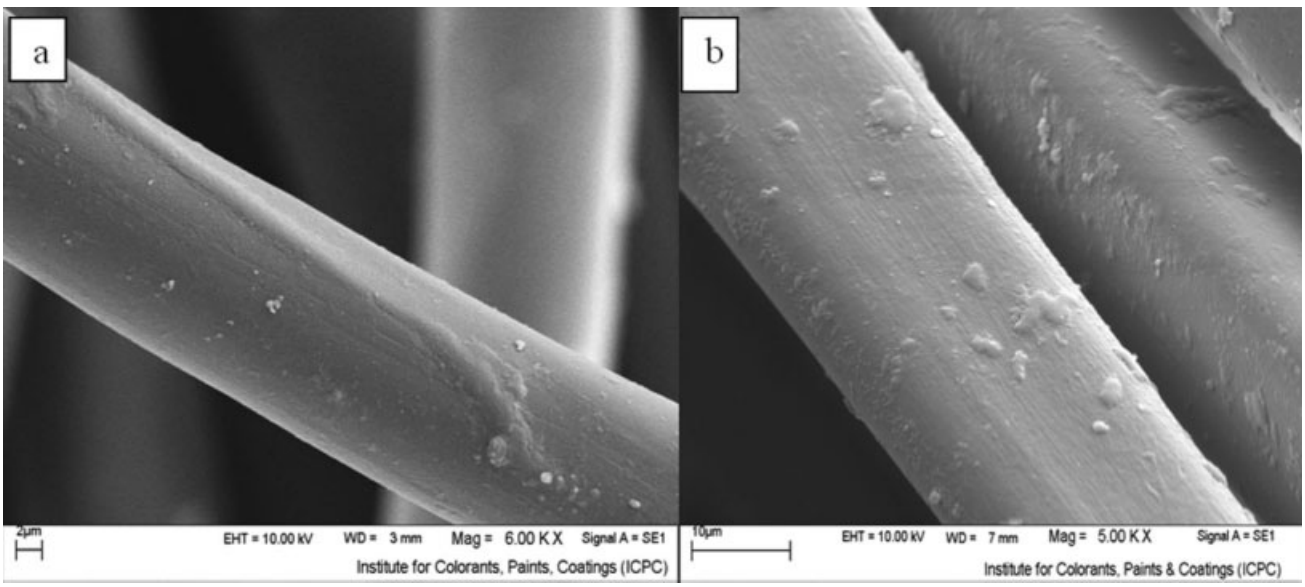

Figure 3 SEM images of (a) untreated nylon 6 fiber at 6.00 KX and (b) nylon 6 fiber treated with lipase enzyme 1% o.w.f. at 5.00 KX.

observer and illuminant D_{65}. The L^* is the color coordinate, which represents the lightness of samples and can be measured independently of color hue. Any decrease in the lightness of samples could be concluded as the more color absorption into the fiber. The a^* stands for the horizontal red–green color axis and the b^* represents the vertical yellow–blue axis. The C^* represents brightness and dullness of the samples. Any increase in the C^* of samples could be concluded as more brightness of the fiber.[22,47]

Determination of color fastness

The wash-fastness properties of the samples were measured according to ISO 105-C01. The color hue changes of the fabric and the degree of staining on the adjacent fabrics were measured after drying. For light-fastness measurements, the samples were exposed to the daylight for 2 and 7 days according to the daylight ISO 105-B01, and the changes in the color (fading) were assessed compared to a blue scale.

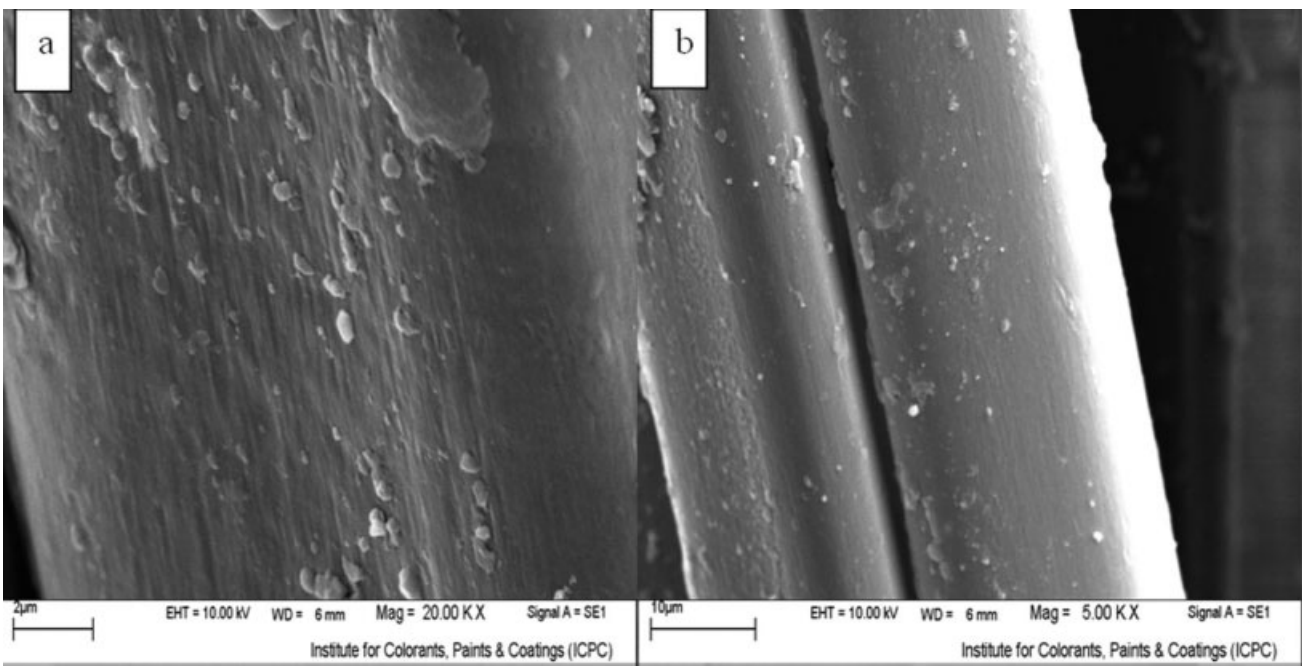

Figure 4 SEM images of nylon 6 fiber treated with lipase enzyme. (a) Nylon 6 fiber treated with lipase enzyme 6% o.w.f. at 20.00 KX. (b) Nylon 6 fiber treated with lipase enzyme 6% o.w.f. at 5.00 KX.

TABLE III
Tensile Properties of Untreated and Enzyme Treated Nylon 6 Fabrics

Enzyme (%)	Initial modulus (g/tex)	Extension at break (%)	Tenacity (g/tex)
0	30.4 (0.4)	37.4 (1.3)	6.80 (0.2)
1	27.1 (0.3)	35.1 (1.1)	6.55 (0.1)
2	25.2 (0.6)	36.5 (1.2)	6.01 (0.1)
4	24.0 (0.6)	33.4 (1.0)	5.88 (0.1)
6	22.9 (0.5)	31.1 (0.5)	5.80 (0.2)

CV was less than 5%.

RESULTS AND DISCUSSION

Structural information

The infrared spectrum of untreated and enzyme treated PA 6 fabrics were shown in Figures 1 and 2. The *N*—H stretching and bending vibrations in polyamide are usually appeared at 3100–3500 cm^{-1} and 1550–1640 cm^{-1}, respectively, depending on the type of amide (primary and secondary), chemical environment (solid and liquid) and intramolecular or intermolecular hydrogen bonds. The C=O stretching vibration band appears in the normal region between 1640 and 1670 cm^{-1}, which usually overlaps with *N*—H bending.[52]

Figure 1 assigned *N*—H stretching of a secondary amide at 3435 cm^{-1} as expected for PA 6. The bands at 1550–1650 cm^{-1} are also characteristic of *N*—H stretching vibration which overlaps with C—O vibration. The band at 1715 cm^{-1} is due to C—O stretching of secondary amide which is shifted from 1640 cm^{-1}. This band shifting confirmed the resonance between molecules in polyamide chains.[52]

Figure 2 revealed that the intensity of broad band frequency at 3433 cm^{-1} is decreased which is due to breakage of some peptide groups after enzyme treatment. The bands at 1580 and 1239 cm^{-1}, relative to the *N*—H and C—N stretching vibrations, indicates the presence of C—*N*—H chemical bonds in the product of enzyme treated PA 6. The presence of intensified bands at 1711 cm^{-1} and 723 cm^{-1} relating to C=O vibration and NH bending are clearly due to hydrolysis of polypeptide chains.

The assignments of FTIR spectra of untreated and treated PA 6 with lipase enzyme clearly suggest that the substrate has undergone severe chemical hydrolysis especially at amide (—CONH—) functional groups.

Dyebath exhaustions

Table I shows the exhaustion values for the untreated and enzyme treated PA 6 dyed with acid and disperse dyes. They showed higher exhaustion on the sample treated with 1% enzyme solution compared to untreated one. Any increase in the enzyme concentration resulted in the increase in dyebath exhaustion. This could be possibly due to hydrolysis of polyamide chains after treatment with lipase which produces the fiber with more functional groups for acid dye attraction. Any increase in enzyme concentration causes increase in fiber hydrolysis.[36,42,43]

Moisture regain

Results of the moisture absorbance test for the untreated and enzyme treated PA 6 are summarized in Table II. The moisture regain of untreated PA 6 fabric increased with 1% enzyme solution compared to untreated one, and it was intensified with the increase in enzyme concentrations to 2%, 4%, and 6%.

The moisture transport from the skin to the outer environment through clothing materials, often referred to breathability of the fabric, is an important factor in human comfort.[53–55] Enzyme hydrolyzes the polyamide chains in the paracrystalline and amorphous regions and, hence, renders the region more accessible for water penetration. This could be an advantage of enzyme treatment to improve the breathability of PA 6 fabrics.

Results for moisture regain measurements are in agreement to the results obtained from the dyebath exhaustions.

TABLE IV
Color Coordinates of Untreated and Enzyme Treated Acid and Disperse Dyed Nylon 6 Fabrics

Dyes used for dyeing (1%)	Enzyme (%)	*L**	*a**	*b**	*C**	*h*
Acid dye	0	41.13 (1.2)	−1.68 (0.01)	−27.34 (1.5)	27.39 (1.0)	266.47 (5.8)
	1	40.00 (1.3)	−1.87 (0.02)	−27.52 (1.9)	27.59 (1.4)	266.10 (6.5)
	2	38.64 (1.5)	−1.61 (0.02)	−27.52 (1.6)	27.62 (1.2)	266.65 (8.2)
	4	38.03 (1.5)	−1.58 (0.02)	−27.24 (1.2)	27.29 (1.1)	266.67 (1.3)
	6	37.50 (1.1)	−1.44 (0.02)	−27.35 (1.1)	27.39 (1.4)	266.97 (2.9)
Disperse dye	0	44.15 (1.6)	15.65 (0.3)	3.17 (0.1)	16.19 (0.5)	14.91 (1.5)
	1	43.95 (1.3)	15.06 (0.4)	3.07 (0.5)	16.37 (0.3)	11.53 (1.5)
	2	43.38 (1.3)	15.99 (0.4)	3.36 (0.4)	16.90 (0.5)	15.97 (1.5)
	4	41.25 (1.9)	15.58 (0.5)	3.31 (0.4)	16.92 (1.5)	12.00 (1.5)
	6	40.63 (1.5)	15.16 (0.7)	3.79 (0.8)	17.62 (0.1)	14.05 (1.5)

Standard deviations in parentheses.

Microscopic characterization

The SEM images of nontreated PA 6 fibers and samples treated with 1%, 2%, 4%, and 6% lipase solutions are shown in Figures 3 and 4. Figure 3(a) shows the surface of untreated PA 6 fiber. It can be seen that the untreated fiber has relatively smooth surface. After treating fibers with 1% lipase solution, the fibers did not show observable changes [Fig. 3(b)]. The high magnification SEM image of the fiber treated with 6% solution of enzyme shows etched surfaces, which indicates surface hydrolysis of the fiber in comparison with the untreated fiber, respectively [Fig. 4(a,b)]. Furthermore, hydrolysis of PA 6 surface can be highly responsible for improvement of wettability and dyeability.

Tensile strength test

Table III shows that the modulus, tenacity, and extension at break values for the sample treated with 1% enzyme were lower than untreated sample. A greater decrease in tenacity and extension at break can be seen by an increase in the enzyme concentration.

It seems that enzyme penetrates into the fiber resulting hydrolysis molecular chains. Increase in enzyme concentration caused more hydrolysis and structural damage, which was confirmed by other authors.[35,48,49]

Hydrolysis of the fiber was observed after tensile strength test, as well as the dye absorption to the fibers. It can be suggested that the physical structure of the substrate has a significant effect in both acid and disperse dyes absorption. The enzymes can attack preferentially the amorphous or less-ordered regions than the crystalline or more-ordered regions because of the enzymes capability of migration into these less-ordered regions compared to the more-ordered ones. Increase in disperse dyes absorption after enzyme treatment could be the result of enzyme hydrolysis occurred in amorphous regions of substrate which is in good agreement with the tensile strength results.[35,42,43,48]

TABLE V
Wash Fastness Properties of Untreated and Enzyme Treated Acid and Disperse Dyed Nylon 6 Fabrics

Dyes used for dyeing (1%)	Enzyme (%)	Wash fastness	Staining on wool	Staining on cotton
Acid dye	0	3	3-4	4
	1	4	4-5	4-5
	2	4-5	4-5	4-5
	4	4-5	4-5	4-5
	6	4-5	4-5	4-5
Disperse dye	0	3-4	3-4	4-5
	1	4-5	4-5	4-5
	2	4-5	4-5	4-5
	4	5	4-5	4-5
	6	5	4-5	4-5

TABLE VI
Light Fastness Properties of Untreated and Enzyme Treated Acid and Disperse Dyed Nylon 6 Fabrics

Dyes used for dyeing (1%)	Enzyme (%)	After 2 days	After 7 days
Acid dye	0	5-6	5-6
	1	5-6	5-6
	2	5-6	5-6
	4	5-6	5-6
	6	5-6	5-6
Disperse dye	0	4	4
	1	4-5	4-5
	2	5	5
	4	5	5
	6	5	5

Colorimetric measurements

The L^*, a^*, b^*, C^*, and h values of enzyme treated acid and disperse dyed PA 6 fabrics and untreated one are given in Table IV. The lightness (L^*) value decreased for the sample treated with 1% enzyme followed by more decrease as the enzyme concentration was increased. There was relatively small change in a^*, b^*, C^*, and h for enzyme treated nylon dyed with acid dyes. Decrease in L^* values could be due to more acid and disperse dyes penetrations into the enzyme treated samples, which are in favor of the previously discussed results of the exhaustion values. There were some increase in C^* value for enzyme treated disperse dyed PA 6 fabrics which were in contrast with those of acid dyed samples with relatively small changes. Increase in C^* values contributes to increase in brightness of the samples which is an important factor in textile products. This could be considered as an advantage of lipase treatment to improve the quality of PA 6 fabrics.

Color fastness

The results obtained from the wash and light fastness tests are given in Tables V and VI. Enzymatic pretreatment improved the wash fastness for the acid and disperse dyed samples, and decreased staining on PA 6 and cotton. Acid and disperse dyes penetrated more easily into the enzyme treated samples, which confirmed the results of exhaustion and colorimetric values.

After exposing the samples to daylight for 2 days, samples treated with enzymes and dyed with disperse dye indicated better light fastness than untreated one. Many factors influence the lightfastness of dyes, including the chemical state of the dye, the physical state of the dye within the fiber, the

fiber substrate, dye concentration in the fiber, dye environmental factors, the source and intensity of illumination, and the presence of UV absorbers and application of other finishing materials after dyeing.[56–65] The decrement of dye concentration is a function of the irradiation time. On the other hand, the light fastness increases with the increase in dye concentration in fiber. The dissipation of absorbed energy of light is easier when more dye is absorbed in the fiber, and consequently the photo-degradation process is slower. Enzyme treatment caused to increase the dye aggregation into the fiber and the shades to become darker. As a result, these aggregates are less vulnerable to fading action caused by sunlight.[66–68]

With an extension of the duration of exposure to the daylight for 7 days, no more fading was observed. The color fading of the samples was limited to a certain period of exposure to daylight.

CONCLUSIONS

PA 6 was treated with 1%, 2%, 4%, and 6% lipase solutions for 80 min. Results of the UV-Vis spectrophotometry analysis on the remaining dyebath solutions showed that the enzyme treatment increased the absorption of the acid and disperse dyes into the fiber. These results are similar to the results obtained from the moisture regain. The color measurement tests confirmed that the L^* values decreased with the treatment of enzyme, and also that any increase in the percentage of enzyme in the solution caused an increase in the amounts of L^*. The wash fastness was improved for enzyme treated acid and disperse dyed samples. In the case of light fastness properties, it was increased for samples treated with lipase and dyed with disperse dye. Enzymatic treatment catalyzed the hydrolysis of polyamide chains and amide functional groups. Tensile strength results confirmed that the amorphous and paracrystalline regions can be more accessible to the enzymatic hydrolysis allowing the dye and water molecules to penetrate more easily into the fiber.

At present, the industrial application of enzymes focuses on some major goals, the first being "biopolishing" to improve surface, making the material softer and the colors brighter. However, strength reduction of the enzymatic reaction limits its industrial application. The results presented in this article prove that lipase can be a promising alternative for nylon bio-finishing processes at an industrial level, since it is an effective way for improving dye absorption and can be an environmental friendly option to the conventional chemical treatments. This process needs to be further characterized for its complete understanding and optimization for textile industry applications.

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