IMPROVING SURFACE ENERGY AND HYDROPHILIZATION OF POLY(ETHYLENE TEREPHTHALATE) BY ENZYMATIC TREATMENTS

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Abstract: In order to increase the hydrophilicity and adhesion of poly (ethylene terephthalate) (PET) fabrics it was studied the action of three types of enzymes (Texazym PES sp5, *Aspergillus niger* and *Aspergillus oryzae*) applied at different incubation times and concentrations. This processes aims to modify morphologically and chemically the superficial structure of the polymeric materials (PET), forming new carboxyl, hydroxyl and other polar groups at the surface, in order to increase adhesion and hydrophilicity. The increase in the hydrophilicity of the fabric was evaluated by measuring the contact angle being the best results obtained for the Texazym PES (87.45º), much smaller than the non-treated fabric (122.95º); and by the wicking height, which revealed an important improvement in the hydrophilicity. The formation of carboxyl and hydroxyl groups was evaluated by a staining procedure with a cationic and reactive dye, respectively. It was also confirmed by the increasing in the polar component of the surface energy, determined by the Qwens-Wendt method. The higher surface energy and thus, the higher adhesion properties, were obtained for the esterase Texazym, using 0.12U during 90 minutes. The surface morphology of the non-enzymatic-treated and enzymatic-treated samples was analyzed by scanning electron microscopy (SEM) showing no degradation of fibers treated under the selected optimum conditions. In contrary, this method showed an important surface cleaning action by removing some undesirable polyester oligomers.

1 INTRODUCTION

Advances of biotechnology in textile industry have brought new products and processes for specialty applications as for instance in biomedical materials.

Polymers and textiles are usually used as films and foils for packaging, protective coating, material for biomedical and sealing applications because of their superior bulk properties, such as transparency, high resistance, strength, good thermal resistance, etc. But these excellent characteristics are often unsuitable for biomedical applications due to their low surface energies. Therefore, surface treatments are usually necessary to improve surface wetting and adhesion properties (Inagaki *et al,* 2001), (Yang and Gupta, 2004), (Guebitz and Cavaco-Paulo, 2008), (Huemann *et al,* 2006).

The synthetic fibers, in particular, polyester made from poly(ethylene terephthalate), (PET) have a reduced number of polar groups (hydroxyl and carboxyl groups) capable to establish hydrogen bonds with water, reflecting in its weak capacity to absorb water also related to its high degree of cristalinity. This property can be changed appealing to chemical methods as, for example, the alkaline treatment. This method can, however, damage not only to the fibers but can also be harmful to the environment. In this way, alternative processes, simultaneously ecological, efficient and safe, have been studied.

Earlier studies demonstrated that the application of lipases, cutinases and esterases to synthetic fibers, help increasing hydrophilicity through the hydrolysis of ester bonds, under moderate conditions (low concentration and low reaction time at room temperature), accompanied by a slight reduction of the resistance to rupture and weight loss (Vertommen et al, 2005), (Heumann *et al*, 2006).

Several studies of enzymatic treatments have been proposed in order to modify the surface properties of polymers such as adhesivity,

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hydrophobicity, oleophobicity, wettability/ hydrophilicity, pilling and static charges, by hydrolysing polymers without affecting the bulk properties, having the advantage of being eco-friendly compared with conventional chemical treatments (Guebitz and Cavaco-Paulo, 2008), (Heumann *et al*, 2006), (Vertommen *et al,* 2005).

Other authors (Heish and Cram, 1998) confirmed that the increasing of the hydrophilicity after modification of polyester with lipases was superior to the one achieved with conventional chemical treatments (alkaline treatment: 3N of NaOH during 2 hours).

Our new approach focus on the surface modification of PET fabrics by enzymatic treatments using three different enzymes (Texazym PES and *Aspergillus niger*, *Aspergillus oryzae*, respectively), in order to form new polar groups (carboxyl and hydroxyl) at the surface, capable to establish hydrogen bonds with water and capable to improve surface adhesion. The study was undertaken to analyze and compare the effect of enzymatic treatments applied in a textile material (100% PET fabric), by studying the morphological and chemical changes at the surface, the mechanical properties and surface energy, in order to establish whether or not the material can be functionalized and its surface adhesion properties can be improved.

2 EXPERIMENTAL

The enzymatic treatment aims to improve the hydrophilicity without harming the mechanical properties of the material. For that purpose, it was investigated a new approach by studying the effect of the three types of enzymes, esterases and lipases (Texazym PES sp5, *Aspergillus niger* and *Aspergillus oryzae*), varying the incubation time and the enzyme concentration.

The chemical modifications were investigated by measuring the contact angle and the wicking height. The indirect determination of the formed carboxyl and hydroxyl groups was measured by staining with a cationic dye (Methylene Blue) and a reactive dye (Reactive Black 5), respectively, and by measuring the surface energy by the Owens-Wendt method. The surface morphological changes were analyzed by scanning electron microscopy (SEM).

2.1 Materials

The substrate (textile material) used in this work was a 100% poly (ethylene terephthalate) fabric, Batavia Twill, with the characteristics indicated in Table 1. The substrate was pre-washed with 1 g/L Plurafac LF 400, at 50ºC during 60 minutes, with mild mechanical agitation (25 rpm). Subsequently, the substrate was rinsed and washed under running water, followed by a thermofixation at 170ºC during 15 minutes.

2.2 The Enzymes

The enzymes selected for this study, were an esterase (Texazym PES sp 5 from inoTEX Ltd.) and two lipases (*Aspergillus* niger and *Aspergillus oryzae* from Sigma). These enzymes were applied according to the literature reviewed under the conditions of pH and temperature indicated by the manufacturer. In Table 2 are presented the principal characteristics of the enzymes used.

It was studied the action of Texazym PES, *Aspergillus oryzae* and *Aspergillus niger*, applied at different concentrations (0.06, 0.09, 0.12, 0.15 and 0.18 U) and different incubation times (30, 60, 90 minutes and 24 hours) with a liquor ratio of 1:25. The enzymatic treatments with Texazym were performed at 30ºC, using 50 mM of sodium acetate buffer solution (pH 5.5). The enzymatic treatments with *Aspergillus niger* and *Aspergillus oryzae* were performed by incubating 2 g of polyester fabric at 45ºC and 40ºC, using 50 mM phosphate buffer (pH 7.0). Immediately before and after treatments all samples were placed in a standard atmosphere (20 ± 2º C, 65% HR) during 24±2 hours.

Table 1: Fabric characterization.

Characterization	Test Method	Value
Warp direction Linear Mass (Tex) Density (Yarns/cm) Diameter (Den)	 NP – 4105 NP – EN 1049-2 NP – 3160	 48.47 21.4 3.3
Weft direction Linear Mass (Tex) Density (Yarns/cm) Diameter (Den)	 NP – 4105 NP – EN 1049-2 NP – 3160	 36.67 32 3.3
Weaving construction	NP EN 1700	Batavia twill
Mass per area (g/m^2)	NP EN 1701	211.65
Composition	NP EN 1808. 2247 and 2248	100% Polyester

2.3 The Dyes

The dyes were selected to fulfill the objectives focalized on assessing the number of hydroxyl and carboxyl groups.

Therefore, a reactive dye Remazol Black B (C.I. Reactive black 5, from DyStar) and a cationic dye (Methylene blue, from Sigma-Aldrivh) were selected. The reason for this choice is essentially the ability to establish bonds with hydroxyl and carboxyl end groups in the polyester fabric, respectively.

Table 2: Characterization of the enzymes.

	Texazym PES sp 5	Aspergillus o.	Aspergillus n.
pH Optimal	5.5	7.0	7.0
Temp. (º)	30	40	45
Origin	*Thermobifida fusca*	*Aspergillus oryzae*	*Aspergillus niger*
Activity	0.6 U/ml	50 U/mg	4 U/g

2.4 Evaluation of the Effectiveness of the Enzymatic Treatments

2.4.1 Determination of the Contact Angle and Surface Energy Estimation

The Dataphysics is composed by a camera, a computer and a monitor which are used to measure the contact angle on the samples. Liquid drops were dispersed on each fabric sample using a micrometer pipette. The image of each drop was captured by the camera connected to a computer, and the captured images were viewed at the monitor. The standard testing methods were according to Tappi T 558 pm-95. The liquid used in the experiment was glycerol and a drop of 5 µl was deposited on the fabric surface and the contact angles were measured. The measurements were performed, after one week since enzymatic application, were repeated eight times and the average was calculated. Afterwards, surface energies were determined according to the Owens-Wendt approach (Owens and Wendt. 1969). This method takes into account the dispersive and polar components of the surface energy. Using different test liquids, in this case water and glycerol, it is possible to calculate the solid surface energy as the sum of the polar and dispersive contributions. Constant values for the test liquids used for contact angle measurements are as follows:

Water: $\gamma = 72.8$ mJ/m^2; $\gamma_s^d = 21.8$ mJ/m^2; $\gamma_s^p = 51.0$ mJ/m^2;

Glycerol: $\gamma = 64.0$ mJ/m^2; $\gamma_s^d = 34.0$ mJ/m^2; $\gamma_s^p = 30.0$ mJ/m^2.

2.4.2 Wicking Rate

The determination of the wicking rate by measuring the rising height was according to DIN 53924 vertical wicking tests and performed after one week since the enzymatic application. Samples of 3cm × 10 cm were prepared and were suspended in a standard atmosphere (20 ± 2º C. 65% HR) for 24±2 hours. The samples were then placed in a solution 0.05 % w.o.f. of dye (Methylene Blue), and immersed at a height of 1 cm. After 10 minutes the samples were removed and the rinsing height was measured up. It was used a solution of dye instead of water to facilitate the reading and the measurement of the rinsing height.

2.4.3 Determination of Carboxyl End Groups

The carboxyl end groups were determinate after dyeing the samples at 50º C with a cationic dye (Methylene Blue. 0.5 % (w.o.f.)) during 20 minutes. Subsequently, the samples were washed in hot and cold water and then dried in an oven at 40º C during 24 hours. The dyed samples were analyzed in a reflectance measuring apparatus (Spectraflash 300 Datacolor, LAV/Spec. Incl., d/8. D65/10º). This procedure aims to evaluate the increase or decrease in the intensity of color (by determining the value of K/S), in order to evaluate the formation of carboxyl groups. The relative color strength (K/S values) were established according to the Kubelka-Munk equation (1), where K and S stands for the absorption and scattering coefficients and R stands for the reflectance value, respectively (Shah and Gandhi, 1990; Pandiyaraj and Selvaranjan, 2008):

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

An increase in the value of K/S, when compared to the non-treated sample, indicates an increase of carboxyl groups known to react with this type of dye.

2.4.4 Determination of Hydroxyl End Groups

The hydroxyl end groups in the non-treated and treated samples were determinate by a dyeing procedure performed at 60º C with a reactive dye (Remazol Black B) during 90 minutes. The dye bath contains 2% w.o.f. of dye, 20 mg.ml^{-1} of Na_2CO_3

and 60 mg.ml^{-1} of Na_2SO_4, pH 11, with a liquor ratio 1:100 and a mechanical agitation of 40 rpm. Subsequently, the samples were washed in hot and cold water and then dried in an oven at 40 ºC during 24 hours. After dyeing, the samples were analyzed in the reflectance measuring apparatus above described to evaluate the increase or decrease in the intensity of color, in order to evaluate the formation of hydroxyl groups. An increase in the K/S values indicates an increase in the formation of hydroxyl groups which react covalently with this type of dye.

2.5 Quality Control Test

2.5.1 SEM Analysis

The surface morphology of the treated polyester fabric was observed using a scanning electron microscope. SEM analysis was performed in all samples after one week since the application of the enzymatic treatments, using a HITACHI S2700 Electron Microscope and an EMITECH-K550 gold evaporator.

2.5.2 Determination of the Resistance to Abrasion

The Martindale evaluation system was used for measuring the resistance to abrasion (mechanical properties) of the non-treated and treated samples. The standard testing method was according to IWS TM 112. In this method, the samples are tested under a weight of 9 KPa and run until the rupture of two yarns.

3 RESULTS AND DISCUSSION

3.1 Evaluation of the Effectiveness of the Enzymatic Treatments

The results for the effect of the enzymatic treatments, over the contact angle and the hydrophilicity are indicated in Figures 1 and 2. The results for the contact angle (Figure 1) showed a slight decrease for all enzyme concentration, but the most significant decrease was observed with Texazym PES, when compared to the non-enzymatic treated sample (Control). For the others enzymes (*Aspergillus niger* and *Aspergillus oryzae*) it can be also observed a slight decrease. However, for higher concentrations of enzyme and incubation times a significant decrease of contact angle in relation to the control can be observed, being in accordance with the results obtained by other authors (Hsieh e Cram. 1998).

After a comparative analysis, we can conclude that either the use of *Aspergillus oryzae* or an *Aspergillus niger* made possible to achieve a lower contact angle, hence greater hydrophilicity for polyester fabric. However the Texazym PES led to the better results. Definitely, by applying the Texazym PES with a concentration of 0.12 U during 90 minutes it can be achieved a contact angle of 87.45º; by applying the *Aspergillus niger* with a concentration of 0.15 U during 60 minutes a contact angle of 101.50º, is obtained.

The use of *Aspergillus oryzae*, (0.12 U during 60 min) also allows to achieve lower values for the contact angle (109.03º). The more favourable conditions to decrease the contact angle seens to be using the Texazym PES with a concentration of 0.12 U during 90 minutes. For lower incubation times (30 to 60 minutes) there are no important changes in the contact angle values.

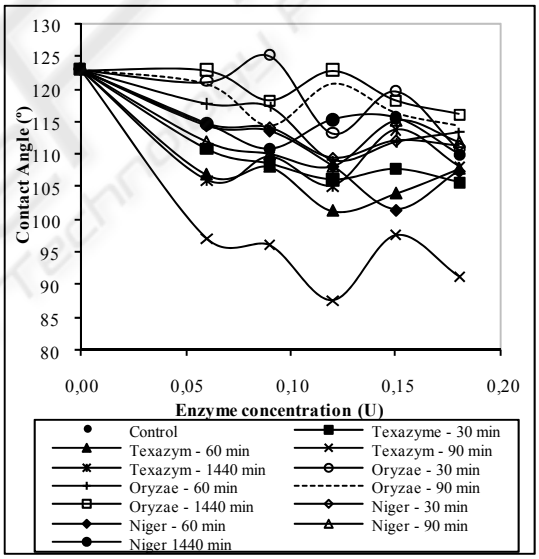

Figure 1: Values of contact angle for all enzymes.

The results of hydrophilicity obtained by the effect of capillary are illustrated in Figure 2. Their analysis indicates that the best results are achieved using the Texazym PES, followed by the use of the *Aspergillus niger*. In those circumstances it's obtained a wicking height of 5.40 cm and 4.60 cm, respectively. Other studies revealed that the application of other enzymes promoted similar results, however with a lower wicking height (≈ 4 cm) (Alisch-Mark et al, 2006). In agreement with the results of the contact angle and hydrophilicity, the use of *Aspergillus oryzae* leads to lower values

in the capacity of absorption of water by capillarity effect (Figure 2).

The results obtained by the analysis of the carboxyl end groups (Figure 3), formed by the application of Tezaxym PES for any concentration and incubation time, showed a significant increase detected by the reaction with the cationic dye, when compared to the other enzymes and the control.

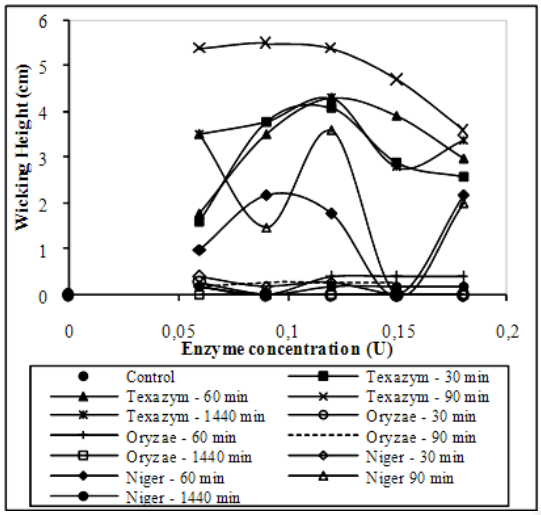

Figure 2: Values of hydrophilicity (expressed by the wicking height).

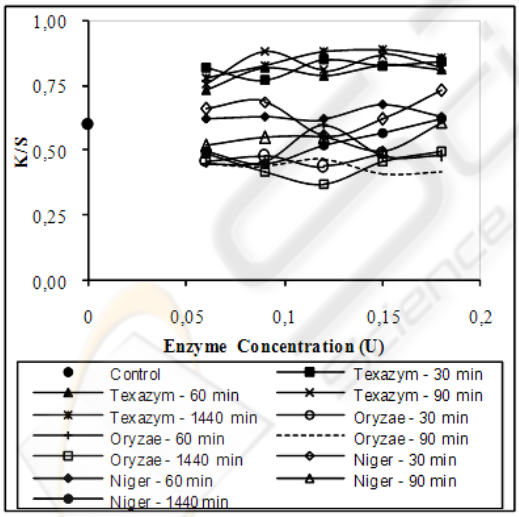

Figure 3: Values of K/S (620 nm), after dyeing with cationic dye.

The more favourable conditions to the grafting of these groups are by applying a concentration of 0.12 to 0.15 U for any incubation time. These results are in accordance with the results obtained before (contact angle and hydrophilicity), showing that the presence of carboxyl groups favours the establishment of hydrogen bonds with water. An application with *Aspergillus oryzae* seems to be less desirable with regards with the formation of these functional groups, reflecting up in the lower capacity of the treated fabrics to absorb water.

The formation or not of the hydroxyl groups, using different enzymes, can be indirectly measured by the higher or lower intensity of color that results from the reaction of a reactive dye with the hydroxyl groups (-OH) by the formation of covalent bonds. After analyzing the results presented in Figure 4, it can be concluded that a higher formation of the hydroxyl groups is achieved by using the Texazym PES, whichever the incubation time, comparing with the others enzymes and with the control. This feature is extremely important with respect to future applications of this enzyme in the textile industry, where the incubation time is a cruel factor in textile treatments.

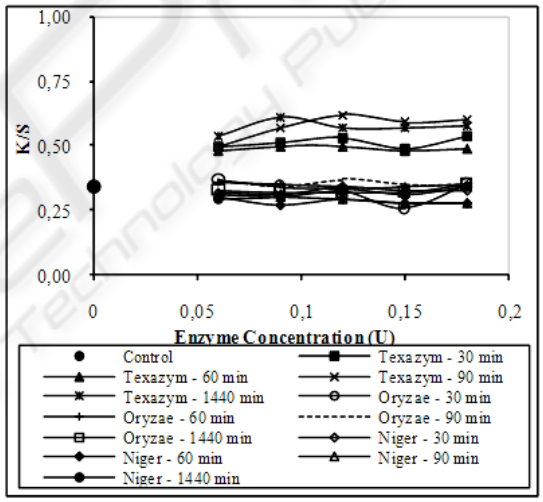

Figure 4: Values of K/S (600 nm), after dyeing with reactive dye.

To better elucidate which were the best conditions for each enzyme, considering the best results of the contact angle and wicking height, the selected optimum conditions for each enzyme are presented in Table 3. The best results regarding the lower contact angle and higher wicking height are obtained for the Texazym PES us1ing a concentration of 0.12 U during 90 minutes, followed by the *Aspergillus* niger using 0.15 U, 60 minutes and finally, by the *Aspergillus oryzae* using 0.12U during 60 minutes. In those conditions, an application with Texazym PES yielded a rinsing height of 5.40 cm, which is a very good value when compared with the ones obtained by other authors (Alisch-Mark *et al*, 2006). In this study they

achieved a rinsing height of 4.2 cm (approximately) after 48 hours of incubation with *T.fusca* (16 U). Our results revealed an important achievement since much lower incubation time and enzyme concentration was used.

However, the other results regarding the quality control parameters are important to better define the optimal conditions overall.

Table 3: Optimal conditions for different operating parameters.

	Contact Angle (glycerol) (º)	Rising Height (cm)	Cationic Dye K/S	Reactive Dye K/S
Control	122.95	2.7	0.60	0.34
Texazym PES (0.12 U, 90min)	87.45	5.40	0.81	0.62
Asperg. niger (0.15 U, 60 min)	101.50	4.60	0.50	0.28
Asperg. oryzae (0.12U, 60 min)	109.03	0.40	0.60	0.34

The total surface energy, the dispersion component and polar component of the fabrics were calculated according to the Owens-Wendt approach (γ_s^p. γ_s^d. γ_s are the polar component, the dispersion component and the total surface energy, of fabric, respectively) and are indicated in Tables 4 to 6.

It is clear that the total surface energy increases with incubation time and enzyme concentration. The values of surface energy obtained for the control was 0.60 mJ/m^2 for the polar component, 3.28 mJ/m^2 for the dispersion component and 3.88 mJ/m^2 for the total surface energy. Analyzing the results, an important increase of the polar component for the Texazym PES is verified when compared with the other enzymes. The higher value (187.31 mJ/m^2) was obtained to the incubation time of 1440 minutes with a concentration of 0.15U. However, good values for the polar component can be obtained for lower incubation times (183.85 mJ/m^2, 153.52 mJ/m^2), when a concentration of enzyme between 0.12U to 0.18 U is used during an incubation time of 90 minutes. Considering the other enzymes, much lower values for the polar component are obtained: 30.36 mJ/m^2 (using 0.18 U during 60 min for the *Aspergillus niger*), and 11.47 mJ/m^2 and 10.04 mJ/m^2 using 0.12 U during 30 minutes and 0.15 U during 60 minutes, respectively, for the *Aspergillus oryzae*.

Table 4: Surface energy determination for different operating parameters of Texazym PES.

Time (min)	Concentration of enzyme (U)	γ_s^p (mJ/m^2)	γ_s^d (mJ/m^2)	γ_s (mJ/m^2)
30	0.06	22.49	1.9	29.4
	0.09	67.88	15.9	83.78
	0.12	99.57	29.15	128.72
	0.15	97.6	29.76	127.36
	0.18	82.13	20.29	102.41
60	0.06	31.02	1.78	32.8
	0.09	81.07	21.81	102.88
	0.12	98.54	24.12	122.66
	0.15	110.76	32.74	143.5
	0.18	102.32	32.31	134.62
90	0.06	61.00	6.32	67.32
	0.09	122.07	29.78	151.85
	0.12	183.85	63.43	247.38
	0.15	153.52	39.21	192.73
	0.18	141.68	29.69	171.36
1440	0.06	45.98	5.70	51.68
	0.09	117.12	42.4	159.53
	0.12	147.85	54.21	202.06
	0.15	187.31	91.66	278.97
	0.18	145.94	57.2	203.14

Table 5: Surface energy determination for different operating parameters of *Aspergillus niger*.

Time (min)	Concentration of enzyme (U)	γ_s^p (mJ/m^2)	γ_s^d (mJ/m^2)	γ_s (mJ/m^2)
30	0.06	2.67	2.76	5.43
	0.09	3.9	1.94	5.84
	0.12	0.1	11.34	11.43
	0.15	0.00	11.55	11.55
	0.18	14.95	0.02	14.97
60	0.06	0.52	6.49	7.01
	0.09	0.88	5.84	6.71
	0.12	0.04	15.78	15.82
	0.15	0.45	25.27	25.72
	0.18	30.36	1.77	32.13
90	0.06	2.44	2.94	5.37
	0.09	3.11	3.82	6.93
	0.12	0.28	10.81	11.09
	0.15	1.79	3.62	5.41
	0.18	5.82	1.39	7.21
1440	0.06	5.31	1.01	6.38
	0.09	9.47	0.42	9.89
	0.12	21.49	1.47	22.97
	0.15	3.03	2.19	5.22
	0.18	0.42	8.99	9.42

It is mainly due to the incorporation of polar groups like carboxyl and hydroxyl on the fabric

surface, verified by other authors (Pandiyaraj and Selvarajan, 2008) and in our present study.

Table 6: Surface energy determination for different operating parameters of *Aspergillus oryzae.*

Time (min)	Concentration of enzyme (U)	γ_s^p (mJ/m^2)	γ_s^d (mJ/m^2)	γ_s (mJ/m^2)
30	0.06	18.48	2.11	20.59
	0.09	4.85	0.07	4.92
	0.12	3.24	2.66	5.09
	0.15	11.47	0.17	11.64
	0.18	0.92	6.98	7.89
60	0.06	2.66	1.97	4.63
	0.09	6.74	0.29	7.03
	0.12	0.05	12.22	12.27
	0.15	10.04	0.22	10.26
	0.18	1.91	4.07	5.98
90	0.06	6.68	0.06	6.74
	0.09	0.00	10.31	10.31
	0.12	8.77	0.01	8.78
	0.15	5.49	0.72	6.21
	0.18	0.95	5.31	6.26
1440	0.06	1.18	2.21	3.39
	0.09	3.16	1.48	4.64
	0.12	5.69	0.07	5.76
	0.15	2.05	2.39	4.43
	0.18	1.54	3.61	5.15

The change in the polar component verified for all enzymatic treatments, when applying different enzyme concentrations and incubation times, affects the total surface energy as a function of enzymatic operating parameters. These results show that the main contribution to the increase in surface energy is due to the polar components which can incorporate with moisture through hydrogen bonds. Thus, good wettability is obtained when the values of polar component is high (Pandiyaraj and Selvarajan, 2008), being the most favorable conditions the one's for the enzymatic treatment with Texazym PES.

3.2 Quality Control Tests

With the test of resistance to abrasion was intended to assess whether the mechanical properties of polyester fabrics would have suffered any significant change after the treatment with enzymes (Texazym PES, *Aspergillus niger* and *Aspergillus oryzae*). These results were referenced in Table 7.
After their evaluation it can be seen that there weren´t verify important losses in the resistances to abrasion using any enzymatic treatment when compared with the control. The loss of around 4% in the resistance is perfectively safe for the textile.

Table 7: Values of the resistance to the abrasion in the optimal conditions for each enzymatic treatment.

	Resistance to abrasion (cycles)
Control	24000
Texazym PES (0.12 U. 90min)	23000
Aspergillus niger (0.15 U. 60 min)	23000
Aspergillus oryzae (0.12U. 60 min)	23000

The SEM analysis aims to observe any surface changes on fibers after the enzymatic treatment. The images presented are referred to the control and to the optimum conditions of application of the Texazym PES. The others enzymes didn't revealed any changes when compared to the control. Looking at images of Figures 5 and 6 it can be seen that there is no degradation of the surface of the fibers under study. In contrary, the use of the Texazym PES applied under the optimum conditions seems to be the cause of a greater effect of cleaning since it can be seen the presence of a smaller number of particles deposited on the fibers (oligomers). This effect of total or partial elimination of them is of utmost importance, especially in what concerns to adhesion and dyeing properties.

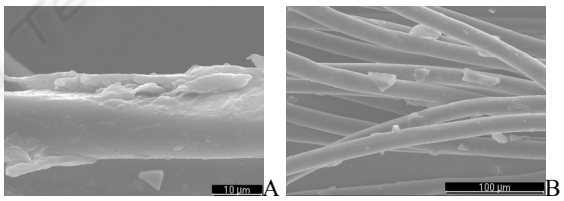

Figure 5: SEM micrographs of Control samples (A-magnification: 2500x, B-magnification: 500x).

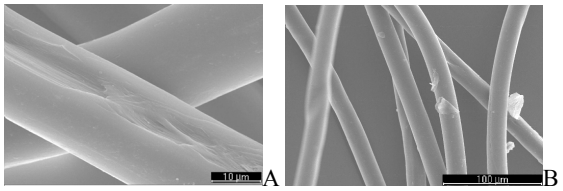

Figure 6: SEM micrographs (Texazym PES, using 0.12 U during 90 min).

4 CONCLUSIONS

The effect of the different enzymatic treatments, incubation time and enzyme concentration on the

hydrophilization of PET fabrics and surface energy increasing was analyzed. Depending on the enzymatic process used, the wettability of PET fabrics can be significantly improved. It was found the formation of carboxyl and hydroxyl polar groups by the Texazym PES action. The enhancement of polar groups on the fabric surface was confirmed with a cationic dye (Methylene blue) and a reactive dye (Remazol black B). Thus, improvement in adhesion properties can be expected.

Our results revealed an important achievement since much lower incubation time and enzyme concentration was used, comparing to previous investigations.

The increasing in the hydrophilicity and surface energy of PET fabrics are also known to have importance in the increasing in adhesion of cells and tissues, which is a very important property for permanent biomedical implants.

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