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Cellulase immobilization onto the reversibly soluble methacrylate copolymer for denim washing

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ABSTRACT

Cellulase treatment of denim fabrics is an environmentally friendly way for producing desired worn look. In this work, the enzymatic treatments of the denim fabrics, i.e., bio-washing, using native cellulase and cellulase immobilized with reversibly soluble copolymer (Eudragit S-100), immobilized-cellulase, have been investigated. According to the analyses of the lightness (CIE *L* value), color strength (*K*/*S* value) and color variations, at a cellulase concentration level of 6% o.w.f., the denim fabrics treated with the immobilized cellulase showed decoloration and color effect close to the native cellulase. However, the immobilized cellulase treatment of the denim fabrics showed lower weight loss and considerably higher tensile strength than those treated with the native cellulase. Both the native and immobilized cellulases improved the crystalline indice and the apparent crystallite size of the fiber sample compared with the control ones. The amorphous portion of the cellulose suffered more hydrolysis by the native cellulase than the immobilized cellulase. Scanning electron microscope pictures (SEM) and digital pictures further indicated that the immobilized cellulase can efficiently remove indigo dyestuffs on the surfaces of the denim fabrics without the problem of excessive damage to the fibers.

1. Introduction

Denim is a rugged cotton twill textile, in which the weft passes under two or more warp threads. It is characteristic of any indigo denim that only the warp threads are dyed, whereas the weft threads remain plain white. As a result of the warp-faced twill weaving, one side of the fabric shows the blue warp threads, the other side shows the white weft threads. Jeans are very popular cotton casual dresses made from denim around the world due to their advantages including special worn look. Traditionally, denim jeans have been washed with pumice stones to achieve a desirable worn look and increase the denim's softness and flexibility. However, the use of natural pumice stones in denim garment washing has some unavoidable disadvantages. For example, the overload of the pumice stones can result in severe physical damage to equipment and garments, and the particulate material can also clog the machine drainage passages (Heikinheimo, Buchert, Miettinen-Oinonen, & Suominen, 2000).

Cellulase treatment of cotton fabrics is an environmentally friendly way for improving the property of the fabrics (Belghiht, Ellouz-Chaabouni, & Gargouri, 2001; Cavaco-Paulo, Almeida, & Bishop, 1996). During the last two decades, cellulases were widely used in textile wet processing, such as bio-washing of denims to achieve the fascinating worn look or bio-polishing of cotton fabrics to eliminate the fuzz. The worn look of blue jeans can be obtained by non-homogenous removal of indigo dyes trapped inside the cellulosic fibers by the cooperative action of enzymes and mechanical action (Mamma, Kalantzi, & Christakopoulos, 2004). Nowadays, the bio-washing of denim fabrics with cellulase has replaced the traditional stonewashing due to its superior garment quality with significantly increased capacity of the laundry (Belghiht et al., 2001). However, it should be noted that cellulase used in bio-washing often result in severe strength losses of the denim fabrics. Some research had shown that the hydrolytic reaction catalyzed by cellulase is not limited to the fiber surface, because cellulase can easily diffuse into the fiber which leads to certain strength loss of the cellulosic fabric. To minimize this problem, enlarging the cellulase molecule by chemical modification could limit its activity on the cellulosic fiber surface, and the diffusion of an enlarged enzyme molecule into the interior of the cellulosic fibers is significantly inhibited (Ibrahim, EL-Badry, Eid, & Hassan, 2011; Lenting & Warmoeskerken, 2001).

Eudragit S-100, a copolymer of methacrylic acid and methyl methacrylate (135,000 molecular weight), has been extensively used to immobilize a variety of enzymes by covalent and non-covalent methods because of the advantages of this polymer. The polymer precipitates at low pH around 4.5 and is completely soluble in aqueous solutions with pH values over 5.5. This makes it the best choice for carrying out bio-catalytic reactions in soluble phase in the most favorable pH range (Fujii & Taniguchi, 1991;

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Rodrigues, Cabral, & Taipa, 2002; Shen, Rushforth, Cavaco-Paulo, Guebitz, & Lenting, 2007; Smith, Schroeder, Guebitz, & Shen, 2010). Silva et al. had immobilized protease on Eudragit S-100 to solve the problem of protease penetration into the core of wool fiber (Silva, Zhang, Shen, & Cavaco-Paulo, 2006; Silva, Gübitz, & Cavaco-Paulo, 2006). In our former work, a commercial preparation of cellulase has been covalently immoblized onto Eudragit S-100, and a Eudragit–cellulase conjugate with a high molecular weight has been obtained (Yu, Yuan, Wang, Fan & Wang, 2012). About 80% of the activity of the immobilized cellulase was retained after immobilization. The immobilized cellulase retained 52% of its original activity after five cycles of repeated uses, showing high reusability (Yu, Yuan, Wang, Fan & Wang, et al. 2012). In the present study, we further study the effects of the immobilized cellulase on denim washing to assess their application potential in textile industry.

2. Experimental

2.1. Materials

Acidic Cellulase Suhong 989N (CMCase 92 U/mL) was kindly supplied by Novozymes (Suzhou, China). Eudragit S-100 (composed of a 1:2 copolymer of methacrylic acid and methyl methacrylate) was supplied by Degussa-Hüls, S.A. (Shanghai, China). Indigo-dyed denim fabric was supplied by Xinglong company (Henan, China). The fabric weight per area was 475 g/m^2. All other chemicals were analytical grade.

2.2. Immoblization of cellulase onto Eudragit S-100

The cellulase was covalently linked to Eudragit S-100 by carbodiimide coupling 1.5% (w/v). Eudragit S-100 solution was prepared in phosphate buffer with pH 7.2. Carbodiimide coupling agent solution (0.2%, in w/v) was added into the polymer solution and stirred for 10 min. Then the cellulase solution (1%, 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 acetate buffer to precipitate the Eudragit–cellulase conjugate. 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. The precipitate was dissolved in 0.02 M phosphate buffer (pH 7.2) and reprecipitated with acetic acid solution (pH 4.5). Finally, the Eudragit–cellulase precipitate was redissolved in 100 mL of 0.3 M Tris–HCl buffer (pH 7.2) for use (Yu et al., 2012a).

2.3. Enzymatic treatment of denim fabrics

The native cellulase treatment of the denim fabric was carried out in a SW-12A Launderometer (Wenzhou Darong Company, China), a thermostatically controlled water bath system in which stainless steel containers can be mounted. The containers were rotated at a speed of 50 rpm. The denim fabrics were treated with different dosages of cellulases (2%, 4%, 6% o.w.f.) in 0.2 M acetate buffer at pH 5.5, a liquor-to-fiber ratio of 20:1, 50 °C for 60 min. The mechanical agitation was simulated by using 10 stainless steel balls of 5 mm diameter. After cellulase treatment, the denim fabrics were rinsed with deionized water to wash away abraded fibers attached on the fabrics, followed by deactivation of residual cellulase on the fabrics in deionized water at 80 °C for 10 min. After that, the denim fabrics were dried at 70 °C. Above experiment was also carried out using the covalent conjugate Eudragit–cellulase under the same treatment conditions and activity units as the native cellulase. Each experiment was repeated three times.

2.4. Measurement of color

Color characteristics of denim fabrics were measured by a Color-Eye 7000A spectrophotometer (Ceretag Macbeth Company, America) under the illuminant D65 using a 10° standard observer. The fabrics were folded twice to ensure opacity and were measured five times. The color strength was denoted by the K/S value (Ghoranneviss et al., 2007):

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

where K is absorption coefficient, depending on the concentration of colorant; S is scattering coefficient, caused by the dyed substrate; R is reflectance of the colored sample.

In addition, CIE color coordinates, L^* (lightness and darkness), a^* (redness and greenness) and b^* (yellowness and blueness) were also tested.

2.5. The weight loss and tensile strength of denim fabrics

The weight loss was calculated as the difference in the weight of samples before and after the cellulase treatments according to the following equation:

$$\text{Weight loss}(\%) = \frac{W_1 - W_2}{W_1} \quad (1)$$

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

The tensile strength of the denim fabrics was determined by the strip method according to ASTM D1682-64 using a YG(B)026D-250 Tensile Strength Tester (Wenzhou Darong Company, China). Samples were tested five times and the values were averaged.

2.6. Structure and surface morphology studies

The crystalline nature of cotton fabrics was determined by a D8 X-ray diffractometer (Bruker Company, Germany) using copper Kα radiation (λ = 0.154 nm). The angles scanned were 3–80° at 4°/min.

The crystalline indice (CrI) of the cellulosic samples were calculated from the X-ray diffraction patterns by the following equation (Heinze & Liebrrt, 2001):

$$\text{CrI}(\%) = \frac{I_{002} - \text{I}_{am}}{I_{002}} \quad (2)$$

where I_{002} is the peak intensity from the (0 0 2) lattice plane (2θ = 22.5°) and I_{am} is the peak intensity of amorphous phases (2θ = 18.3°).

Apparent crystallite size was estimated according to Scherrer equation (Heinze & Liebrrt, 2001):

$$\text{Crystallite size} = \frac{0.89\lambda}{\beta \cos\theta} \quad (3)$$

where λ is the wavelength of the incident X-ray (0.154 nm), θ is the Bragg angle corresponding to the (0 0 2) plane, and β is the half-height width of the peak angle of the (0 0 2) reflection.

The surface morphology of the untreated and treated denim samples was studied using a Quanta-200 scanning electron microscope (FEI Company, Netherlands). Platinum was sputtered onto the fabric sample as a conducting material to analyze the sample.

3. Results and discussion

3.1. Decoloration efficiency

The effect of the native and immobilized cellulase treatment on the CIE L value of the denim fabric was shown in Fig. 1. CIE L value of

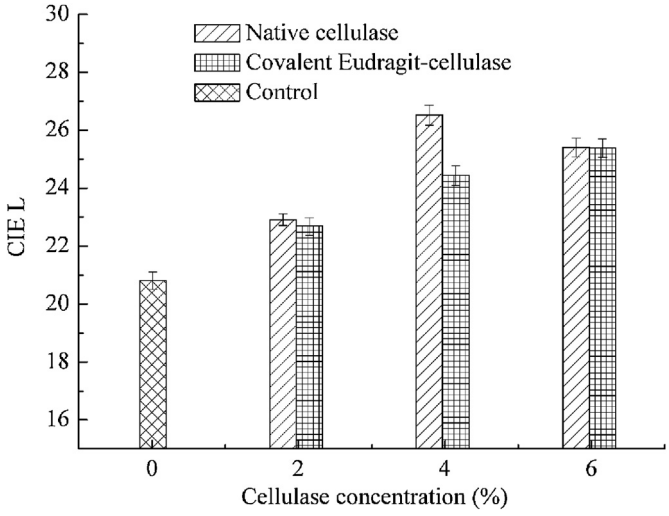

Fig. 1. Effect of enzymatic treatment on the CIE *L* values of the denim fabrics.

the denim fabric is used to quantify the decoloration efficiency after washing treatments. The higher CIE *L* value, the more decoloration of the indigo dyes (Pazarlioglu, Sariisik, & Telefoncu, 2005). At a cellulase concentration level of 6% o.w.f., the denim fabric treated with the native and immobilized cellulases showed CIE *L* values of 25.40 and 25.38, respectively. This result reveals that the enzymatic treatments with the native and immobilized cellulases produced similar decoloration efficiency for the denim fabrics.

3.2. Color properties of denim fabrics

The effect of the cellulase treatment on fabric color was monitored on the right side of the denim fabrics by measuring the color parameters with a Color-Eye 7000A spectrophotometer. The appearance color strength (*K*/*S*) and color parameters of cellulase-treated denim fabrics are shown in Table 1. The *K*/*S* value is linearly related to the concentration of the dyestuffs on the surfaces of the fabrics, which could be used to characterize the color retained on the fiber surfaces after cellulase treatment. It could be seen that the enzymatic treatment caused *K*/*S* values of the denim fabrics to decrease. It is because the surface fibers on denim fabrics are hydrolyzed by cellulases and the weaken fibers are further removed by the frictional forces between the treated fabrics. The indigo dyestuffs on the fabrics are also dislodged together with the removed cotton fibers. At 6% o.w.f. cellulase concentration, the denim fabrics treated with the immobilized cellulase show K/S value closed to the native cellulase. The results indicate that the denim washing with the immobilized cellulase could produce color effects for the denim fabrics close to washed with the native cellulase.

The effects of enzymatic treatment on CIE *a* and *b* values of the denim fabrics are also listed in Table 1. Both CIE *a* and *b* values of the denim fabrics were decreased after the enzymatic treatments. The treatments of the denim fabrics with the native and immobilized cellulases presented similar change of color parameters. The results also mean that the denim washing with the native and immobilized cellulases can achieve the similar faded effects for the denim fabrics.

3.3. Backstaining

The degree of backstaining was determined by the measurement of *K*/*S* and CIE *L* values of the reverse side of the denim fabrics. Backstaining of the reverse side of denim fabrics is used as an indication of the degree of backstaining of the fabric (Pazarlioglu et al., 2005). The higher *K*/*S* value and lower CIE *L* value, the higher degree of the backstaining. The *K*/*S* and CIE *L* values of the reverse side of the denim fabrics are shown in Table 1. It seems that the backstaining degree marginally increases with increasing cellulase concentration, regardless of whether it is the native or immobilized cellulase. At 2% and 4% o.w.f. cellulase concentration, the backstaining degree of denim washing with the native cellulase is higher than the denim washing with the immobilized cellulase. That could be because, at 2% and 4% o.w.f. cellulase concentrations, the amount of indigo in washing bath from denim washing with the native cellulase was more than the denim washing with the immobilized cellulase. At a cellulase concentration level of 6% o.w.f., the denim washing with the native and immobilized cellulases led to similar degree of backstaning.

3.4. Fiber damages of denim fabric

Almost in all textile processes, including denims washing, it is particularly important to minimize weight and strength losses of the fabrics.

Fig. 2 shows the weight loss of the denim fabrics treated with the native and immobilized cellulases. It is demonstrated that weight loss was increased with the increasing cellulase concentration. At 4% and 6% o.w.f. cellulase concentrations, the denim fabrics treated with the native cellulase showed the weight losses of 7.88% and 9.20%, respectively. However, using exactly same treatment conditions and activity units, the immobilized cellulase treatment resulted in the weight losses of merely 4.84% and 5.53%, respectively, indicating less damage to the denim fabrics. The hydrolytic attack of the immobilized cellulase was more concentrated on the surface of the cellulosic fiber due to its larger molecule size and volume, which enabled the hydrolysis process of the denim to be efficiently controlled.

The effect of enzymatic treatment on the tensile strength of the denim fabrics was shown in Fig. 3. The results show that the tensile strength of all denim fabrics decreases significantly after they were exposed to cellulase treatment particularly at higher concentrations regardless of the used enzyme. Tensile strength of the denim

Table 1
The color strength and color parameters of cellulase-treated denim fabrics.

	Cellulase concentration (%)	The right side of the fabrics			The reverse side of the fabrics	
		K/*S*	CIE *a*	CIE *b*	*K*/*S*	CIE *L*
Control	0	17.35	1.44	−9.86	2.15	46.71
Native cellulase	2	16.20	0.67	−11.89	2.82	45.66
	4	13.52	−0.22	−11.67	2.95	44.92
	6	13.82	−0.16	−11.75	3.18	44.05
Immobilized cellulase	2	16.40	−0.76	−11.86	2.59	46.94
	4	15.30	−0.45	−11.82	2.71	46.43
	6	13.90	−0.18	−11.46	3.14	44.31

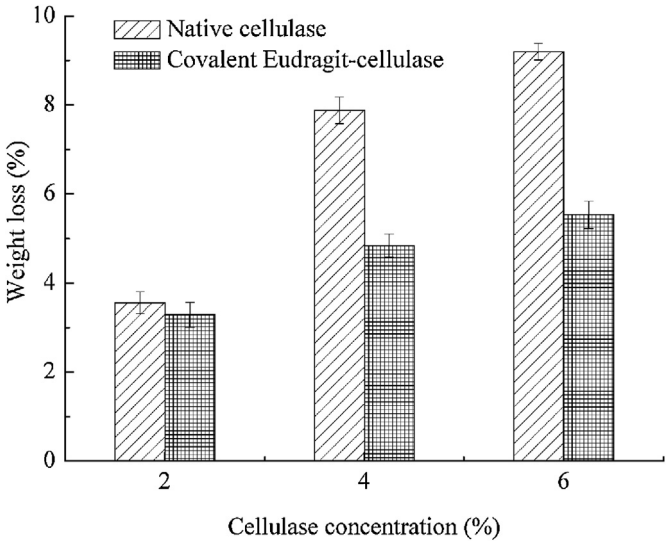

Fig. 2. Effect of enzymatic treatment on the weight loss of the denim fabrics.

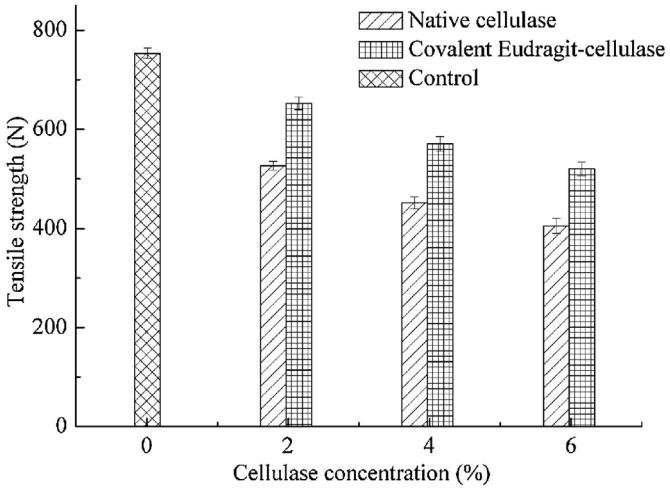

Fig. 3. Effect of enzymatic treatment on the tensile strength of the denim fabrics.

fabrics treated with the immobilized cellulase is higher than that of the native celluase-treated ones. At a cellulase concentration level of 6% o.w.f., the denim fabric treated with the immobilized cellulase achieved a tensile strength of 515 N, which is remarkably higher than that of the native cellulase-treated one (423 N). This is because the fact that the hydrolytic attack of the immobilized cellulase would focus on the surface of the denim fibers, causing considerably less damage to the denim fabrics. Taken as a whole, the denim fabric treated with the immobilized cellulase showed lower weight loss and considerably higher retained tensile strength than those treated with the native cellulase. This is a very important result of a cellulase product, since a significant effect should be obtained without significant fabric damage, and this can be achieved by enlarged cellulase molecule.

3.5. X-ray diffraction

Fig. 4 shows typical X-ray diffraction intensity profiles of the denim fabrics. The peaks located near 2θ = 14.8, 16.4, 22.5, and 34.5, which are the characteristics of 1 0 1, 1 0 $\bar{1}$, 0 0 2, and 0 4 0 reflections of cellulose I, were measured. The changes in the crystallinity index (CrI) and apparent crystallite size of the denim fabrics due to cellulase treatment are shown in Table 2.

The cellulases improved the CrI and the apparent crystallite size of the fabric compared with the control samples. With the same initial activity of the cellulases in water phase, CrI of the denim fabrics treated with the native cellulase is higher than the immobilized cellulase treated ones, which indicates that the amorphous portion of the cellulose suffered more hydrolysis when using the native cellulase. The results also revealed that the crystalline regions of the cellulose are difficult to hydrolyze by the native and immobilized cellulases. The increased CrI and the crystal size of cellulose are good evidence that the amorphous portion of the cellulose was more readily hydrolyzed by the cellulases than the crystalline portion.

3.6. SEM micrograph

Fig. 5 shows the SEM images of the denim fabrics treated with the native cellulase and the immobilized cellulase. The enzymatic degradation of the cotton fibers treated with the native cellulase was significant (Fig. 5b–d). That led to significant weight and tensile strength losses of the denim fabrics. In contrast to this, as shown in Fig. 5e–g, the denim fabrics treated with the immobilized cellulase suffered less damage due to the localization attack on the surfaces of the fibers caused by the immobilized cellulase.

3.7. Digital photograph

The worn look of denim fabrics is obtained by non-homogeneous removal of the indigo dye trapped inside the fibers by the cooperative action of cellulase and mechanical factors. Mechanical action is essential for color removal from both sides of the fabric during enzymatic washing. It was simulated by using 10 stainless steel balls of 5 mm diameter in our experiments. Fig. 6 shows the appearance of the denim fabrics treated by the native and immobilized

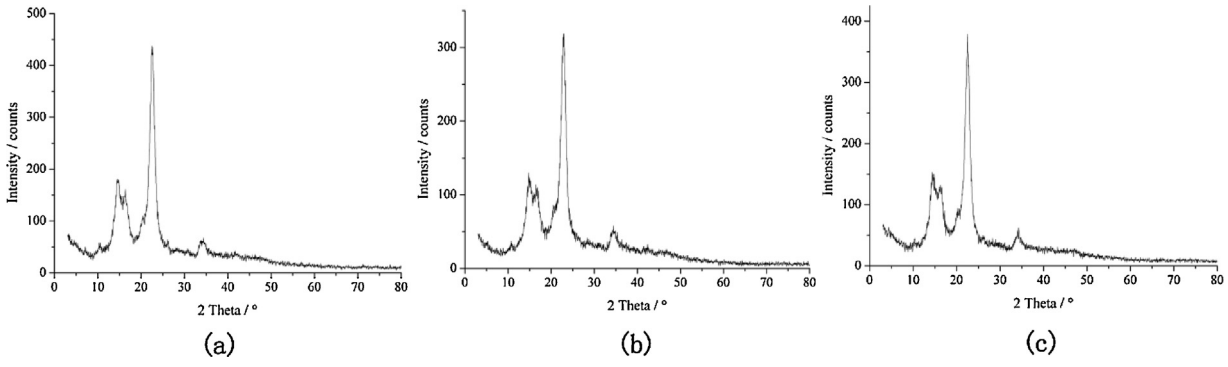

Fig. 4. X-ray diffraction of denim fabrics.

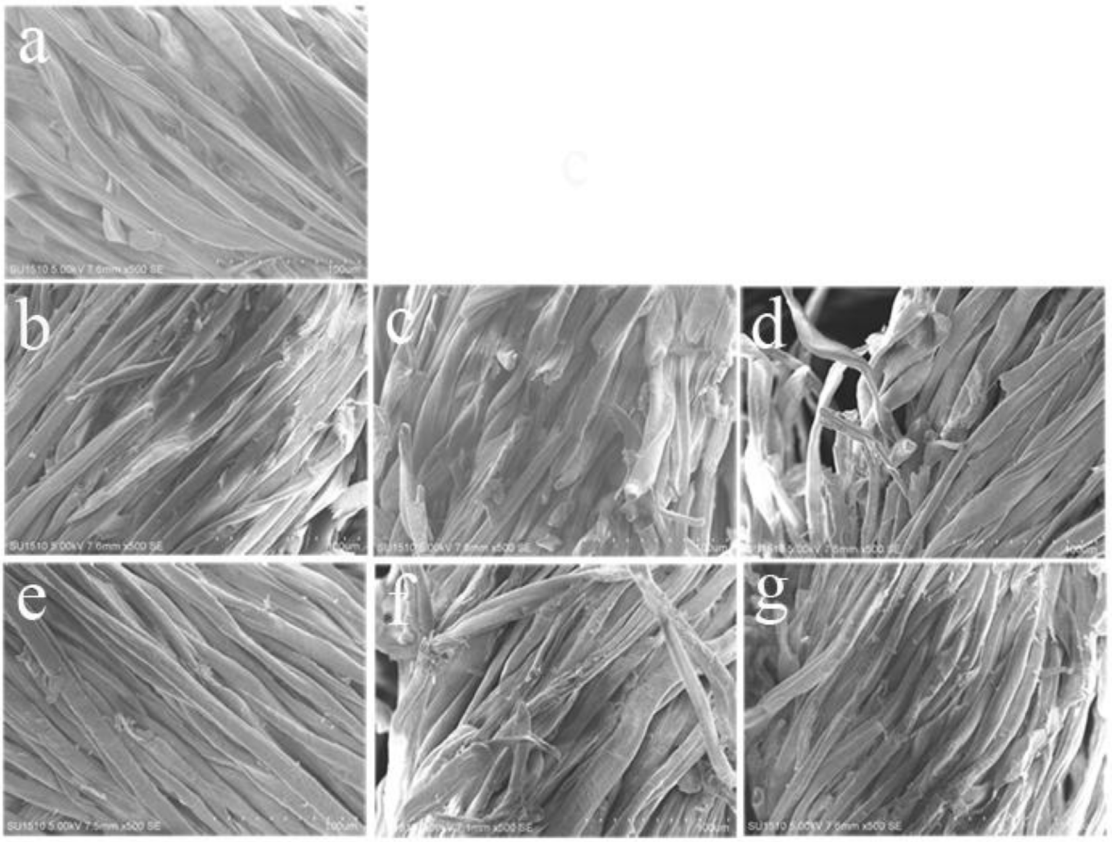

Fig. 5. SEM morphology of denim fabrics treated with (a) buffer (blank), (b) 2% o.w.f. N, (c) 4% o.w.f. N, (d) 6% o.w.f. N, (e) 2% o.w.f. I, (f) 4% o.w.f. I, (g) 6% o.w.f. I (N-native cellulase, I: immobilized cellulase).

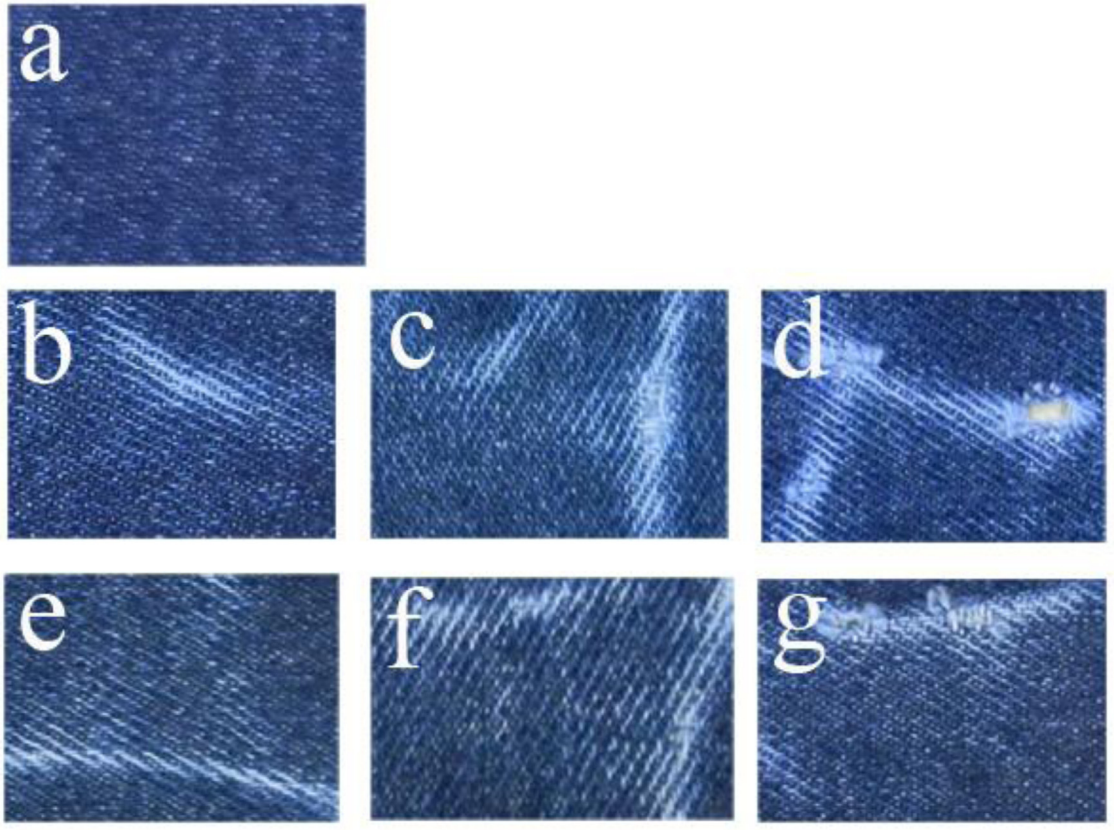

Fig. 6. Photograph of denim fabrics treated with (a) buffer (blank), (b) 2% o.w.f. N, (c) 4% o.w.f. N, (d) 6% o.w.f. N, (e) 2% o.w.f. I, (f) 4% o.w.f. I, (g) 6% o.w.f. I (N, native cellulase; I, immobilized cellulase).

Table 2
The results of X-ray diffraction for different denim fabrics.

Sample	I_{am} $2\theta = 18.3^\circ$	I_{101} $2\theta = 14.8^\circ$	$I_{10\bar{1}}$ $2\theta = 16.4^\circ$	I_{002} $2\theta = 22.5^\circ$	I_{040} $2\theta = 34.5^\circ$	CrI (%)	Crystallite size (nm)
Untreated	71	180	159	425	66	83.29	5.79
Native cellulase treated	52	127	113	321	55	83.80	5.93
Immobilized cellulase treated	60	150	131	368	61	83.70	5.92

cellulases. Both native and immobilized cellulases gave good decloloration results, where some faded streaks are observed. However, at higher levels of cellulase dosages (4% and 6% o.w.f. cellulases), the denim fabrics treated with the native cellulase suffered more damage, and even some holes appeared on the denim fabrics as shown in Fig. 6 c and d.

4. Conclusions

In the washing processing of denim fabrics using cellulase, excessive hydrolysis of the cellulosic fibers and subsequent severe weight and tensile strength losses of the fabrics is undesired. To minimize these adverse effects, one option is the modification of the enzyme to locate the enzymatic hydrolysis on the surface of the fiber. Covalent Eudragit–cellulase, immobilized enzyme which has a higher molecular weight, can efficiently remove indigo dyestuffs on the surfaces of the denim fabrics without severe damage to the mechanical properties. Therefore, the immobilized cellulase has great potential in the bio-washing of denim fabrics.

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