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The Impact of Egyptian Thermophylic Cellulase on The Dyeability of Natural and Recovered Cellulosic Fabrics

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BIOREMEDIATION using enzymes is an important tool in textile industry; therefore the goal of the present study is to subrogate the pretreatment of natural (cotton) and recovered (viscose) cellulosic fabrics with microbial acidothermophilic cellulase produced by the native Egyptian fungus Aspergillus terreus RS2. The produced enzyme was examined at two activity levels (10 and 20U/mL) in order to reduce the effluent load that was produced from scouring and bleaching processes and to upgrade a naturally adequate transaction for water and power economy. The effectiveness of the enzymatic pretreatment under the optimum conditions on raw, scoured and bleached cellulosic fabrics has been proved as the results indicated an increase in the color intensity of the treated fabrics in compare to the untreated one for different classes of reactive dyes based on Anthraquinones and Double azo. The fastness properties of the pretreated dyed fabrics were implemented. The contact angle for the pretreated cellulosic fabrics, tensile strength, Scanning electron microscopy and FTIR analyses were performed.

Keywords: Cellulosic fabrics, Thermophilic cellulase, Reactive dye, One bath.

Introduction

Cellulosic fibers are considered as one of the most used fibrous raw materials in the textile industry. Cotton and regenerated (viscose) cellulosic fibers are put through various wet processing treatments to improve its dyeability. Different processes have been evolved in the last decades in which the fiber properties and quality are mainly influenced by the used process [1]. In the traditional pretreatment processes, the raw fabrics have to undergo a series of chemical treatments before it turns into a final fabric. The chemicals used for all these processes are absolutely toxic and environmentally hazardous [2]. Consequently, the industrial application of enzymatic pretreatment has been grown up rapidly [3]. Pretreatment of cellulose before coloring can offer a facial and effective technique for enhancing color fiber affinity [4]. Utilization of enzymes is considered as an example of white industrial biotechnology [2], achieving cost reduction, biodegradability, saving the consumption of energy, water and raw-materials, improving the product quality and potential process integration [3].

Latterly, cellulases that are the key enzymes in the hydrolysis of cellulose have become the third class of enzymes used in textile and laundry industry [4]. Cellulases are ubiquitous in nature as they are produced by the aid of wood degrading fungi and bacteria as a part of the energy transfer and the carbon cycle and commercially, filamentous fungi of Trichoderma and Aspergillus species are the main source for their production [5]. The main application of cellulases in textile

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industry is the removal of surface fiber fibrils from cellulosic fabrics to avoid pilling [6], improve fabric appearance, the ageing of indigo-dyed denim garments [1,7] and brighter luminosity of colors that's known as bio-polishing [1,3,8]. Although several studies have been previously examined the application of cellulases in cotton bio-polishing, the yielded results are contradictory [9]. The aim of the present study is to examine the effect of the Egyptian enzymatic treatment of 100% cotton and viscose fabrics on various physical properties, improve its dyeability with reactive dyes and determine the effectiveness of pretreatment of dyed fabrics on fastness properties.

Materials and Methods

Cellulosic fabrics preparation

Raw100% cotton and viscose woven fabrics were procured from Modern Company for Textile Industries, Shubra El Khema , Cairo, Egypt. The fabrics were scoured with (2g/L) nonionic detergent solution (Hocstapal CV from Clariant, Egypt) with a liquor ratio of 1:25 at 60°C for 45 min then rinsed twice in cold tap water and dried at room temperature. Scoured cotton fabric was then subjected to conventional H_2O_2 bleaching in a lab-jigger using 2% (o.w.f) H_2O_2, 6% (o.w.f) sodium meta silicate, 0.7% (o.w.f) NaOH and 0.05% (o.w.f) non-ionic detergent at 85°C for 2h at pH 10.5-11.0. After bleaching, the treated fabric was thoroughly washed under running water followed by neutralization with 1% acetic acid solution for 15 min before further washing with hot and normal running water. Finally, the washed fabric sample was dried in air [10].

Chemicals and dyes

Potato dextrose agar (PDA) medium was purchased from Merck, Darmstadt, Germany. Carboxymethyl cellulose (CMC) was purchased from Sigma-Aldrich, Saint Louis, USA. Dinitrosalicylic acid (DNS) was purchased from Panreac, Barcelona, Spain. The applied dyes are a commercial sample; C.I. Reactive Blue 19 (molecular structure: anthraquinones) and C.I. Reactive Red 120) (molecular structure: Double azo class) Supplied from DyStar Coloures , Frankfurt-Germany. The structure was as shown in figure 1. All other chemicals and reagents used were laboratory grad.

Enzyme production

Preparation of rice straw

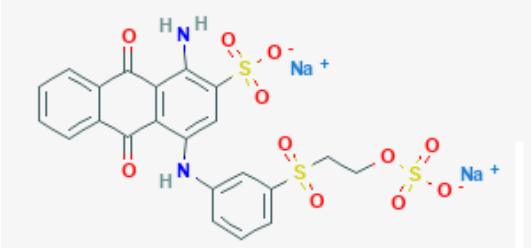
Rice straw samples were collected after air drying from fields situated in Al Sharqiya governorate, Egypt. The samples were cut into pieces, ground using standard grinder and used directly without further pretreatment.

Microorganism and culture conditions

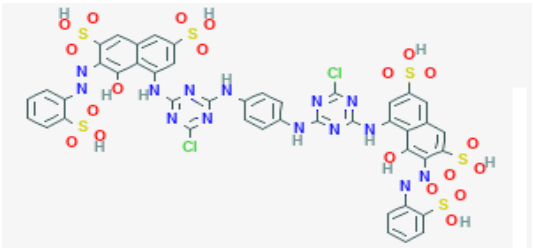
Solid state fermentation (SSF) technique for rice straw using *Aspergillus terreus* RS2 (Accession no. MN368221) was performed for the production of the enzyme. The fungus was initially cultivated on potato dextrose agar slants and incubated at 30°C for 7days. After cultivation, spore suspension was prepared by scratching of each slant with 10 mL distilled water containing 0.1% tween 80 and the fermentation process was performed [11]. Briefly, in 250mL Erlenmeyer flask 2mL of the spore suspension were used to cultivate 3.75g of rice straw (with initial moisture content of zero percentage) moistened in the ratio 1:3 (biomass to moistening agent ratio) with modified moistening agent composed of (g/L) $(NH_4)_2SO_4$; 10, KH_2PO_4; 2, $CaCl_2$; 0.3, $MgSO_4.7H_2O$; 0.3 and adjusted to pH 7, then incubated at 30°C for 8days. At the end of the fermentation process, the enzyme was extracted by adding 50mL of distilled water to each flask, shaking at 150 rpm and 30°C for 1hr then centrifuged at 5000rpm for 10min. The culture free supernatant was air dried then subjected to further analysis [12].

Enzyme activity

The enzyme activity was determined by DNS method [13] in a reaction mixture consisted of 500µL of 1% carboxymethyl cellulose (dissolved in 0.05M acetate buffer, pH 5) and 500µL of the suitable dilution of the enzyme solution. After



C.I. Reactive Blue 19



C.I. Reactive red 120

Fig. 1: Molecular structure of dyes

incubation at 50°C for 30min, 2.5mL of DNS was added to stop the reaction and the color developed after boiling for 10min was measured at 540nm. One unit of the enzyme was defined as the amount of enzyme that released 1µmol of glucose per minute under the assay conditions.

Effect of pH and temperature on the enzyme activity and stability

To evaluate the effect of pH on the produced enzyme, the enzymatic activity was determined at different pH range from 3.5 to 5 using 0.05M acetate buffer and from 5.5 to 7 using 0.05M phosphate buffer. The stability of the enzyme at the optimum pH was determined by estimating the residual activity of the enzyme after different pre-incubation periods ranged from 1 to 6h. The activity of the enzyme without pre-incubation was considered as 100% activity.

The effect of the temperature on the enzyme activity was estimated at various temperatures (40 to 70°C) at the optimum pH. Thermal stability was studied by determining the residual activity of the enzyme at the optimum conditions every hour up to 6h after its pre-incubation at temperatures range from 50 to 65°C. The activity of the enzyme without pre-incubation was considered as 100% activity.

Enzymatic treatment of cellulosic fabrics

Treatment condition

Raw 100% cotton, scoured and bleached fabrics were treated with aqueous solutions of the produced cellulase at two activity levels (10 and 20U/ml) in stopper glass bottles using a liquor ratio of 1:25 at 65˚C for 60min. The aqueous solutions bath was adjusted to the optimum activity pH of the produced enzyme using acetate buffer. The temperature of the treatment bath was raised to 90ºC for 15 min for the deactivation of the added enzyme. Finally, the enzyme treated fabric samples were thoroughly washed with normal running water and dried in air. The pretreated and untreated cellulosic fabrics were dyed separately with the previously mentioned reactive dyes by exhaustion technique using an Infra colour Dyeing Machine (Mumbai, Maharashtra, India).

Dyeing procedure

The fabrics were immersed in dyeing bath containing the reactive dye (1%) with Glauber's salt (30g/L) as an electrolyte that assist the exhaustion of dye. The dyeing baths were heated steadily (2°C/min) from room temperature until the dyeing temperature was achieved at 60°C, after 30min Na_2CO_3 (15 g/L) was added as a fixing agent. The dyeing process was carried out at 60ºC for 60 min in a fabrics-to-liquor ratio of 1:40. As the dyeing was completed, a good wash must be applied to remove the extra and the unfixed dyes in a soaping bath containing 3g/l non-ionic detergent (Triton X-100) at 60°C for 30min. Finally, the dyed fabrics were dried at room temperature.

Dyeing and treatment in one bath

Raw 100% cotton and viscose fabrics were treated with the enzyme (10U/mL) then dyed with 1% C.I. Reactive red in the same bath. The enzyme treatments were carried out using a liquor ratio of 1:25 at 65˚C for 60 min, the reactive dye (1%) was added by exhaustion technique using an Infra colour Dyeing Machine (Mumbai, Maharashtra, India).

Analysis

Color strength (K/S)

Color strength (K/S Value) was measured on a Minolta Spectrophotometer Hunter Lab Universal Software Ultra scans (USA). The values were calculated using the following "KUBELKA-MUNK" equation:

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

where K is the absorption co-efficient, R is the reflectance of the dyed sample and S is the scattering co-efficient at the wavelength of maximum absorption.

Fastness Testing

The color fastness of the dyed fabrics to wash before and after treatment as well as the fastness fabrics to perspiration was determined in accordance to the AATCC test method 69, 23(1993)

Mechanical Properties

The tensile testing (tenacity and elongation %) of the fabrics before and after treatment was evaluated using an Instron Tensile Tester (USA) according to ASTM D 76 Standard Specification for Textile Testing Machines.

Infrared spectroscopy

Infrared spectra were recorded on FTIR Nicolet 5 DX Spectrophotometer. The samples were examined as 1.5% KBr pellets.

The contact angle

The contact angle was measured using the OCA 15EC Contact angle model produced by the company of Data Physics Instrument Gmbh (The

Data Physics Instruments GmbH headquarters in Filderstadt, Germany).

Scanning electron microscopy

The surface morphology of the treated fibers was examined using scanning electron microscopy (SEM; Model JSM-5600LV, Jeol, Tokyo, Japan).

Result and Discussion

Enzyme activity

Under the optimum fermentation conditions previously described by Ismail and Hassan, [11], the fungus *Aspergillus terreus* RS2 produced high cellulase activity (9.5U/mL in fresh culture filtrate and 1000U/g of dried form) using rice straw as a sole carbon source. Generally, the production of cellulases is very expressive owing to their various biotechnological applications. However their high cost, low titer of their production and low thermal stability of the produced enzymes remain the most significant barriers to their industrial applications [14-16]. Therefore, microbial production of cellulase on local agriculture wastes represents a promising future for industrial applications with high environmental impact.

Effect of pH and temperature on the enzyme activity and stability

The produced enzyme possessed optimum activity at pH 4.5 using 0.05M acetate buffer at which the enzyme retained its complete activity for more than 6h. By increasing the pH, the enzyme activity decreased and at pH 7 the enzyme possessed about 40% of its initial activity (Figure 2). This result is consistent with other researches concerned with the production of cellulases by other *Aspergillus* species, expressing the optimum activity in acidic condition [17-19].

Measurements of the enzyme activity as a function of the temperature of the reaction indicated that the enzyme was optimally active in temperature range from 55 to 65°C. By increasing the temperature to 70°C, the enzyme lost about 40% of its initial activity (Figure3). The examination of the thermal stability of the enzyme at temperatures range from 50 to 65°C indicated that the enzyme retained about 90% of its activity at 60°C up to 6h and by increasing the temperature to 65°C, it retained more than 75% of its activity after 6h (Figure 4). Optimum temperature for *Aspergillus terreus* cellulase has been indicated [17] to be 50°C at which the enzyme retained 99% of its maximum activity after pre-incubation for 150min. Also Sharma *et al*., [20] reported that the produced enzyme was optimally active at 50°C and the enzyme retained 90% of its activity after pre-incubation for 300min at 60°C. Moreover, it was reported that 70°C as the optimum temperature for the produced enzyme without the indication of its thermal stability [18]. The high thermal stability of the produced enzyme under acidic conditions indicated in the current study suggests its possible exploration in various biotechnological applications.

Enzymatic treatment of cellulosic fabrics

Initially, the effect of different enzyme activity (10 and 20U/mL) on the treatment of 100% cotton fabrics was examined (Table 1). The results of the color intensity indicated noticeable enhancement in the treated fabric compare with the untreated one without the detection of a palpable difference between the enzyme added units. Moreover the dyed treated raw fabrics gave approximately the same result of the dyed treated bleached fabrics. So the enzymatic treatment using the produced cellulase can subrogate the chemical treatments

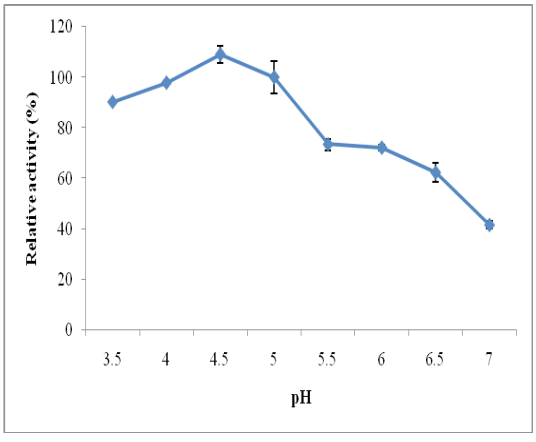


Fig. 2.The effect of pH (pH 5 is the control)

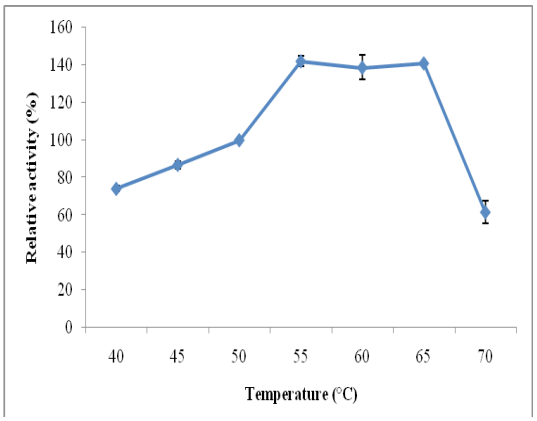


Fig. 3. The temperature effect (50°C is the control) on the activity of the produced enzyme.

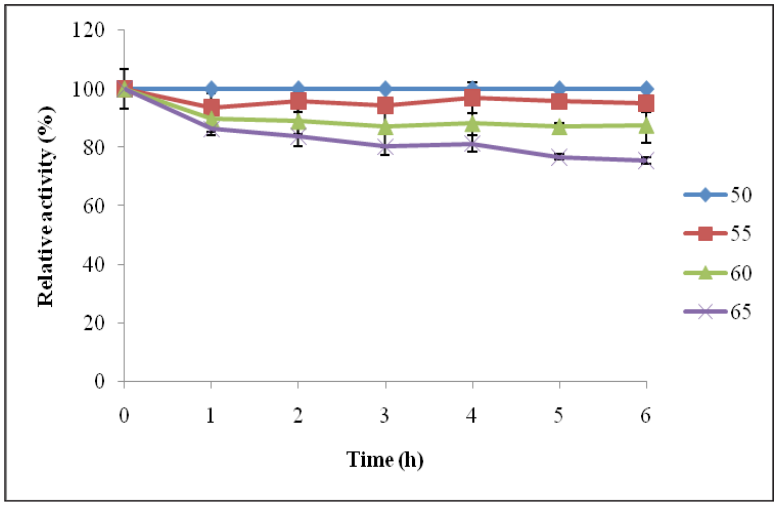


Fig. 4. The relation between the relative activity and thermal stability of the enzyme .

TABLE 1: Effect of treatment with different enzyme activity on color intensity of 100% cotton fabrics

Samples	K/S	
	Red120	Blue19
Raw	1.60	1.44
Raw &Treated with enzyme *	2.66	2.18
Raw &Treated with enzyme **	2.91	2.20
Scoured	1.60	1.60
Scoured &Treated with enzyme *	2.63	2.05
Scoured& Treated with enzyme **	2.63	2.08
Bleached	3.01	2.19
Bleached& Treated with enzyme *	3.63	3.11
Bleached& Treated with enzyme **	3.67	3.15

Enzyme activity * =10U/mL **= 20U/mL

of the scouring and the bleaching steps. This result may be attributed to the elimination of the superficial microfibrils of the cotton fibers under the controlled hydrolysis processes using cellulases, leaving the surface of the fibers free and do not hamper the water permeability in compare to the chemical hydrolysis [3].

The effect of the produced cellulase (10U/mL) on viscose fabrics was also studied (Table 2). The obtained results indicated an increase in the color intensity of the treated fabrics in compare to the untreated one. It is evident from Table 2 that the increase in color shade (K/S) was obtained for both of the treated fabrics, manifesting the effect of the produced enzyme on cotton fabrics, with higher extent for 100% cotton than that of viscose fabrics. As for dyes, the results for the treated fabrics which dyed with C.I. Reactive red 120 were better than C.I. Reactive Blue 19 that may be attributed to their molecular structure.

Dyeing and treatment in one bath

Raw 100% cotton and viscose fabrics were treated with the produced cellulase (10U/mL) using a liquor ratio of 1:25 at 65˚C for 60 min by exhaustion technique using an Infra colour Dyeing Machine (Mumbai, Maharashtra, India). Then dyeing process was carried out in the same bath using C.I. Reactive red 120 in 1:50 liquor ratio. The results shown in Table 3 indicated an obvious enhancement in the color strength for both of the treated fabrics in compare to the untreated one.

Fastness properties

The fastness properties of both untreated and treated cotton and viscose fabrics, dyed with Reactive Blue19 and Red120 were illustrated in Table (2, 3). No huge changes were observed for fastness properties of washing, perspiration and

TABLE 2: Color intensity and fastness properties of dyed treatment cotton and viscose fabrics with reactive dyes

Samples	Dyes	K/S	Fastness properties									Light
			Washing			Perspiration						
						Alkali			Acid			
			W	C	Alt.	W	C	Alt.	W	C	Alt.	
Raw cotton		1.60	3	2-3	3-4	4	3-4	4	4	3-4	4	3-4
Raw cotton &Treated with enzyme *	C.I. Reactive red 120	3.46	3	3	3-4	4	3-4	4	4	4	4	4
Raw viscose		2.84	3	2-3	3-4	4	3	4	4	3-4	4	3
Raw viscose &Treated with enzyme *		3.46	3	2-3	3-4	4	3-4	4	4-5	3-4	4	3
Raw cotton	C.I. Reactive Blue 19	1.44	4	4-5	4	4	4	4	4	3-4	4	4-5
Raw cotton &Treated with enzyme *		2.52	4	4-5	4	4	4	4	4	3-4	4	5-6
Raw viscose		1.75	4	4-5	4	4	4	4	4	4	4	5
Raw viscose &Treated with enzyme *		2.52	4	4-5	4	4	4	4	4	4	4	4-5

Where: Alt. = alteration, W = staining on wool, C = staining on cotton

TABLE 3: Color intensity and fastness properties of dyed treatment cotton and viscose fabrics with reactive dyes in one bath

Samples	K/S	Fastness properties									Light
		Washing			Perspiration						
					Alkali			Acid			
		W	C	Alt.	W	C	Alt.	W	C	Alt.	
Dyed raw cotton	4.26	1-2	2	3-4	4	2-3	4	4	2-3	4	3-4
Dyed treated raw cotton	5.6	1-2	1-2	3-4	4	3	4	4	2	4	3-4
Dyed raw viscose	4.08	2	1-2	3-4	3-4	2-3	4	4	2	4	3
Dyed treated raw viscose	5.18	2	2	3-4	3-4	2-3	4	4	2-3	4	3

Treatment: produced cellulase with 10U/mL **Dyeing:** C.I. Reactive red 120

light of the treated samples. Also the results of the fastness properties of the one bath treated fabrics (Table 3) were ranged from good to very good without the detection of much difference between the untreated and treated samples.

Tensile strength, elongation, roughness and yellowness index

The results shown in table 4 indicated that the tensile strength for the enzymatic treated cotton fabrics was decreased to 8.879kg f/mm^2 compared with 14.184kg f/mm^2 for the untreated samples. Moreover, the enzymatic treatment of viscose decreased the tensile strength from 5.088kg f/mm^2 for the untreated fabrics to 3.88kg f/mm^2. The elongation percentage of the treated fabrics was increased in compare to the untreated one. However, an observed improvement was indicated in roughness and yellowness index of the enzymatic treated fabrics in compared to the untreated one.

Infrared spectroscopy

Infrared spectroscopic analysis for both of untreated cotton and viscose fabrics were compared to treated fabrics with 10U/mL of the produced cellulase (Figure 5). Some changes in the IR spectra were observed after the enzymatic treatment of the cotton fabrics. Shrinkage in some peaks was observed for the treated fabrics in compare to the untreated one, especially the peak at 4000-3200cm^{-1} that attributed to the free –NH valence vibration in amide group and the peak at 3650-3200cm^{-1} that attributed to the free OH in addition to the peak at 1281-1051cm^{-1} refereed to C-OH.

Contact angle:

Wetting is the capacity of a fluid to continue reaching with a strong surface that coming about because of the intermolecular associations. The level of wet (wettability) is controlled by a force balance among adherent and coherent powers

TABLE 4: Tensile strength, elongation %, roughness and yellowness index of untreated and treated cellulosic fabrics.

Samples	Tensile strength (kg f/mm^2)	Elongation %	Roughness µm	Yellowness Index
Raw cotton	14.184	23.7	22.1	23.0
Treated raw cotton	8.879	30.2	17.6	13.62
Raw viscose	5.088	25.5	20.8	11.58
Treated raw viscose	3.88	35.9	15.8	8.13

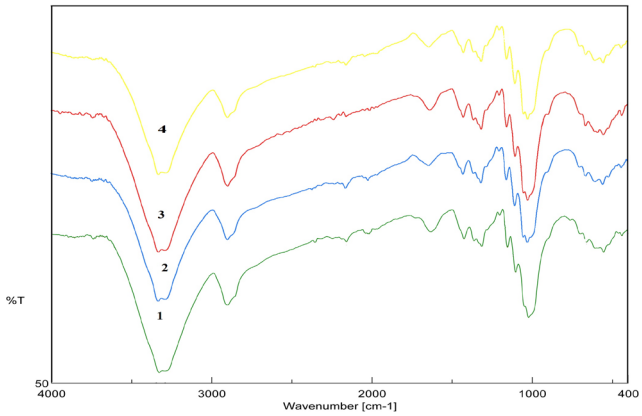


Fig. 5: IR spectra of (1:untreated cotton), (2:treated cotton), (3:untreated of viscose) and (4: treated of viscose)

[21]. The contact point (θ), as set in Figure 6, is the edge at which the liquid–vapor interface meets the solid–fluid interface. The angle between the fabric surface and the water molecule decides the behavior of the wetted fabric. In the current study, the contact angle of the treated fabrics was less than that of the untreated one. It may be attributed to the efficiency of the used enzyme to access the cellulose content leaving the surface of the fibers free and do not hamper the water permeability in compare to the chemical hydrolysis.

Cotton and cellulose fibers are more homogeneous than wool and are richer in hydroxyl groups. Moreover, the dyeing of cotton with anionic dyes, i.e., direct and reactive, request a high concentration of electrolytes in dyeing bath to reduce negative charges on the fiber surface and to promote the exhaustion of dyes [22]. So the treatment of cotton fabrics using the produced cellulase may be regarded as an acceptable substitute in studies of the perspiration transport properties of fabrics.

Scanning electron microscopy

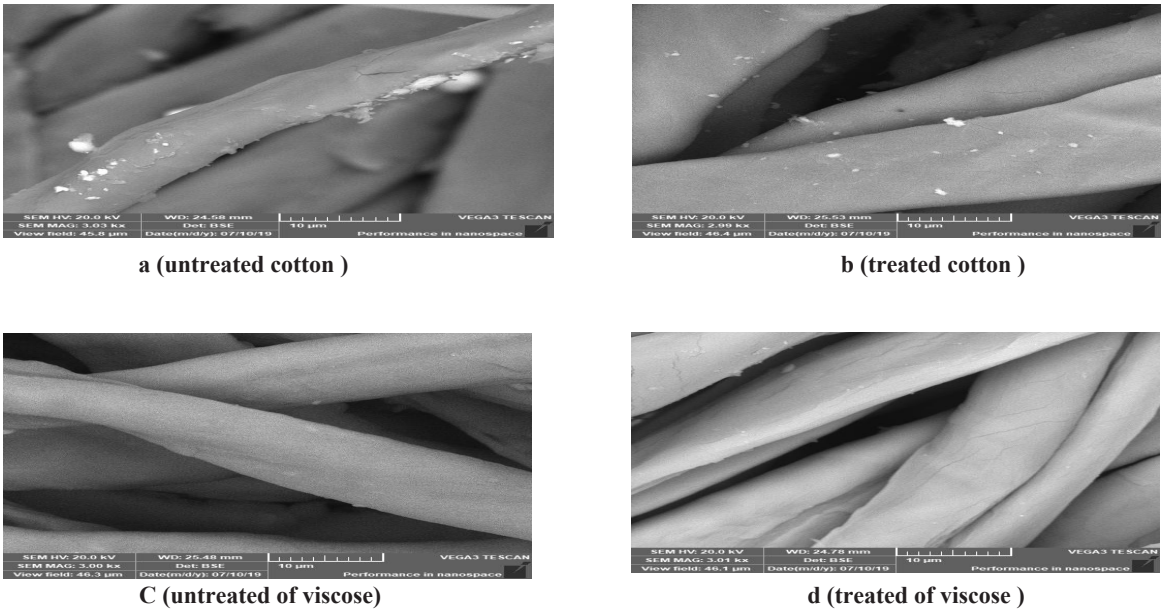
Cellulosic fabric was scanned under scanning electron microscope to illustrate the effect of the enzymatic treatment on their surface features and the results were shown in Figure 7 (a, b, c, d). The results indicated that the treatment of fabrics with the produced cellulase removed the unveiled the primary wall of the cotton fibre. This gave rise to facilitate dyeing process**.**

Conclusion

Although commercial cellulases are available, the production of cellulases with special characteristics is still challengeable. So the present work describe a promising future for ecofriendly enzymatic pretreatment of natural (cotton) and recovered (viscose) cellulosic fabrics with microbial acidothermophilic cellulase produced by the native Egyptian fungus *Aspergillus terreus* RS2. Under the optimum fermentation conditions using rice straw as a sole carbon source, high cellulase activity (9.5U/mL in fresh culture filtrate and 1000U/g of dried form) was achieved. The produced enzyme was applied to reduce the effluent load that was produced from scouring and bleaching processes and to upgrade a naturally adequate transaction for water and power

(cotton untreated) *(cotton treated)* *(viscose untreated)* *(viscose treated)*

Fig. 6: The data of contact angle



a (untreated cotton) **b (treated cotton)**

C (untreated of viscose) **d (treated of viscose)**

Fig 7: Scanning electron microscopy (a, b, c and d)

economy. The effectiveness of the enzymatic pretreatment under the optimum conditions of the produced enzyme on raw, scoured and bleached cellulosic fabrics indicated an increase in the color intensity of the treated fabrics in compare to the untreated one for different classes of reactive dyes. The fastness properties of the pretreated dyed fabrics were implemented without the detection of palpable differences among the staining values between the treated and the untreated fabrics. The contact angle for the pretreated cellulosic fabrics, tensile strength, Scanning electron microscopy and FTIR analysis was performed.

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تأثير السليولاز المستخلص محليا على صباغة الأقمشة السليلوزية الطبيعية والمستعادة

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تعد المعالجة الحيوية للأقمشة السليلوزية الطبيعية (القطنية) و المستعادة (فسكوز) باستخدام إنزيم السليولاز الناتج من الفطريات المصرية من الطرق المهمة في صناعة النسيج. وذلك من أجل تقليل النفايات السائلة الملوثة للبيئة الناتجة عن عمليات الغسيل والتبييض. كما أظهرت النتائج في ظل الظروف المثلى للمعالجة زيادة في شدة اللون للأقمشة المعالجة مقارنة بالأخرى غير المعالجة عند صباغتها بالصبغات النشطة. كما تم قياس وتقييم زاوية التلامس و الميكروسكوب الماسح الإلكتروني وتحليل العناصر بالاشعة السينية .FTIR