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Carbohydrate Polymers 72 (2008) 178–184
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Effect of enzymatic treatment on cotton fiber dissolution in NaOH/urea solution at cold temperature

Ying Wang [a,b], Yulin Zhao [b], Yulin Deng [a,b,*]

[a] School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA 30332-0620, USA
[b] Institute of Paper Science and Technology, Georgia Institute of Technology, Atlanta, GA 30332-0620, USA

Received 6 June 2007; received in revised form 31 July 2007; accepted 2 August 2007
Available online 17 August 2007

Abstract

In this communication, the dissolution behavior of enzyme pretreated cotton fibers in NaOH/urea solution is reported. The experimental results indicated that although the crystallinity of cotton linter almost did not change during the enzymatic pretreatment, the solubility of cellulose in cold NaOH/urea solution increased from 30% for original cotton fibers to 65% for enzymatic treated fibers, which was mainly attributed to the reduction of cellulose's molecular weight by the enzymatic treatment. Moreover, the dissolution time was also greatly shortened by the enzymatic pretreatment. The results suggest that the effect of crystallinity of the cellulose on the cellulose dissolution in NaOH/urea solution is much less than that of molecular weight. It was found that the temperature plays a dominating role to the cellulose fiber solubility in NaOH/urea solution.

Keywords: Cellulose; Crystallinity; Enzyme; Dissolution; Molecular weight

1. Introduction

Cellulose, as an environmentally friendly and renewable biomaterial, constitutes around 1.5×10^{12} tons of the total annual biomass production. Cellulose has basic molecular unit of $C_6H_{10}O_5$ and is linked in the form of β-1,4-glucan. Each chain unit of cellulose contains three hydroxyl groups which make cellulose a hydrophilic material. However, cellulose is hard to be dissolved in aqueous solutions due to the existing of large quantities of inter- and intra-molecular hydrogen bonds and considerable van der Waals forces between the non-polar groups.

Cellulose is a material that does not melt at the temperature lower than its degradation temperature. Strong intra- and inter-molecular hydrogen bonds in cellulose prevent its molecules from dissolution in most common solvents. Although there are a number of approaches to produce regenerated cellulose, such as viscose rayon, cuprammonium cellulose, Lyocell fibers, the market is shrinking due to the environmental and economic feasibility concerns of these methods. The high cost in recoverying organosolvents also hinders their further applications in large scale. For these reasons, cellulosic materials are regarded as unmoldable materials. Because of the un-moldable identity, wood and cotton fibers are difficult to be refabricated as other thermosetting and thermoplastic polymers. If an effective, economic and environmentally friendly cellulose dissolution method can be developed, a new platform for producing moldable cellulosic intermediate materials will be created, which provides new opportunities for using cellulosic materials as a renewable and sustainable engineering polymers.

Sodium hydroxide is a simple chemical that can swell cellulose in a certain concentration, and even can dissolve cellulose at high NaOH concentration. The dissolution mechanism is that soda hydrates can penetrate the amorphous area of cellulose, and destruct the neighboring crys-

* Corresponding author. Address: School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA 30332-0620, USA. Tel.: +1 404 894 5759; fax: +1 404 894 4778.
E-mail address: yulin.deng@chbe.gatech.edu (Y. Deng).

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doi:10.1016/j.carbpol.2007.08.003

talline regions. It was found that for low to moderate degree of polymerization (DP) of cellulose, the maximal solubility occurs with 8–10% soda solution. Cellulose dissolution in aqueous NaOH was studied with particular attention on the roles of crystalline form and molecular weight in other's works (Isogai & Atalla 1998). However, NaOH alone has never been used as a solvent for dissolving cellulose in industrial applications because of the chemical degradation of cellulose, high soda concentration and chemical recovery problems.

In recent years, researchers found that sodium hydroxide with urea at cold temperature can dissolve cellulose better than sodium hydroxide alone. Zhang's research group investigated the cellulose dissolution chemistry in NaOH/urea solution, and found that certain compositions of NaOH/urea and NaOH/thiourea are good solvents for cellulose (Cai & Zhang, 2005; Zhang, Ruan, & Gao, 2002; Zhou, Qin, Liu, & Zhang, 2006; Zhou, Zhang, & Cai, 2004; Zhou, Zhang, Cai, & Shu, 2002; Zhou, Zhang, Li, Wu, & Cheng, 2005). According to their research, the sodium hydroxide and urea aqueous solutions are non-derivatizing solvents for cellulose to form true solution. The proposed mechanism is that NaOH hydrates–urea hydrates–free water–cellulose form a special complex in the solution. NaOH destroys inter- and intra-hydrogen bonds between cellulose molecules and urea hydrates function as hydrogen bonds donor and receptor between solvent molecules and prevent the reassociation of cellulose molecules, thus leading to molecular dissolution of cellulose. Laszkiewicz (1998), Isogai and Atalla (1998) and Zhou et al.'s works (2004 and 2006) suggested that there exists an upper DP limit of cellulose for each solution system, and those cellulose with a higher DP value beyond the corresponding upper limit cannot be dissolved in the solution.

Cellulase, an enzyme, can be used to cut high molecular weight cellulose. Bhat and Bhat (1997) did a comprehensive review on cellulose degrading enzymes and their potential industrial application. Rahkamo et al. (1998) applied enzyme treatment on hardwood to dissolve pulp and improve the solubility in 9% of NaOH solution. The effect of *Trichoderma* cellulases on the fine structure of a bleached softwood kraft pulp was studied by Ramos, Nazhad, and Saddler (1993) and Ramos, Filho, Deschamps, and Saddler (1999). All the enzymes used in their study, including Celluclast, have high hemicellulose activity and can remove most of the residual hemicellulose from softwood kraft pulp. In the process of enzyme hydrolysis, the reduction of cellulose DP can only be observed at the early stages induced by a random attack of endoglucanases at amorphous regions. Cellobiohydrolases (CBHs) can remove cellobiose residues from the end of cellulose chains, therefore, can gradually, but only slightly, decrease the degree of polymerization. Ramos and his coworkers did not find much effect of enzyme treatment on cellulose crystallinity, but they suggested that the crystallinity change can be observed after longer incubation time. The enzyme prefers to attack the crystalline at (002) diffraction plane (Cao & Tan, 2005), so it can be applied to cut the length of crystal.

It is well known that molecular weight, crystal size and crystal packing pattern will affect the dissolution of polymer in a solution. However, it is not clear if the molecular weight and crystal properties have the same effect on the dissolution using NaOH alone and NaOH/urea solution. It is also unknown how high the dissolution degree can be achieved with NaOH/urea solution after enzymatic pretreatment. In this study, enzyme was utilized as a tool to tailor cellulose molecules to the desired molecular weight to facilitate their dissolution in NaOH/urea solution. We will show the advantages of the two-stage cotton fibers dissolution using enzyme and NaOH/urea solution. The comparisons with NaOH/urea treatment and NaOH treatment alone will be discussed.

2. Experimental

2.1. Materials and chemicals

The cellulose material used in this study was cotton linters (Procter & Gamble Company). Celluclast 1.5 L enzyme is a gift from Novozymes North America, Inc. It has 60 filter paper units (FPU per gram of liquid and a density of 1.1989 g/ml. Urea, in crystal form, is from Aldrich and NaOH in 50% solution is from VWR. The chemicals were used as received.

2.2. Sample treatments

For NaOH treatment, the cotton linters were mixed with various concentrations of NaOH aqueous solutions to make a 2% slurry at room temperature, and then put into freezer at −15 °C for 72 h. After the samples were solid frozen, they were taken out from the freezer and stirred at room temperature for 10 min after the thaw of the solid. The dissolved part and undissolved part were separated by centrifuge at 7000 rpm. The residue part was washed with deionized (DI) water adequately and then oven dried and stored in a desiccator. The dissolved part was regenerated by neutralizing alkaline with hydrochloric acid, then oven dried and weighted.

For NaOH/urea treatment, the cotton linters were mixed with 6% NaOH and 4% urea at room temperature to make a 2% cellulose slurry, then put into freezer at −15 °C for various time. The frozen samples were taken out and subjected to the same treatment as in NaOH treatment.

Cotton linters were used in enzymatic pretreatment and dissolution experiments. The linters were first mixed with, pH 4.5, acetate buffer to make a 5% slurry, then pre-heated to 50 °C through water bath, after that, celluclast was added at the dosage of 0.4 ml/g substrate. The reaction was carried out with mixing under 200 rpm. The treating time was varied from 0.5 h to 4 h. After the treatment, the reaction was immediately stopped by submerging into boiling water and followed by filtration and extensive washing with DI water. The washed samples were vacuum oven dried for 48 h at 60 °C. Then the samples were stored

in a desiccator and were subjected to NaOH/urea treatment later.

2.3. Characterization

The dissolution degree of cellulose was defined as the weight of regenerated cellulose divided by original oven dried weight of cellulose

$$S = W_r/W_o \times 100\% \quad (1)$$

where S is solubility degree, W_r is the weight of regenerated cellulose, and W_o is the original weight of cellulose.

The molecular weight of cellulose residue was calculated through the correlation between DP and viscosity using the equation (Immergut, Ranby, & Mark, 1953)

$$DP^{0.905} = 0.75[\eta] \quad (2)$$

and the viscosity was measured according to Tappi Test Method T230 om-94.

The crystallinity and crystallite size were characterized using Powder X-ray Diffraction (XRD). The Bragg angle was scanned from 5 to 40. The X-ray source is CuKα with wave length of 0.154 nm.

3. Results and discussion

3.1. Solubility

Isogai and Atalla (1998) reported that 9% NaOH solution could dissolve cellulose with DP lower than 200. Zhang's group claimed that cotton linters with DP lower than 400 can be rapidly dissolved in 6% NaOH/4% urea within 5 min after freezing for 12 h (Cai & Zhang, 2005). However, for the common wood fibers and cotton linters which have much higher DP, i.e. above 800, considerable amount of cotton residues were observed by naked eyes after NaOH/urea treatment. The solubility of the cotton linter (DP of 850) was shown in Fig. 1.

Fig. 1 is the effects of treating time and temperature on the solubility of cotton linter in NaOH/urea solution. After 24 h treatment by soaking the cotton liner (DP of 790) samples in the solutions at different temperatures, the solubility is only about 3% for room temperature treatment and 5% for 4 °C temperature treatment, while at −15 °C, the solubility was dramatically enhanced to 31%. A significant effect of soaking time on the dissolution degree was also observed for NaOH/urea up to 48 h treatment at −15 °C. However, the longer treatment time beyond 48 h could not further improve the solubility, which suggests the up limitation of the dissolution under this condition. The highest solubility obtained in 6% NaOH/4% urea solution is 35%, which means 65% of cotton linters were undissolved. The limit of the dissolution degree may be due to crystallinity, crystal size, and DP of cellulose. In this study, various DPs of cellulose were obtained by enzyme pretreatment of cotton linter.

The yield of cellulose after enzyme hydrolysis was measured by the weight loss of cotton linter. The data were shown in Fig. 2. The yield dropped quickly to 82% during the first hour of treatment, then went to a much slower rate, suggesting that there are two different underlying mechanisms. The yield was about 95% after half an hour treatment.

The effects of enzyme pre-treatment on the solubility of cellulose in NaOH/urea solution were studied and shown in Fig. 3. In 2.5 h and 4 h of NaOH/urea treatments, the solubility increased from around an estimated 5–30% and 60%, respectively. The estimated 5%, which was read from Fig. 1, was based on the same NaOH/urea treatment time both for enzyme pretreated and un-pretreated samples. The maximal solubility was increased from 30% to 65%. The experiment results showed that, the solubility dramatically enhanced with enzyme pretreatment and the dissolution time largely shortened comparing with the data without enzyme pretreatment. It was also shown that it was also shown that

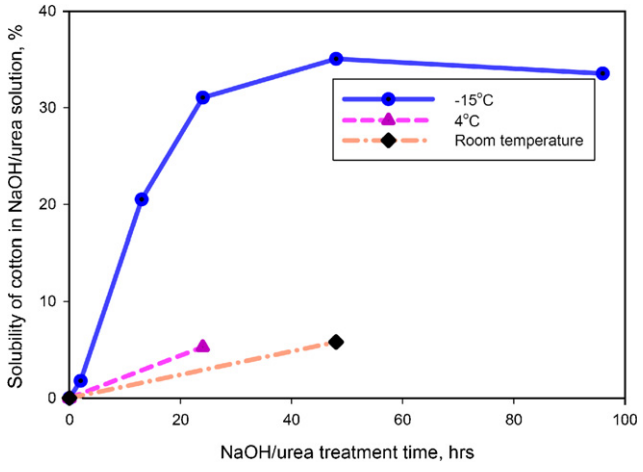

Fig. 1. Solubility of cotton linter in NaOH/urea solution.

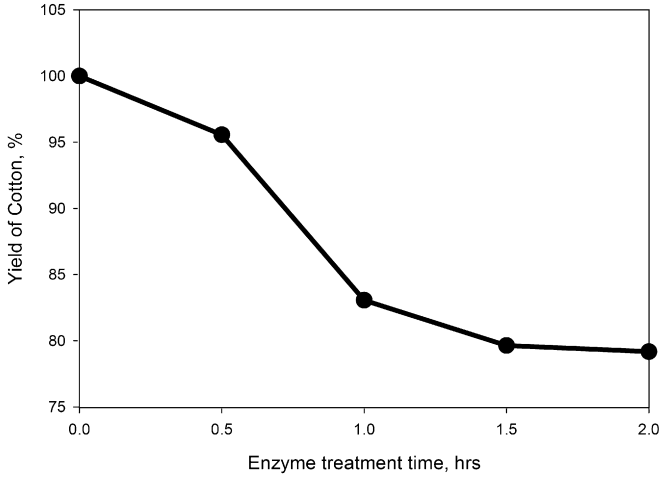

Fig. 2. Yield of cotton linter after enzymatic hydrolysis.

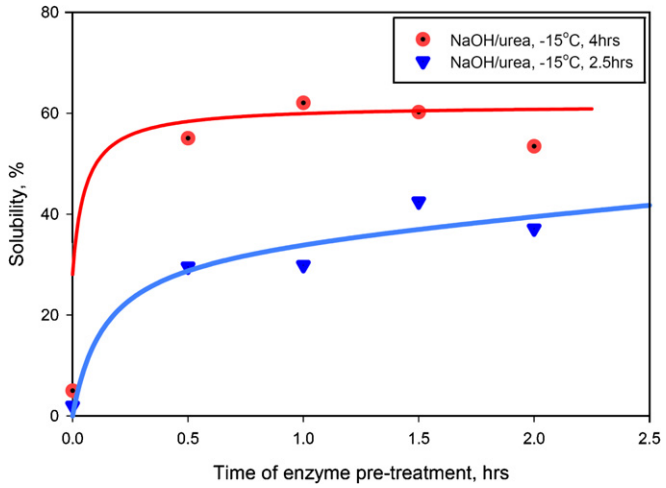

Fig. 3. Solubility of enzyme pretreated cotton linter in NaOH/urea solution as a function of enzymatic hydrolysis time.

cellulose solubility was increased rapidly during the first hour of enzymatic pretreatment, and then changed slowly up to 2 h of treatment time.

The reason for reaching the maximum solubility in a very short pretreatment time is not clear.

3.2. Morphology change

Fig. 4 shows the morphology change of cotton linter due to enzyme and NaOH/urea treatment observed by polarized optical microscope. Enzyme treatment did not change the shape of fiber except smoothening the fiber surface by removing fibrillars (shown in Fig. 4a and b) and shortening the fiber length. However, the treatment of the mixture of cotton linter and NaOH/urea solution at frozen temperature remarkable changed the fiber shape and size. Clearly, the freezing treatment of cotton linter and NaOH/urea solution still plays a dominating role in the fiber dissolution even after enzyme pretreatment, as compared in Fig. 4c and d. The fibers were highly swollen and most of the cellulose crystals have diminished (Fig. 4d).

3.3. Crystallinity and crystal size

Crystallinity obtained from Powder X-ray Diffraction data is calculated according to Martin and Segal, Creely, Martin, and Conrad (1959) method. This method is fast and easy. It uses the height of (200) peak and the minimum between (200) and (110) peaks, assuming that Intensity of (200) represents both crystalline and amorphous part while

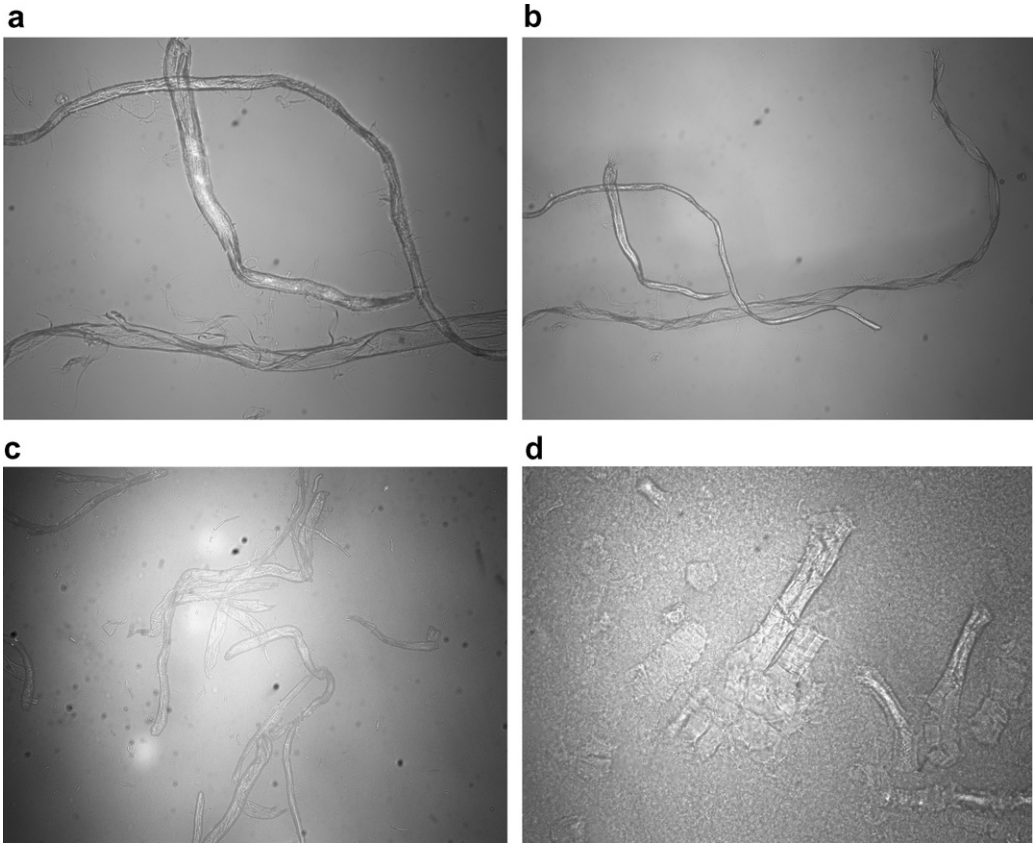

Fig. 4. Morphology change of cotton fibers: (a) cotton linter; (b) enzyme treatment; (c) enzyme pretreatment followed by NaOH/urea treatment at room temperature; and (d) enzyme pretreatment followed by NaOH/urea treatment at cold temperature.

the minimum intensity at the mentioned location is for amorphous part only.

$$\mathrm{CrI} = \left(I_{(200)} - I_{(\mathrm{am})}\right)/I_{(200)} \quad (3)$$

where CrI is the crystallinity index, $I_{(200)}$ is the intensity at (200) peak ($2\theta = 22.7°$) and I_{am} is the intensity at the minimum between (110) peak and (200) peak.

Commercial cellulose fibrous from Sigma was used as standards to test the repeatability and accuracy of crystallinity analysis by XRD. Good repeatability was obtained with crystallinity of $93.3 \pm 0.2\%$.

Fig. 5 was XRD spectra of pure Sigma cellulose (a), enzyme treated bleached kraft softwood fiber (b), undissolved (c) and regenerated bleached kraft softwood fiber (d) from direct treatment of NaOH/urea solution. Pure Sigma cellulose is in cellulose I crystal type, same as native cellulose, and enzyme treatment did not change the crystal type, however after NaOH/urea treatment, the crystal type changed from cellulose I to cellulose II for both the undissolved residue and the regenerated cellulose. The crystal size was calculated using Scherrer's equation (Nieduszynski & Preston, 1970):

$$L(h\,k\,l) = k\lambda/(B\cos\theta) \quad (4)$$

where L is the crystal dimension at the (h k l) lattice plane of diffraction, λ is the wavelength of X-ray, k is Scherrer's constant, and B is the half width of peak.

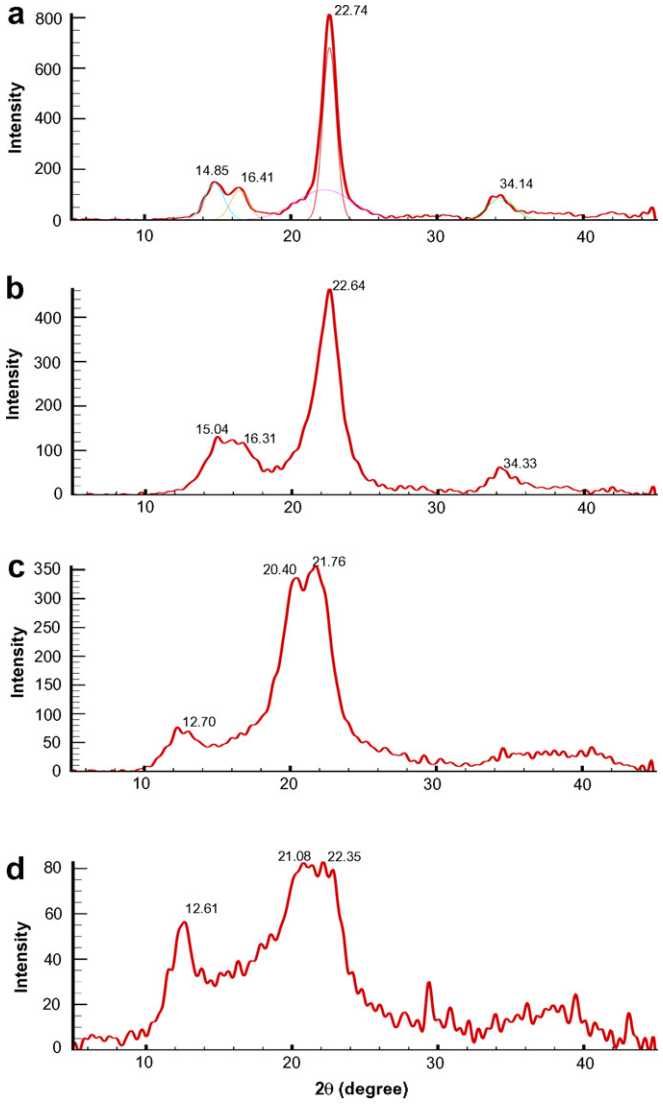

Fig. 5. Crystal types of cellulose by XRD (a) is pure cellulose from Sigma; (b) is enzyme treated bleached softwood fiber; (c) and (d) are undissolved and regenerated bleached softwood fiber from NaOH/urea solution, respectively.

The DP and crystallinity index of cellulose from different sources and their solubility in NaOH/urea solution were summarized in Table 1. It can be seen from Table 1 that pure Sigma cellulose, which has the lowest DP and the highest crystallinity index, gave the highest solubility among all the samples. Enzyme treatment did not obviously decrease the crystallinity of cotton linter, but did improve the solubility significantly. Ramos (Ramos, Filho, Deschamps, & Saddler, 1999) also found that for high molecular weight wood fiber, enzyme treatment did not obviously affect crystallinity. Based on the results of our experiments, it can be concluded that DP of cellulose plays a more important role in cellulose dissolution in NaOH/urea solution than cellulose crystallinity does, and cellulose with high crystallinity does not necessarily lead to low solubility.

The XRD spectra of assorted treated cotton linters were shown in Fig. 6, where we can see that for enzyme treated sample, the basic shape of spectra in (a) did not change except a slight increase of the intensity of the (200) peak, corresponding to a slight increase of crystallinity. This can be explained by that the endoglucanase component in Celluclast attacks the amorphous area of cellulose, leaving this area exposed for CBHs to react, but this process is relatively slow so that we can only observe small increase of the crystallinity after 2 h of enzymatic treatment (see Table 2). The effect of enzyme treatment on NaOH and NaOH/urea treated cotton residues was investigated and no crystal type change was found as shown in Fig. 6b, comparing with NaOH and NaOH/urea treated cotton residues. For short time NaOH/urea treatments, crystal type change was not observed. However, for the samples under 72 h of treatments by different concentrations of NaOH, the crystal type change from cellulose I to cellulose II was observed and the peaks shifted to lower Bragg angles,

Table 1
Crystallinity Index (CrI) and solubility in NaOH/urea solution for different fibers

Fiber	DP	CrI (%)	Solubility (%)
Cotton linter	850	91.6 ± 0.5	20–30
Bleached SW fiber	1090	82.5 ± 0.5	20–30
Sigma cellulose fibrous	216	93.3 ± 0.2	>90
Enzyme treated cotton linter	620–680	90.9 ± 0.6	>60
Regenerated Softwood from NaOH/urea solution		66.0 ± 0.5	

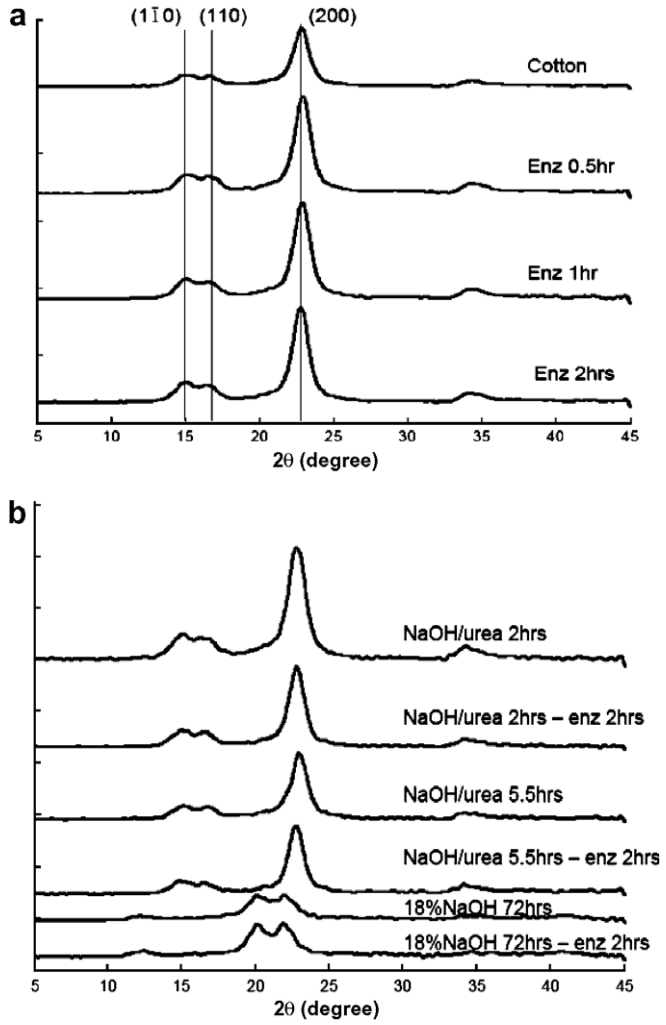

Fig. 6. Crystal structure change of cotton linter prepared by (a) enzyme treatments; (b) NaOH, NaOH/urea, NaOH–enzyme and NaOH/urea–enzyme treatments.

suggesting that the crystallite sizes decreased with the NaOH treatments. The calculated data were summarized in Table 2, showing the d-spacing of crystal lattices, crystal dimensions perpendicular to (1$\bar{1}$0), (110), and (200) lattice planes and the crystallinity index for cotton linters. The d-spacing and crystal dimension of these diffraction planes did not change for enzyme treated cotton linter and also for NaOH/urea treatment of up to 5.5 h. As a comparison, for 6% and 14% NaOH freezing treatment after 72 h, d-spacing changed from 0.592, 0.532, and 0.389 to 0.722, 0.436, and 0.402 for cotton treated by 6% NaOH, suggesting that crystal type has changed from cellulose I to cellulose II. Meanwhile, crystal size apparently decreased.

3.4. Degree of polymerization

The effect of enzyme treatment on the DP of cellulose was manifested in Fig. 7a. At the initial state of enzyme hydrolysis, the reduction of DP was extremely obvious. However, cellulose DP dropped slowly for the extended treatment duration.

Comparing with enzyme treatment, the effects of NaOH and NaOH/urea treatment on DP of cellulose were also investigated (Fig. 7b and c). The time required to reduce cellulose DP to 650 by the enzyme treatment was 2 h. However, it took 24 h for the NaOH/urea approach to achieve the same reduction of cellulose DP. This showed that cellulose DP reduction by enzyme treatment was much faster than that by NaOH/urea treatment. The effect of NaOH concentration on cellulose DP was shown in Fig. 7c. With higher NaOH concentration, greater cellulose degradation was observed.

The effect of molecular weigh on the solubility of is shown in Fig. 7d. It is shown that the solubility decreased as the degree of the polymerization increased for the cellulose treated by NaOH/urea solution for 2.5 and 4 h.

Although both enzyme and NaOH/urea treatments can degrade cellulose, their roles in the two step treatment are different. Cellulase attacks the cellulose crystal from (002) lattice plane, cutting cellulose molecules. This effect is dominant especially at the initial stage of enzyme treatment, which is shown in Fig. 7a. NaOH/urea solution interacts with cellulose crystal to break the inter- and intra-cellulose hydrogen bonds to obtain individual cellulose molecules. The enzyme pretreatment reduces the length of cellulose crystal, thus let it be easily penetrated by NaOH and urea solution.

Table 2
Crystallinity and crystal size change of cotton with enzyme treatment

Samples	d-spacing (nm)			Crystallite dimension (nm)	CrI (%)
	(1$\bar{1}$0)	(110)	(200)	(200)	
Cotton	0.592	0.532	0.389	6.97	91.6 ± 0.5
Enzyme 0.5 h	0.586	0.529	0.387	6.54	90.5 ± 1.0
Enzyme 1 h	0.593	0.534	0.389	7.47	90.9 ± 0.4
Enzyme 1.5 h	0.595	0.535	0.391	6.82	91.1 ± 0.5
Enzyme 2.0 h	0.595	0.535	0.390	6.82	91.2 ± 0.3
NaOH/urea, −15 °C, 2 h	0.594	0.537	0.389	6.80	89.8 ± 0.5
NaOH/urea, −15 °C, 5.5 h	0.589	0.531	0.388	6.28	90.1 ± 0.5
6% NaOH −15 °C, 72 h	0.722	0.436	0.402	4.96	82.6 ± 0.5
14% NaOH −15 °C, 72 h	0.722	0.442	0.406	5.34	83.1 ± 0.5

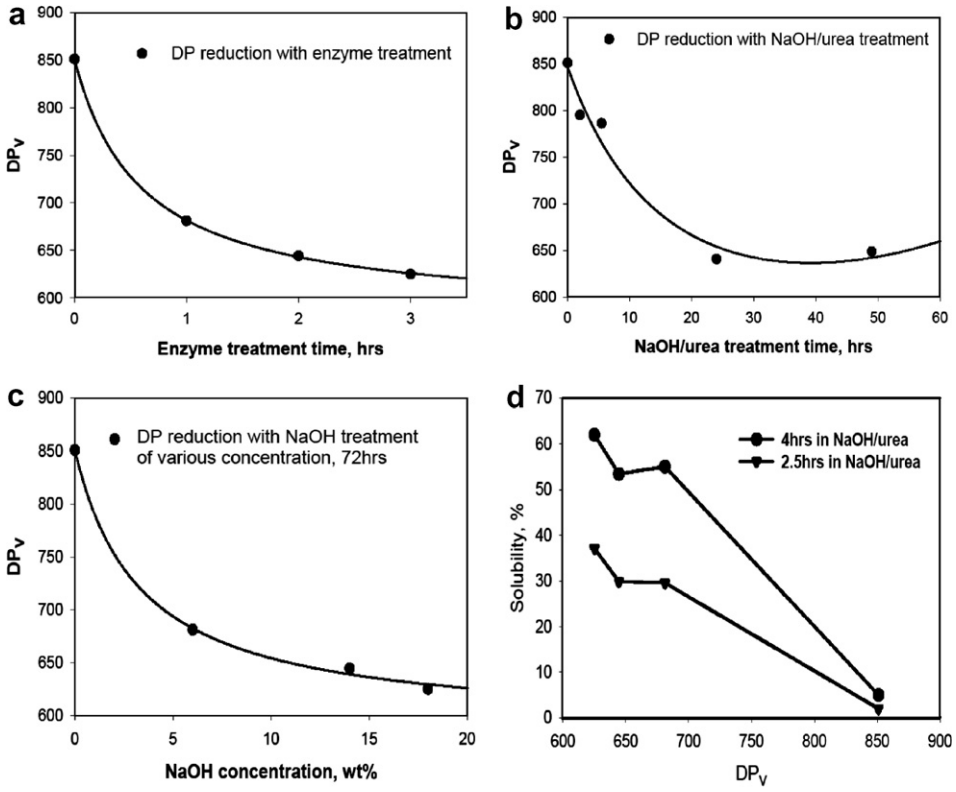

Fig. 7. DP changes with (a) various time of enzyme treatment at the dosage of 0.4 ml enzyme per gram of oven dried cotton fiber; (b) various time of 6% NaOH/4% urea treatment at 2% solid contents of cotton fiber in solution; (c) various concentrations of NaOH treatment at 2% solid contents of cotton fiber in solution for 72 h; (d) the solubility changes in 6% NaOH/4% urea solution as a function of degree of polymerization tailored by enzyme pretreatment as in (a).

4. Conclusions

Enzyme pretreatment greatly enhanced the dissolution degree of cellulose in NaOH/urea solution with much shorter time. Enzyme treatments did not change crystal type and crystal size, slightly increased crystallinity of cellulose, but reduced the molecular weight rapidly. High crystallinity did not necessarily result in low solubility, or at least crystallinity alone could not explain the difficulty of cellulose dissolution.

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