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A Promising Approach for Bio-finishing of Cotton Using Immobilized Acid-cellulase

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Abstract: In this research, cellulases were immobilized on Eudragit S-100 to minimize the tensile strength loss of cotton fabric caused by the enzymatic hydrolysis. About 76 % of the enzyme activity and 81 % of the amount of protein were recovered after the immobilization process, and the immobilized cellulase exhibited good reuse ability. The immobilized cellulase had the better adsorptive performance on cotton than the free cellulase. In addition, the results revealed that the catalytic efficiency of the immobilized cellulase on cotton was degradation, perhaps because the diffusion of the enlarged cellulase molecules is significantly inhibited in the interior of the cotton fiber. Moreover, the cotton fabric treated with the immobilized cellulase showed less weight and strength losses. SEM pictures further indicated that the cotton fabric treated with the immobilized cellulase suffered less damage.

Keywords: Cellulase, Hydrolysis, Adsorption, Immobilization, Cotton

Introduction

In recent years, varieties of enzymes are used in textile wet processing to improve qualities of fabric and to avoid environmental pollution [1-5]. For example, cellulases have been widely used to produce the special worn look for denim fabric and to eliminate fuzz on the surface of cotton fabric [6-12].

The structure of cotton fiber is composed of crystalline and non-crystalline (amorphous) regions, and both regions can be hydrolyzed by cellulases. Many capillary voids include two forms to be distributed in the interior of cotton fiber. The first form is gross capillaries such as the cell lumina, pit apertures, and pit-membrane pores with diameters ranged from 20 nm to 10 or more microns. The second form is cell wall capillaries such as the spaces among microfibrils, which are less than 20 nm in diameter. Diameters of cellulase molecules commonly range from 2.4 to 7.7 nm, with an average of 5.9 nm. Thus, the enzymatic attack can not only be limited to the fibre surface, because cellulase molecules can diffuse readily into the gross capillaries, and then penetrate into the interior of the fiber [13]. The significant weight and tensile strength losses to the cotton fiber can be caused by the hydrolysis in the interior of the fiber [14]. Enlarging the molecular size of cellulases will focus the hydrolytic attack more relatively to the surface of the fiber, because the permeation of an enlarged enzyme molecule is inhibited in the interior of the fiber. It can be expected that the tensile strength loss of the fabric should decrease because less cellulose molecules in the interior of the fiber will be hydrolyzed (Figure 1).

Eudragit S-100, a copolymer of methacrylic acid and methyl methacrylate with a molecular weight of 135000, has been widely used as a carrier for enzyme immobilization because of its unique reversibly soluble-insoluble property. The solubility of this copolymer can be controlled by adjusting the pH value of the aqueous solutions. The copolymer is soluble above pH 4.8 and precipitates below pH 4.6. Some studies had indicated that the solubility profile of the immobilized enzymes became similar to that of Eudragit S-100 when enzymes were bonded to Eudragit S-100 [15-18]. Thus, the immobilized enzymes can be used in their soluble forms during the enzymatic reactions, and be recovered in their insoluble forms after the reactions by altering the pH of reaction mixture [19].

In our previous study, cellulases were immobilized on Eudragit S-100 using a crosslinking method [20]. The mechanism of the immobiliation reaction is illustrated in Figure 2. In this research, the adsorptive and hydrolytic performances of the free and immobilized cellulases on cotton fabric were investigated. During the enzymatic reaction, the immobilized cellulase was as soluble as the free

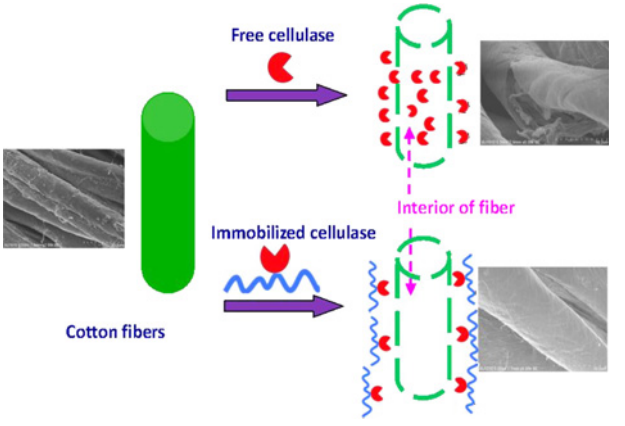

Figure 1. Action of free and immobilized cellulases on cotton.

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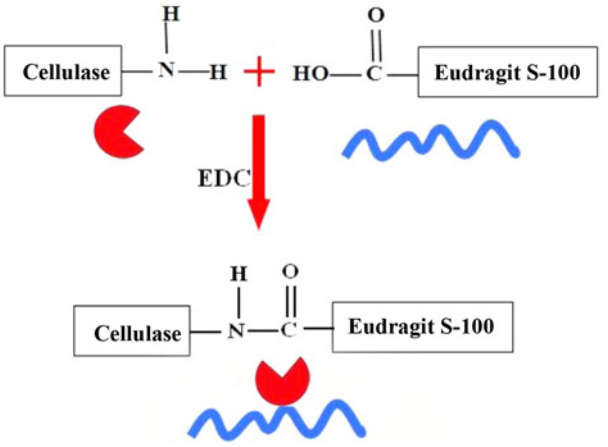

Figure 2. Coupling reaction between cellulase and Eudragit S-100.

cellulase. The enzymatic treatments for cotton fabric using the free and immobilized cellulases were studied to evaluate the effects of the treatments. The purpose of this research is to provide some useful information guiding the possible bio-treatment of cotton fabric using the immobilized cellulase.

Experimental

Materials

The enzyme used in the experiments was an acidic cellulase Suhong 989N with a CMCase activity of 92 U/m*l*, and was supplied by Novozymes (Suzhou, China). Eudragit S-100 was kindly donated by Degussa-Hüls, S.A. (Shanghai, China). All other chemicals used were of analytical grade. The woven cotton fabric (150 g/m^2) was supplied by Wuxi No.1 Cotton Textile Company (Wuxi, China).

Immoblization of Cellulase onto Eudragit S-100

The cellulase was covalently linked to Eudragit S-100 by a carbodiimide coupling procedure. Eudragit S-100 solution (5 %, in w/v) was prepared in phosphate buffer with pH 7.2. Carbodiimide solution (0.2 %, in w/v) was added into the polymer solution with stirring for 10 min. Then the cellulase solution (5 %, in v/v) was added. The mixed solution was kept under stirring for 12 h at room temperature. After this, the pH of the mixture was decreased to 4.5 with 0.1 M HCl solution to precipitate the immobilized cellulase. The precipitate was centrifuged (11,000×g) at room temperature for 10 min, and washed by resuspending in 0.01 M acetate buffer (pH 4.5) for 10 min. Then the precipitate was separated by centrifugation (11,000×g, 10 min) again. Finally, the immobilized-cellulase precipitate was redissolved in 0.2 M acetate buffer at pH 5.5 for further use.

Determination of Protein Concentration and Cellulase Activity

Protein concentrations of the cellulases were determined by Lowry method using bovine serum albumin as the standard.

The cellulase activity was measured by using 1 % (w/v) CMC (carboxymethylcellulose) as the substrate. The amount of generated glucoses was measured by a UV/Vis spectrophotometer at 546 nm using 3,5-Dinitrosalicylic acid (DNS) agent as the color indicator. One unit of cellulase activity was defined by the amount of enzyme, which produced 1.0 μmol of reducing sugar from the substrate per minute [21].

The protein recovery yield, activity recovery yield, and stability index were calculated based on equation (1), (2), and (3) as follows:

$$\text{Activity recovery yield (\%)} = \text{activity recovered / activity loaded} \times 100 \quad (1)$$

$$\text{Protein recovery yield (\%)} = \text{protein recovered / protein loaded} \times 100 \quad (2)$$

$$\text{Stability index} = \text{activity recovery yield / protein recovery yield} \quad (3)$$

Enzymatic Hydrolysis of Cotton Fabric

The hydrolysis of cotton fabric by the cellulases was carried out at 50 °C, pH 5.5, with a liquor-to-fiber ratio of 20:1 in 100 m*l* stoppered Erlenmeyer flasks. The free and immobilized cellulases were used with the same CMCase activity. After the cellulase treatments, the reaction mixtures were centrifuged (4000 g, 10 min) to separate the supernatant and the insoluble cotton. The control fabric samples were treated under the same conditions without the cellulases.

The amount of soluble reducing sugars was measured by a UV/Vis spectrophotometer at 546 nm using 3, 5-Dinitrosalicylic acid (DNS) agent as the color indicator. The experiments were carried out in triplicate, and the results were averaged.

Determination of Kinetic Parameters of Enzymatic Reaction

Michaelis-Menten constant (K_m and V_{max}) values of the free and immobilized cellulases were determined by a series of enzyme assays at varying concentrations of the cotton fabric samples. K_m values were calculated according to Lineweaver-Burk plots [22].

Weight and Strength Losses of Cotton Fabric

The weight loss was calculated the weight of samples according to the following equation (4):

$$\text{Weight loss (\%)} = (W_1 - W_2) / W_1 \quad (4)$$

where W_1 is the weight of the cotton fabric before cellulase treatment, and W_2 is the weight of the cotton fabric after cellulase treatment.

The tensile strength of the cotton fabric samples was tested using a YG(B)026D-250 Tensile Strength Tester (Wenzhou Darong Company, China). The samples were

tested five times, and the values were averaged. The strength loss was calculated according to the following equation (5):

$$\text{Strength loss (\%)} = (S_1 - S_2) / S_1 \quad (5)$$

where S_1 is the strength of the cotton fabric before the cellulase treatment, and W_2 is the strength of the cotton fabric after the cellulase treatment.

Surface Morphology

The surface morphology of the cotton fabric samples was studied using a SU-1510 scanning electron microscope (Hitachi, Japan). Platinum was sputtered onto the fabric samples as a conducting material to analyze the samples.

Results and Discussion

Covalent Immobilization of Cellulase onto Eudragit S-100

As shown in Table 1, the protein and activity recovery yields of the immobilized cellulase (0 cycle) were 75.6 % and 81.1 % respectively, and 93 % of the molecules of the immobilized cellulase showed their activities. Enzymes usually lost some of their activities after immobilization because of the blocking of catalytic active site by binding of enzymes to polymers [19].

In our previous work, the results showed that the immobilized cellulase was soluble in aqueous solutions above pH 5.2 and precipitated below pH 4.6 [23]. Thus, the immobilized cellulase can be reused. The protein and activity recovery yields of the immobilized cellulase after repeated use are shown in Table 1. The immobilized cellulase retained 37.6 % of the original enzyme activity of the free cellulase after five cycles of repeated uses, and exhibited good reuse ability.

Effect of Immobilization on Cellulase Adsorption

The first step in the enzymatic hydrolysis of cellulosic materials is enzyme adsorption on the surface of the substrates [24]. In order to find the relationship between the adsorption and the initial enzyme concentration, the adsorption experiments were carried out at varying initial cellulase concentrations. As shown in Figure 3(A), the adsorption amount of the cellulase proteins gradually increased, and reached equilibrium at 0.9 mg/m*l* of the initial cellulase concentration. By contrast, the amount of the immobilized-cellulase protein adsorbed was higher than that of the free cellulase. Because several protein molecules of the free cellulase could combine with the carrier to form an immobilized cellulase molecule, and the immobilized cellulase had larger sizes of the molecules than the free cellulase, which led to the fact that the intermolecular force between the immobilized cellulase and cotton was much stronger than that between the free cellulase and cotton.

Figure 3(B) reveals the effect of the contact time on the cellulase adsorption on the cotton fabric with an initial cellulase concentration of 1 mg/m*l*. The results showed that the process of the adsorption consisted of two phases, initial rapid phase and following slower phase. The consequences could be attributed to the existence of lots of accessible adsorption sites on the surface of the cotton fabric at the beginning, and then the amount of available adsorption sites decreased as the increasing amount of the cellulase adsorbed [25].

Table 1. Activity and protein recovery yields of the immobilized cellulase after each reaction cycles

Immobilized cellulase	Activity recovery yield (%)	Protein recovery yield (%)	Stability index
0 cycle	75.6±1.2	81.1±1.8	0.93
1st cycle	69.5±1.5	73.2±1.4	0.95
2st cycle	61.2±0.8	64.8±1.4	0.94
3st cycle	52.9±1.0	57.2±0.8	0.92
4st cycle	45.6±1.2	48.8±1.2	0.93
5st cycle	37.6±1.8	40.4±0.9	0.93

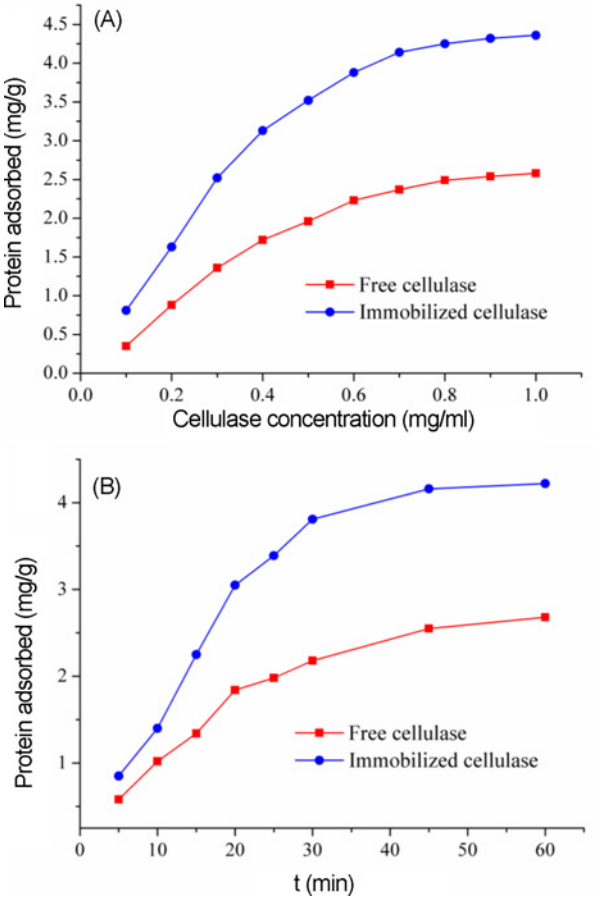

Figure 3. Effect of (A) initial enzyme concentration and (B) contact time on adsorption of cellulases on cotton. Treatment conditions; (A) adsorption temperature 4 °C, adsorption time 1 h, pH 5.5 and (B) adsorption temperature 4 °C, cellulase concentration 1.0 mg/m*l*, pH 5.5.

Hydrolysis of Cotton Fabric with Immobilized Cellulase

The effect of the substrate concentration on the enzymatic hydrolysis is shown in Figure 4. The results indicated that the higher substrate concentration resulted in the higher amount of the reducing sugars produced. However, the substrate concentration had comparatively slight impact on the hydrolytic performance of the immobilized cellulase compared with the free cellulase. The Michealis-Menten constant (K_m) and the maximum apparent initial catalytic rate (V_{max}) are the important constants for assessing the affinity of the cellulases to the cotton fabric. The lower K_m value means the stronger force of attraction between the cellulases and the cotton fabric. The K_m and V_{max} values were obtained from Lineweaver-Burk plots. Two linear equations of the free and immobilized cellulases were y=342258.64x+1249.13 and y=239030.24x+22349.58, respectively. As can be calculated (Table 2), the immobilization procedure showed obvious effects on the K_m and V_{max} values of the cellulase. The K_m value of the immobilized cellulase (10.69 $g \cdot l^{-1}$) was significantly lower than that of the free cellulase (273.97 $g \cdot l^{-1}$). Therefore, the affinity of the immobilized cellulase to the cotton fabric was higher than that of the free cellulase because of the increase of the intermolecular force between the immobilized cellulase and the cotton fabric. Besides, the V_{max} of the free cellulase (80.06×10^{-5} $g \cdot l^{-1} \cdot s^{-1}$) was higher than that of the immobilized cellulase (4.47×10^{-5} $g \cdot l^{-1} \cdot s^{-1}$). The results showed that the catalytic efficiency of the immobilized cellulase on cotton was degradation, perhaps because the diffusion of the enlarged immobilized-cellulase molecules and the hydrolysis were significantly inhibited in the interior of the cotton fiber.

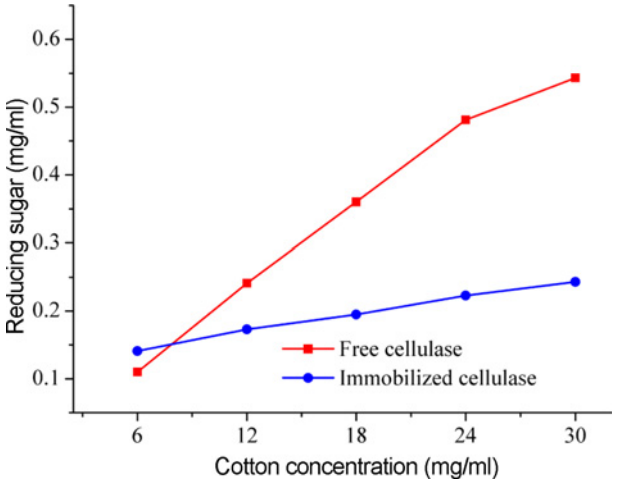

Figure 4. Effect of substrate concentration on the enzymatic hydrolysis of cotton fabrics. Treatment conditions: reaction temperature 50 °C, reaction time 2 h, pH 5.5.

Table 2. Kinetic parameters of the free and immobilized cellulases

Enzyme	K_m ($g \cdot l^{-1}$)	V_{max} ($g \cdot l^{-1} \cdot s^{-1}$)
Free cellulase	273.97	80.06×10^{-5}
Immobilized cellulase	10.69	4.47×10^{-5}

Figure 5 shows the reducing sugars released from the hydrolysis of CMC (carboxymethylcellulose) and cotton fabric by the free or immobilized cellulases with the same CMCase activity of 4.5 U/m*l*. The results revealed that the release rate of the reducing sugars was fast at the initial stage of the reactions, and then the rate decreased as the prolonging of reaction time. As shown in Figure 5(A), the hydrolysis of CMC with the free or immobilized cellulases has the similar consequences. However, using the cotton fabric as the substrate, the hydrolytic rate was slower using the immobilized cellulase than the free cellulase, particularly when the reaction time had gone beyond 12 h (Figure 5(B)). The findings indicated that the structures of the substrates had a stronger influence on the hydrolytic performance of the immobilized cellulase than the free cellulase, and the hydrolysis of the immobilized cellulase on the cotton fabric

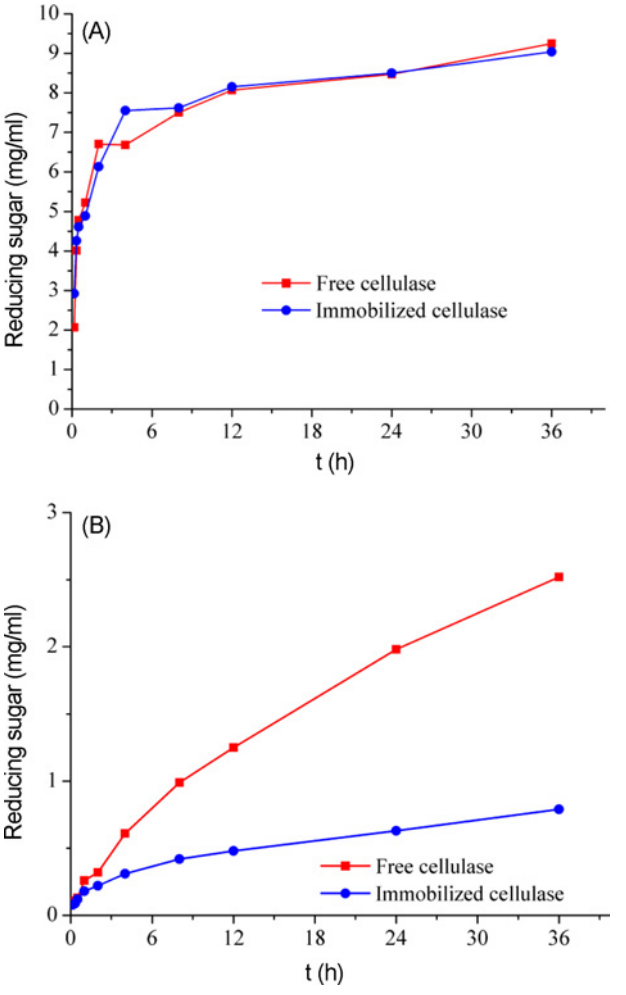

Figure 5. Time curves for the enzymatic hydrolysis of (A) CMC and (B) cotton. Treatment conditions: reaction temperature 50 °C, pH 5.5, liquor-to-fiber ratio 20:1.

Table 3. Effect of immobilized cellulase treatment on weight and strength losses

	Enzyme activity (U/m*l*)	Reaction time (h)	Weight loss (%)	Strength loss (%)
Free cellulase treatment	4.5	2	4.02±0.15	12.14±0.81
		6	9.36±1.06	18.89±1.25
		12	11.66±0.28	22.36±1.32
Immobilized cellulase treatment		2	2.08±0.11	3.25±0.67
		6	4.35±0.73	9.74±1.18
		12	5.18±0.31	13.24±0.96

was inhibited because the hydrolytic attack was refrained in the interior of the fiber.

Weight and Strength Losses of Fabric

Cellulases are used to improve the quality of cellulosic fabrics by removing fuzz fiber and pill from the surface of the fabrics [8]. The cotton fabric samples were treated with the free or immobilized cellulases at 50 °C and pH 5.5. Table 3 lists the weight and strength losses of the cotton fabric samples. The results showed that the cotton fabric treated with the immobilized cellulase showed lower weight and strength losses than those treated with the free cellulase. The cotton fabric samples treated with the free cellulase for 2 h showed the strength loss of 12.14 %, while the immobilized-cellulase treatment resulted in the strength loss of merely 3.25 %. The results meant that the cotton fabric samples suffered considerably less damage by the immobilized-cellulase treatment because the hydrolytic attack of the immobilized cellulase can be concentrated on the surface of the fiber. It is very important for the cellulosic textiles obtaining benign properties without serious fabric damage during the cellulase treatment.

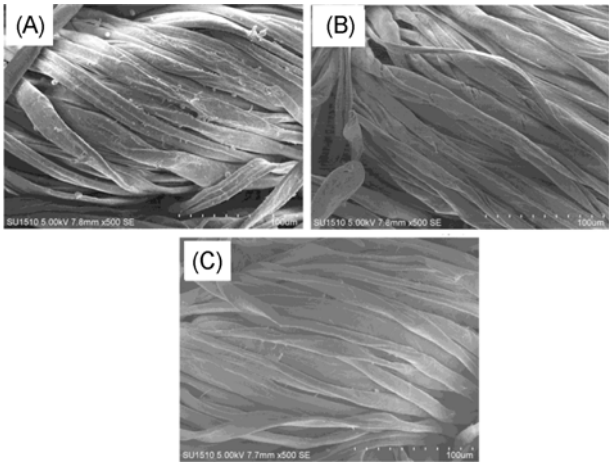

Figure 6. SEM images of cotton fabrics treated with (A) buffer (blank), (B) free cellulase and (C) immobilized cellulase. Treatment conditions: reaction temperature 50 °C, pH 5.5, reaction time 12 h, liquor-to-fiber ratio 20:1.

Surface Appearance of Cotton Fabric

Figure 6(A) shows the surface morphology of the untreated cotton fabric. There are many projecting fuzz on the surface of the cotton fabric. Figure 6(B) and 6(C) show the SEM images of the cotton fabric samples treated with the free or immobilized cellulases for 12 h, respectively. It was obvious that the surfaces of the cotton fabric samples became smoother after the cellulase treatments. However, many cracks appeared on the cotton fiber treated with the free cellulase. The results showed that the fiber treated with the free cellulase suffered more damage than those treated with the immobilized cellulase, which was in accordance with the results of the hydrolysis experiments.

Conclusion

In this research, the enlarged immobilized cellulase was used in the cotton bio-treatment. The immobilized cellulase could be utilized in its soluble form during the enzymatic reaction, and be retrieved in its insoluble form by altering the pH after the reaction. The immobilized cellulase had higher adsorption rate and adsorption amount of protein on the cotton fabric than the free cellulase. Nevertheless, the hydrolysis of the immobilized cellulase was degenerated because the permeation of the immobilized cellulase was inhibited in the interior of the fiber. The weight and strength losses of the cotton fabric treated with the immobilized cellulase were lower than those of the free celluase-treated ones. Therefore, this method can improve the qualities of cotton fabric, while overcoming the problems of the losses of weight and tensile strength. This procedure provides a promising approach for the cotton bio-finishing processes.

Acknowledgements

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