Fibers and Polymers 2014, Vol.15, No.8, 1609-1615 DOI 10.1007/s12221-014-1609-6

A Study of Surface Morphology and Structure of Cotton Fibres with Soluble Immobilized-cellulase Treatment

Yuanyuan Yu, Jiugang Yuan, Qiang Wang*, Xuerong Fan, Ping Wang, and Li Cui

Key Laboratory of Science and Technology of Eco-Textile, Ministry of Education, Jiangnan University, Wuxi, Jiangsu 214122, China

(Received December 29, 2013; Revised March 26, 2014; Accepted March 28, 2014)

Abstract: Our previous studies have demonstrated that cotton fabrics treated with soluble immobilized-cellulase showed considerably lower degradation and higher retention of tensile strength than those treated with free cellulase. It is important to investigate the surface morphology and structure of cotton fibres for understanding the enzymatic degradation. In this study, the effects of the soluble immobilized-cellulase on the surface morphology and structure of cotton fibres were investigated. The ultrastructural changes in the fibre surfaces were inspected using Tapping-Mode Atomic Force Microscopy (TM-AFM) and scanning electron microscopy (SEM), and the results showed that the smooth surfaces could be obtained after the immobilized-cellulase treatment, and no obvious damage was observed on the fibre surfaces. The hydrogen bonds in the certain depth area beneath the fibre surface were investigated using Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy (ATR-FTIR) after the cellulase treatments. Furthermore, the result of fibre accessibility indicated that the hydrolysis occurring in the interior of the cotton fibres was limited during the immobilized-cellulase treatment. Crystalline index (CI) of the cotton fibres treated with free cellulase was slightly higher than that of fibres treated with immobilized cellulase.

Keywords: Soluble immobilized-cellulase, Surface morphology, Cotton, TM-AFM, ATR-FTIR

Introduction

Cellulase treatment is one of the most commonly used wet processing techniques in textile industry, such as bio-washing of denim fabrics or bio-polishing of cellulose-containing fabrics [1-4]. Bio-polishing of cotton fabrics is one of the best-known contemporary cellulase applications for enhancing the fabric appearance by removing fuzz fibres from the fabric surfaces [5-7].

Total cellulases contain three major types of enzymes. The presumed attack mode of total cellulases on cellulose involves the synergistic actions of the cellulase components. Firstly, endoglucanase (EG: EC 3.2.1.4) randomly attacks β-1,4 bonds within the cellulose chains, then exoglucanase (CBH: EC 3.2.1.91) consecutively catalyses β-D-glucosides with a release of cellobiose at the terminal non-reducing ends of the cellulose chains, and finally, glucosidase (EC 3.2.1.21) hydrolyses cello-oligosaccharide and cellobiose into glucose [8,9].

A large amount of gross capillaries with diameters ranging from 20 nm to 10 μm or larger are distributed in the interior of cotton fibres. The diameters of cellulase molecules commonly range from 2.4 nm to 7.7 nm, with an average value of 5.9 nm [10]. Thus, cellulase molecules can readily diffuse into the gross capillaries and penetrate the interior of the fibre, resulting in a significant loss of weight and tensile strength of fibre. One of the approaches to decrease the loss of the tensile strength is to increase the molecular size of cellulase, because the enlarged cellulase can limit the enzymatic attack to the fibre surface. The increase in molecular size of cellulases can be achieved by coupling the cellulases with macromolecules. In our previous work, cellulases were immobilized onto Eudragit S-100, which was an enteric copolymer of methylacrylic acid and methyl methacrylate, by using a 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) crosslinking method [11]. The mechanism of the immobilization reaction is illustrated in Figure 1. Eudragit S-100 can be precipitated at low pH around 4.5, and be dissolved in aqueous solutions at pH values over 5.0. Thus, the immobilized enzymes can be used in their soluble

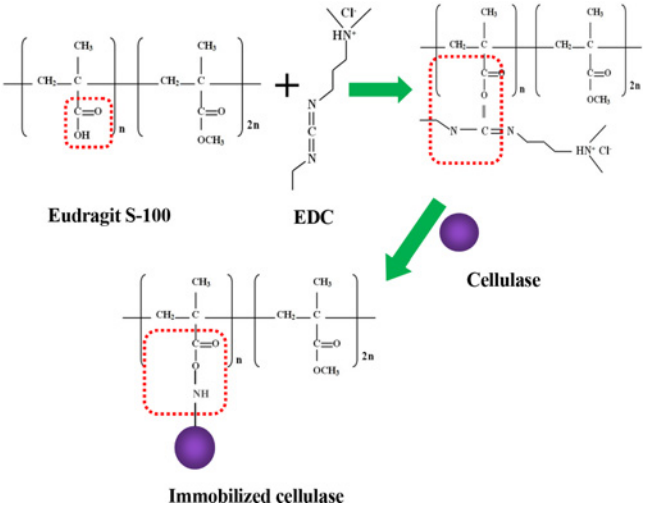

Figure 1. Mechanism of coupling reaction between cellulase and Eudragit S-100.

*Corresponding author: qiang_wang@163.com

form during the enzymatic reaction. The cotton fabrics treated with soluble immobilized-cellulase showed reduced weight loss and considerably higher tensile strength than those treated with free cellulase [12], and the soluble immobilized-cellulase can efficiently remove indigo dyestuffs on the surfaces of the denim fabrics without severe damage to the mechanical properties [13].

To understand the enzymatic degradation of cotton fibres treated with soluble immobilized-cellulase, it is important to investigate the surface morphology and structure of the cotton fibres. In the present investigation, TM-AFM and SEM were applied to observe the ultrastructural changes in the fibre surfaces. The hydrogen bonds in the certain depth area beneath the fibre surface were studied using ATR-FTIR. In addition, the accessibility of the cotton fibres was measured, and the effect of the immobilized-cellulase treatment on the crystallinity of cotton fibres was investigated using X-ray diffraction (XRD).

Experimental

Materials

Acidic cellulases (Suhong 989N, CMCase 92 U/m*l*) were supplied by Novozymes (Suzhou, China). Eudragit S-100 (purity 100 %) was kindly donated by Degussa-Hüls, S.A. (Shanghai, China). Carbodiimide hydrochloride (EDC) was obtained from Aladdin Reagent Company (Shanghai, China). All other chemicals were of analytical grade. The cotton fibres were obtained from Wuxi Cotton Textile Company, China.

Immobilization of Cellulase onto Eudragit S-100

First, 0.2 g of carbodiimide was added to 100 m*l* of Eudragit S-100 solution (1.5 % w/v), and the reaction mixture was stirred for 10 min. Thereafter, 2 m*l* of cellulase solution (17.5 mg protein/m*l*) was added to the mixture, which was then stirred for 12 h at room temperature. After the reaction, Eudragit-cellulase (i.e., immobilized cellulase) was precipitated by lowering the pH value of the reaction solution to 4.5 with 2 M acetic acid. The precipitate was then centrifuged at 11000×*g* for 10 min at room temperature, and washed by resuspending it in 0.01 M acetate buffer (pH 4.5) for 10 min. Finally, the Eudragit-cellulase precipitate was redissolved in 0.2 M acetate buffer at pH 5.5 for further study [11].

Enzymatic Treatments of Cotton Samples

The cotton fibre samples were treated with the free or soluble immobilized-cellulases (CMCase, 4.5 U/m*l*) in 0.2 M acetate buffer (pH 5.5) with a liquid-to-fibre ratio of 20:1 at 50 °C for 2 h. After the cellulase treatments, the samples were rinsed with deionized water, followed by the deactivation of residual cellulases on the samples in deionized water at 80 °C for 10 min. Then, the cotton samples were dried at 60 °C.

Atomic Force Microscopy Analysis

The ultrastructural examination of the fibre surface was performed under a CSPM 4000 Atomic Force Microscope (AFM) (Benyuan, China) by using the fibres immobilized on magnetic stubs. All images were obtained at the tapping mode with the use of the same silicon cantilever at a scanning frequency of 2.0 Hz in air at room temperature. The morphological data (i.e., surface roughness) of the fibres were obtained by analysing the topography images with the built-in software supplied with AFM.

SEM Analysis

The surface morphology of the fibre samples was inspected using a Su-1510 Scanning Electron Microscope (Hitachi, Japan). A thin layer of platinum, which served as a conductive layer, was sprayed onto the fibre sample surface prior to the SEM analysis.

ATR-FTIR Analysis

ATR-FTIR spectra of all samples were recorded with a Nicolet Is10 Infrared Spectrophotometer (Thermo Nicolet, USA) with an ATR attachment. The absorbance measurements were carried out in the range of 650-4000 cm^{-1}.

Accessibility of Cotton Fibres

The accessibility of the cotton fibres was measured by a methylene blue (MB) adsorption assay [14,15].

Cotton fibres (0.2 g) were added to 25 m*l* of MB solution (0.1 g/*l*) with an agitation speed of 200 rpm at 25 °C for 12 h. The MB concentrations were monitored by a UV-2802S spectrophotometer (Unico, China) at 660 nm.

Methylene blue absorption value (MBSV) was calculated for each cotton sample by using equation (1):

$$\text{MBSV (adsorbed MB in mg/cellulose in g)} = (MB_1 - MB_2)/0.2 \quad (1)$$

where MB_1 and MB_2 denote the original amount of MB used for adsorption and the amount of MB in the solution after the adsorption, respectively.

X-ray Diffraction Analysis

The crystallinity of the cotton fibres was analysed using a D8 X-ray Diffractometer (Bruker Company, Germany) with copper Kα radiation (λ=0.154 nm) over the range of 3-40 ° at a scanning speed of 4 °/min.

The crystalline index (CrI) was calculated according to the following equation [16]:

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

where I_{002} is the peak intensity of (002) lattice plane (2θ=22 °) and I_{am} is the peak intensity of amorphous phases (2θ=18 °).

Results and Discussion

Changes of Surface Morphology of Cotton Fibres

AFM can be used to examine samples directly on the nanometer scale in three dimensions. It also has the advantages such as in situ measurements in atmospheric or controlled environments, without special sample preparation [17,18]. TM-AFM can be used to scan samples at very low forces, employing a silicon cantilever probe to gently oscillate and tap the sample surface [17,19]. TM-AFM has been widely used to study the mechanism of cellulose-enzyme interactions [19,20]. Lee *et al.* [19] found that cellulase treatment produced the greatest effect on cellulose destruction.

In our study, TM-AFM was used to observe the surface structure of the cotton fibres treated with free or immobilized cellulases (Figure 2). As shown in Figure 2(A1) and (A2), the untreated cotton fibres assembled from microfibrils along an array of parallel straight lines exhibited apparent rugged surfaces. The surface structure of the cotton fibres showed significant changes after the free or immobilized

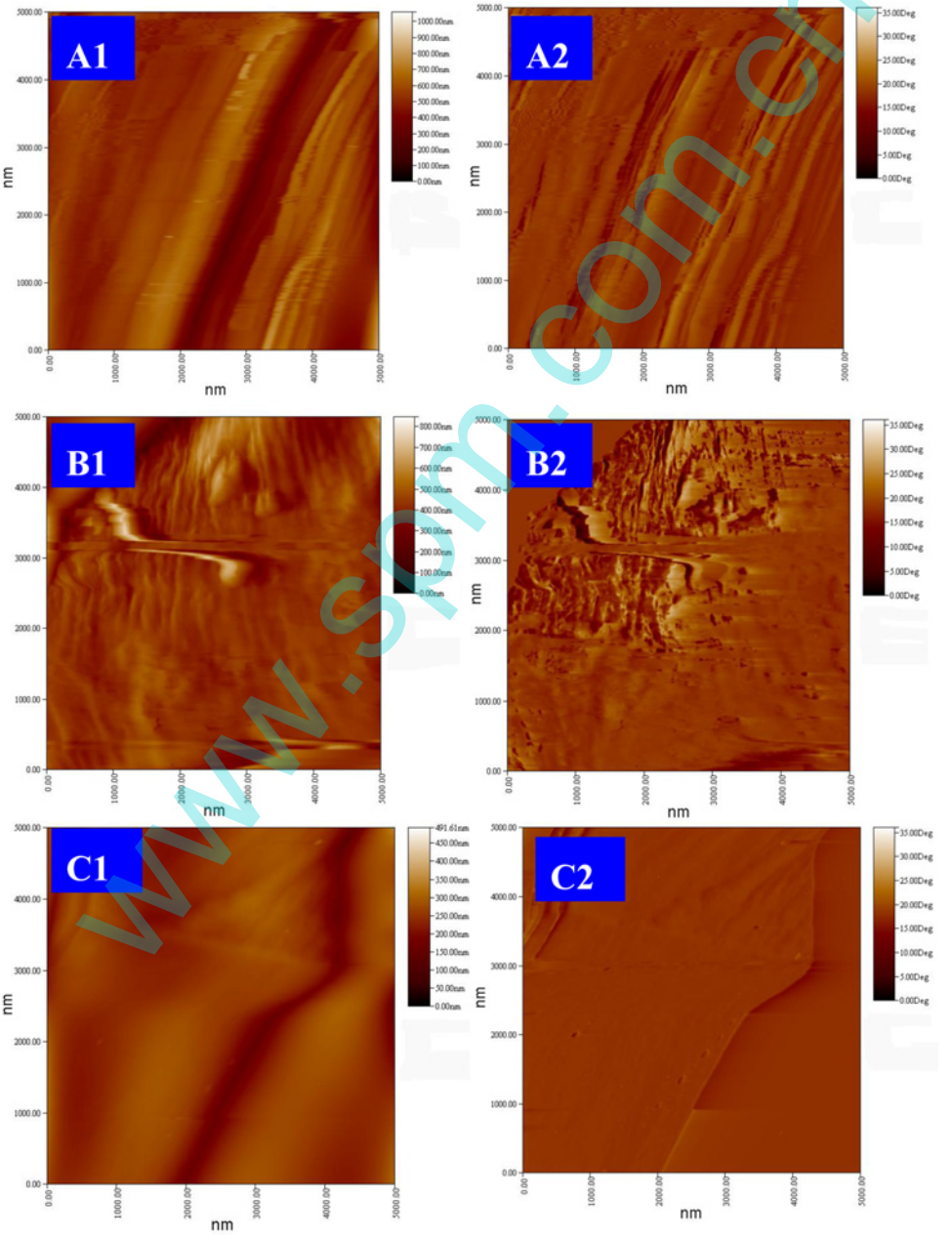

Figure 2. Surface ultrastructure of cotton fibres observed by TM-AFM. Simultaneously acquired topographic images (A1, B1, C1) and phase images (A2, B2, C2); (A) untreated cotton fibres, (B) cotton fibres treated with free cellulase, and (C) cotton fibres treated with immobilized cellulase.

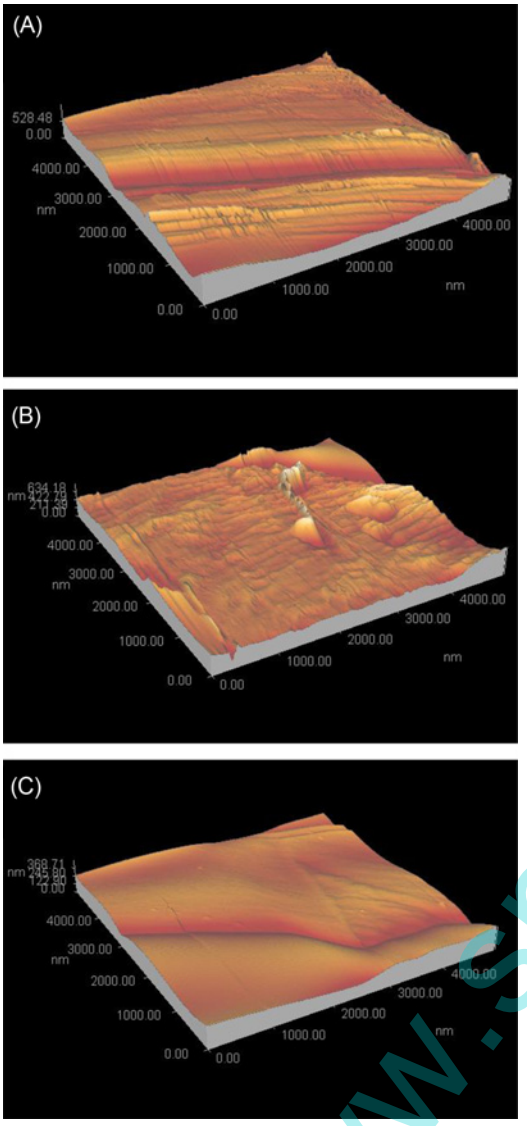

Figure 3. Three-dimensional (3D) topographic images of cotton fibres treated with (A) buffer (control), (B) free cellulase, and (C) immobilized cellulase.

cellulase treatment (Figure 2(B) and (C)). After the free cellulase treatment, the fibre surfaces were partially smooth with disrupted fibre arrangement visible on the surfaces (Figure 2(B)). On the other hand, the smooth fibre surfaces also could be obtained by the immobilized-cellulase treatment, and no obvious structural damage could be observed on the surfaces (Figure 2(C)).

Figure 3 shows 3D topographic images of the cotton fibres treated with the free or immobilized cellulases, from which rough fibre surfaces can be observed. Smoother fibre surfaces could be obtained by the immobilized cellulase treatment as compared with the untreated fibres and with the ones treated by the free cellulase. The surface roughness can be defined as the deviation of the actual surface topography from an ideal smooth surface. Roughness measurements of the fibre surfaces (roughness average and roughness of surface) can be calculated by AFM analyzing software. The values of roughness average and roughness of surface are shown in Table 1. The values of roughness of surface decreased from 86.5 nm to 61.6 nm or to 31.1 nm after the free or immobilized cellulase treatments, respectively. These results demonstrated that the cotton fibres could acquire relatively smooth surfaces after the cellulase treatments, especially using the soluble immobilized-cellulase.

Table 1. Roughness analysis of cotton fibres treated with cellulases

Items	Untreated cotton fibers	Cotton fibers treated with free cellulase	Cotton fibers treated with immobilized cellulase
Roughness average (nm)	67.2	42.3	25.1
Roughness of surface (nm)	86.5	61.6	31.1

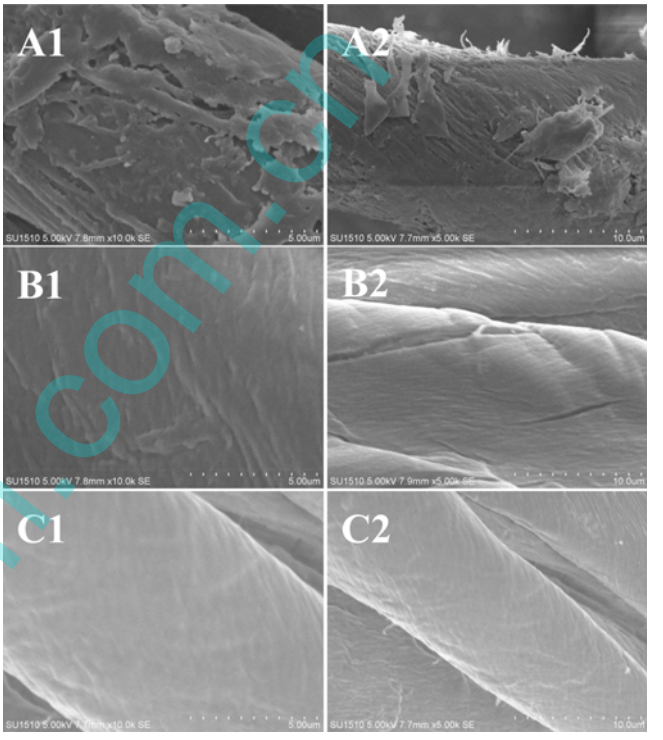

Figure 4. SEM images showing the morphology of cotton fibres treated with (A) buffer (control), (B) free cellulase, and (C) immobilized cellulase.

Figure 4 shows the SEM images of the cotton fibres. The untreated fibres exhibited rough surface features at the micrometre level and a large amount of fuzz fibres projecting from the surfaces. After the hydrolysis by the cellulases, the cotton fibres displayed relatively smooth surfaces, which were in good agreement with the results obtained from AFM analysis. Various pits and cracks existed on the fibre surfaces after the free cellulase treatment, indicating a dramatic

degradation of the cotton fibres due to the treatment. In contrast, it could be seen from the images that the immobilized cellulase treatment resulted in a less pronounced damage to the fibres as compared with the free cellulase treatment.

Changes of Hydrogen Bonds in Cotton Fibre Surfaces

Usually, the characteristics of cotton structure were studied by FTIR using the KBr pellet technique. However, most action of cellulase molecules occurs in the certain depth area beneath the surface of cotton fibre. One advantage of ATR-FTIR over FTIR is ATR-FTIR can detect surface structure in finite depth (0.5-2 μm). In this study, the characteristics of surface structure of cotton fibres were investigated by ATR-FTIR.

The most dominant functional group in cellulose is hydroxyl (-OH), which can form various intramolecular and intermolecular hydrogen bonds. The characteristics of the hydrogen bonds are considered as one of the most influential factors to determine the structure and properties of cellulosic materials. The bands between 3600 and 3000 cm^{-1} in FTIR spectra are representative of OH-stretching vibrations, which could provide a considerable amount of information regarding the flexural vibration of the hydrogen bonds in cellulose. The structure of hydrogen bonding pattern in cellulose is shown in Figure 5 [21]. The intramolecular hydrogen bonds of O(2)H···O(6) and O(3)H···O(5), and intermolecular hydrogen bond of O(6)H···O(3) in the cellulose structure are located at 3460-3410 cm^{-1}, 3375-3340 cm^{-1}, and 3310-3230 cm^{-1}, respectively [22].

Because of the broad and overlapping characteristic absorption peaks of the cotton samples in the range of 3550-3000 cm^{-1} (spectra not shown), an improved spectrum resolution can be obtained for the ATR-FTIR spectra deconvoluted from a background scattering using a Gaussian function for curve-fitting (Figure 6). Table 2 summarizes the characteristics of the hydrogen bonds of the cotton samples derived from the deconvoluted ATR-FTIR spectra.

The total peak areas of the ATR-FTIR spectra in the region of 3550-3000 cm^{-1} decreased after the cellulase treatments, indicating that the relative intensities of the total hydrogen bonds declined in the surface of cotton samples. However, different changes in different types of hydrogen bonds were observed. For the cotton samples treated with the free and immobilized cellulases, the intensity and percentage of the intermolecular hydrogen bond of O(6)H···O(3) decreased, while those of the intramolecular hydrogen bonds of O(2)H···O(6) and O(3)H···O(5) increased. Moreover, the decreases in the intensity and percentage of the intermolecular hydrogen bonds of cotton samples treated with free cellulase were higher than those of the immobilized cellulase treated

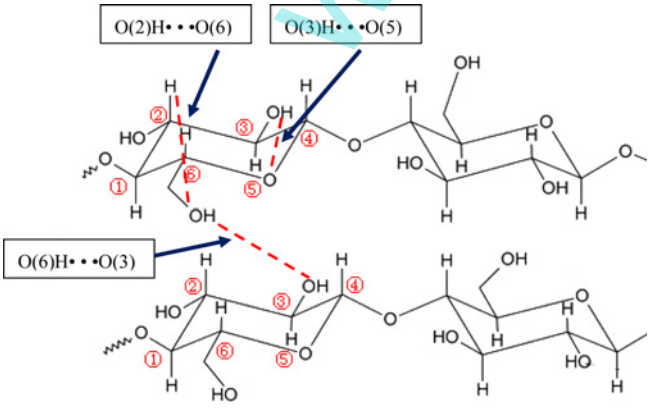

Figure 5. The structure of hydrogen bonding pattern in cellulose.

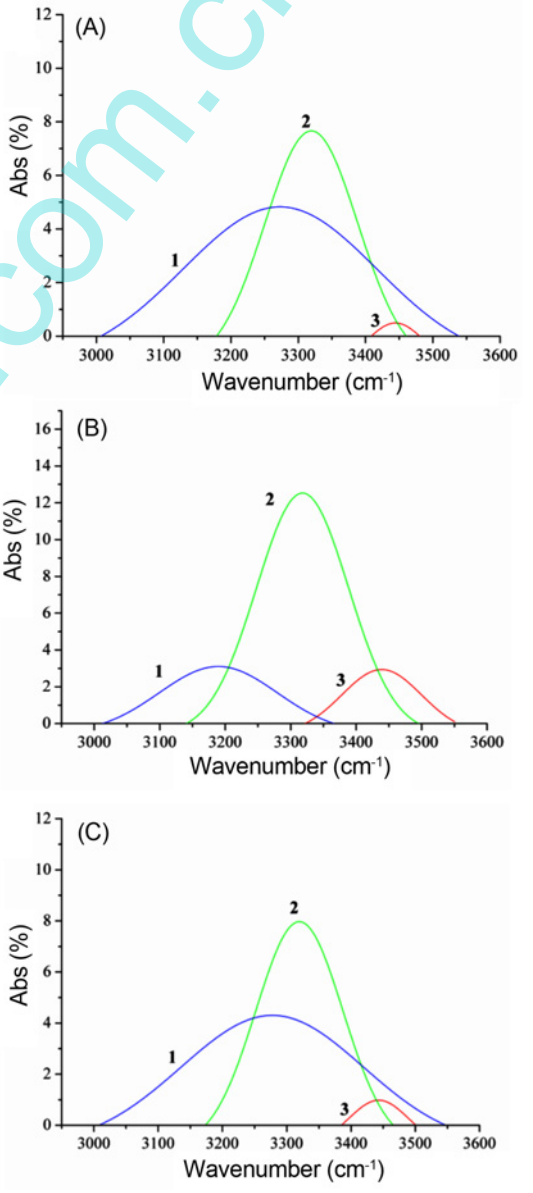

Figure 6. Deconvoluted FT-IR spectra in the range of 3000-3550 cm^{-1} for (A) untreated cotton, (B) cotton treated with free cellulase, and (C) cotton treated with immobilized cellulase. 1: O(6)H···O(3), 2: O(3)H···O(5), 3: O(2)H···O(6).

Table 2. Characteristics of hydrogen bonds of cotton fibres

Sample	Hydrogen bond type	Wavenumber (cm^{-1})	Intensity	Integral area of peak		Percentage (%)
				Total	Individual	
Untreated	O(6)H···O(3)	3272.85	5.90	3803.61	2131.80	56.05
	O(3)H···O(5)	3319.51	8.73		1508.80	39.67
	O(2)H···O(6)	3444.52	1.57		163.01	4.28
Free cellulase treated	O(6)H···O(3)	3189.45	3.64	3656.05	825.69	22.58
	O(3)H···O(5)	3318.16	13.07		2301.77	62.96
	O(2)H···O(6)	3439.05	3.476		528.59	14.46
Immobilized cellulase treated	O(6)H···O(3)	3277.59	5.15	3651.32	1862.67	51.01
	O(3)H···O(5)	3319.65	8.82		1534.28	42.02
	O(2)H···O(6)	3443.12	1.82		254.37	6.97

ones. These results indicated that the damage to the structure of cotton fibres caused by the free cellulase treatment was more substantial than that caused by the immobilized cellulase treatment, because the intermolecular hydrogen bonding plays a dominant role in bonding cellulose molecules together.

Effect of Cellulase Treatments on Accessibility of Cotton Fibres

The determination of the accessibility of the fibres treated with free and immobilized cellulases can be useful for understanding the enzymatic activities on the fibres. The fibre accessibility can provide valuable information about the fine fibre structures in the crystalline and amorphous regions, microfibrillar structure and morphology, the pores, cavities, and spaces between the microfibrils [23,24].

The fibre accessibility was evaluated by a methylene blue (MB) adsorption method. It is assumed that the increase in methylene blue sorption value (MBSV) is primarily due to the increase in the accessibility of cotton fibres. MBSV of the cotton fibres is shown in Figure 7. The results revealed that both free and immobilized cellulase treatments led to the increase in the accessibility of the fibres, and the fibres treated with the free cellulase showed a larger increase in accessibility than those treated with the immobilized cellulase. The major reason for this phenomenon is the cellulase actions in the interior of the fibres, which lead to the increase in the sizes of the pores, cavities, and spaces between microfibrils, i.e., increase in accessibility. This finding has fully demonstrated that the hydrolysis in the interior of the cotton fibres during the immobilized cellulase treatment was less than that during the free cellulase treatment, because the action of immobilized cellulase was limited to the surfaces of cotton fibres.

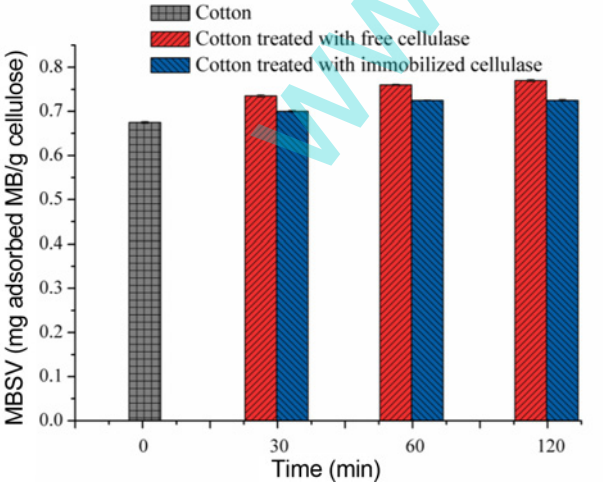

Figure 7. Methylene blue absorption value (MBSV) of cotton fibres without treatment and with enzymatic treatments.

X-ray Diffraction

The crystalline structure of cellulose is one of the most important parameters, which has to be considered for understanding the enzymatic degradation of cellulose. Typical X-ray diffraction intensity profiles of the cotton fibres treated by the free or immobilized cellulases are presented in Figure 8. The measured peaks are located near the diffraction angles

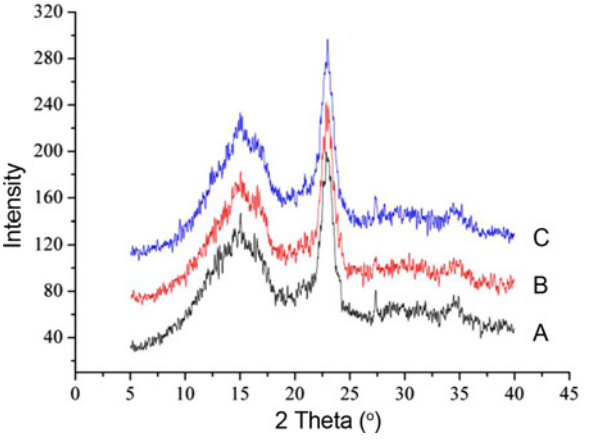

Figure 8. X-ray diffraction patterns of cotton samples; (A) cotton, (B) cotton treated with free cellulase, and (C) cotton treated with immobilized cellulase.

Table 3. X-ray diffraction results of cotton fibres

Sample	I_{am} 2θ=18°	I_{101} 2θ=15°	$I_{10\text{-}1}$ 2θ=16°	I_{002} 2θ=22°	I_{040} 2θ=34°	CrI (%)
Untreated	66	147	130	201	77	67.16
Free cellulase treated	61	143	131	203	68	69.95
Immobilized cellulase treated	70	154	135	218	75	67.89

of 2θ 15, 16, 22, and 34°, which are the characteristics of 101, 10-1, 002, and 040 reflections for cellulose I, respectively. The changes in CrI due to the cellulase treatments are shown in Table 3. A slight increase in the CrI values of the cotton samples was observed after the cellulase treatments as compared with the results of the control samples, because the amorphous portion of the cotton fibres was more readily hydrolysed by the cellulases than the crystalline portion. The CrI value of the cotton fibres treated with the free cellulase was higher than that of the immobilized-cellulase treated ones, implying that the amorphous portion of the cotton fibres was suffered a higher level of hydrolysis by the free cellulase than the crystalline portion.

Conclusion

The surface morphology and structure of cotton fibres treated by free or soluble immobilized-cellulases were studied. As seen from TM-AFM and SEM images, the immobilized cellulase treatment could produce smooth cotton fibre surfaces without causing serious damages to the fibre surface structures. ATR-FTIR was used to investigate the hydrogen bonds in the certain depth area beneath the fibre surface. It was found that the intensity of the intermolecular hydrogen bonding decreased due to the cellulase treatments. The results of accessibility measurement indicated that the hydrolysis of fibre by the immobilized cellulase was limited in the interior of the fibres. From XRD patterns, the decrease in the degree of hydrolysis of the cotton fibres at the amorphous region caused by the immobilized cellulase can be observed.

Acknowledgements

This work was financially supported by National Natural Science Foundation of China (51173071), Program for New Century Excellent Talents in University (NCET-12-0883), Program for Changjiang Scholars and Innovative Research Team in University (IRT1135), the Fundamental Research Funds for the Central Universities (JUSRP51312B), the graduate student innovation plan of Jiangsu Province of China (CXZZ11_0473).

References

1. A. Aly, A. Moustafa, and A. Hebeish, *J. Clean. Prod.*, **7**, 12 (2004).
2. H. Belghiht, S. Ellouz-Chaabouni, and A. Gargouri, *J. Biotechnol.*, **2-3**, 89 (2001).
3. A. Cavaco-Paulo, L. Almeida, and D. Bishop, *AATCC Rev.*, **6**, 28 (1996).
4. M. Hashem and N. Ibrahim, *J. Text. Association*, **11-12**, 63 (2002).
5. L. Hao, R. Wang, J. Liu, and R. Liu, *Carbohyd. Polym.*, **1**, 89 (2012).
6. A. Hebeish and N. Ibrahim, *Colourage*, **4**, 54 (2007).
7. N. Ibrahim, K. El-Badry, B. Eid, and T. Hassan, *Carbohyd. Polym.*, **1**, 83 (2011).
8. L. Hao, R. Wang, J. Liu, and R. Liu, *Carbohyd. Polym.*, **1**, 89 (2012).
9. N. Ortega, M. Busto, and M. Perez-Mateos, *Int. Biodeter. Biodegr.*, **1**, 47 (2001).
10. L. Fan, Y. Lee, and D. Beardmore, "Advances in Biochemical Engineering", Springer-Verlag, Berlin, 1980.
11. Y. Yu, J. Yuan, Q. Wang, X. Fan, and P. Wang, *Appl. Biochem. Biotech.*, **6**, 166 (2012).
12. Y. Yu, J. Yuan, Q. Wang, X. Fan, P. Wang, and X. Sun, *Eng. Life. Sci.*, **2**, 13 (2013).
13. Y. Yu, J. Yuan, Q. Wang, X. Fan, X. Ni, P. Wang, and L. Cui, *Carbohyd. Polym.*, **2**, 95 (2013).
14. E. Hequet, N. Abidi, and J. Gourlot, *J. Cotton Sci.*, **2**, 4 (1998).
15. B. Wu, L. Wang, W. Zhang, Y. Zhao, and P. Gao, *J. Cell. Sci. Technol.*, **12**, 4 (2004).
16. L. Wang, Y. Zhang, P. Gao, D. Shi, H. Liu, and H. Gao, *Biotechnol. Bioeng.*, **3**, 93 (2006).
17. H. Liu, S. Fu, J. Zhu, H. Li, and H. Zhan, *Enzyme Microb. Tech.*, **4**, 45 (2009).
18. S. Hanley, J. Giasson, J. Revol, and D. Gray, *Polymer*, **21**, 33 (1992).
19. I. Lee, B. Evans, and J. Woodward, *Ultramicroscopy*, **1-4**, 82 (2000).
20. I. Lee, B. Evans, L. Lane, and J. Woodward, *Bioresource Technol.*, **2**, 58 (1996).
21. T. Kondo, "Polysaccharides, Structural Diversity and Functional Versatility", 2rd ed., pp.69-95, Marcel Dekker, New York, 2005.
22. D. Ciolacu, J. Kovac, and V. Kokol, *Carbohyd. Res.*, **5**, 345 (2010).
23. C. Caparrós, C. López, M. Torrell, N. Lant, J. Smets, and A. Cavaco-Paulo, *Biotech. J.*, **2**, 7 (2012).
24. V. Ribitsch, K. Stana-Kleinschek, T. Kreze, and S. Strnad, *Macromol. Mater. Eng.*, **10**, 286 (2001).