Alkaline Treatment of Diacetate Fibers and Subsequent Cellulase Degradation

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ABSTRACT: The work investigated the degradation behavior of cellulose acetate (CA) fibers in NaOH solutions in heterogeneous conditions and the effect of alkaline treatment on cellulase degradation of CA fibers. Weight analysis and IR spectra showed that the weight loss of CA fibers immersed in NaOH solution chiefly depended on acetylation. Alkaline treatment promoted the degradation of CA fibers in cellulase solution by reason of deacetylation, especially when the degree of substitution (DS) of CA fibers reached 0.8, cellulase degradation increased most markedly. SEM revealed a smooth surface except some thin holes in the CA fibers after immersion in NaOH solution with a lower concentration because of the formation of alkaline cellulose, and only in a higher concentration such as 5.0*N*, it could be observed that microfibers perpendicular to fiber axis distributed over the surfaces of the fibers. ^{1}NMR spectra suggested that only in a lower NaOH concentration ($\leq$0.25*N*), deacetylation reaction was affected by the reactivities of ester groups at position 2, 3, and 6 in anhydroglucose unit, but did not follow the theoretical trend in the three positions. Moreover, the DS for polymer molecules in CA fibers were dispersive after alkaline treatment in heterogeneous condition and the DS of the product increased during sequent cellulase degradation. This was also demonstrated by the result of IR analysis and X-ray diffraction. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 2466–2474, 2008

Key words: cellulose acetate; cellulase; alkaline treatment; NMR; FTIR; biodegradation

INTRODUCTION

Cellulose acetate (CA) is one of the most important cellulose derivatives because of its wide application in textiles, plastic film, packaging, and cigarette filter tow. The properties of CA depend on its DS, i.e. the average number of acetyl groups per anhydroglucose unit (AGU), and on the substituent distribution at three possible sites of AUG and along the length of cellulose chain.[1] CAs widely used commercially is cellulose diacetate (DS, 2.5) and cellulose triacetate.

In recent years, the efficient reduction or decomposition of the tremendous amount of refuse generated from our daily lives and industrial activities has become a serious problem of public concern. It is generally recognized that degrading polymer waste by microbe system is most favorable for environment protection. However, there exists a long going debate on the question, whether CA is susceptible to biological systems or not. It is generally assumed that CA with a DS of more than 0.76 is not biodegradable,[2] while recently some authors have claimed that CA with a high DS can be degraded by some microorganisms completely. Sakai and coworkers isolated two bacterial strains of *Neisseria sicca*, SB and SC, which is capable of degrading CA textiles (DS, 2.34),[3,4] and Ishigaki et al. reported bacterium Bacillus sp. S2055, which can degrade CA films (DS, 1.7 and 2.5).[5] It was observed in their researches that CA degradation by these microorganisms led to the product of acetate and reducing sugar, therefore the product of esterase and cellulase associated with CA degradation was suggested. However, they also remarked that these bacteria, which could produce esterase capable of deacetylating the CAs, rarely existed in natural environment, and commercial lipase could not be effective for deacetylation of the CAs, possibly on account of the ability of adhesion or substrate specificity.[6] Degradation of commercial CAs by microbiological reaction alone should be difficult and the most important feature of biodegradable materials, degradability, for them has not yet been achieved. Extensive researches have shown that biodegradability of CA depends on DS, that is, the lower the DS, the more biodegradable CA becomes.[7]

Nevertheless, the acetyl groups of CA can also be eliminated as acetic acid by acidic or basic catalysis without the participation of microorganism.[8] It may also be observed that, above all, under acidic or

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basic condition CA is converted into lower DS polymer, and subsequently degraded by microorganism. Hydrolysis of CA by acid or base catalysis in homogeneous system has been actively investigated, and the results indicated that ester bonds are vulnerable to hydrolyze, and glycosidic linkages are rather stable, especially under mild conditions (i.e., at room temperature)[9]; however, there is limited information available on hydrolysis behavior of CA in heterogeneous condition, and especially there is no report about the effect of this hydrolysis on biodegradability of CA. The work investigated the degradation behavior and mechanism of CA fibers in alkaline solution at room temperature, and reported firstly the influence of alkaline treatment on CA degradation by cellulase, which is a ubiquitous microbial enzyme capable of degrading cellulose. In addition, the changes of structure and morphology in these processes were also investigated especially.

EXPERIMENTAL

Materials

Commercial diacetate fibers (CA fibers) with a DS of 2.5 were acquired from Celanese Chemical Industries. Cellulase used in experiment was given by Novozyme, Denmark, with optimum activity at 50°C and pH 4.8, and stored at 4°C before use. Cellulase activity was 6 U/mg based on manufacturer, but not measured. Deionized water was used throughout the experimental. All other chemicals were of reagent grade and used without further purification.

Treatment of CA fibers in alkaline solution

CA fibers about 6 g were immersed in 800 mL of NaOH standardized solutions with various concentrations (0.1–5.0*N*), and were left at 22°C for a given times (1–4 h). The fibers collected were washed extensively with deionized water, and dried in vacuum at 60°C for 24 h. Weight loss of the sample was calculated according to the difference in weight before and after treatment. To ensure the reliability of results obtained, a pair of experiments proceeded at the same time under the same condition.

Subsequent cellulase degradation of the samples obtained by alkaline treatment

Cellulase with a dosage of 800 U/g (based on dry solids) and 80 mL of acetate buffer (pH 4.8) were charged in a 150 mL conical flask. The dried fibers obtained by the above alkaline immersion and about 3 g were used in experimental of cellulase degradation. Cellulase treatment was performed for 24 h in a gyratory water bath at 50°C and 120 rev/min. The degraded samples were washed extensively with deionized water to remove the cellulase and dissolved sugar by filtration. Weight loss of sample was calculated by determining the weight of samples before and after cellulase degradation. To validate the reproducibility of results, a pair of experiments for the same sample was carried simultaneously.

DS determination by chemical method

The acetyl content (the weight percentage of combined acetic acid) was sought titrimetrically according to ASTM D 871-63. DS was calculated with the following formula:

$$\mathrm{DS} = \frac{3.86 \times \%\,\mathrm{acetyl}}{102.4 - \%\,\mathrm{acetyl}}$$

where %acetyl is the acetyl content.

Scanning electron microscopy

The samples were coated with gold film in order to observe the surface morphology and microstructure. The instrument was a JEOL JSM-5600LV electron microscopic with an accelerating voltage of 15 kV.

FTIR spectroscopy

Original CA fiber and the fibers obtained by alkaline treatment were sliced into thin chips with an Y172 Fiber Microtome by turning control screw about 0.5 pitch every time, and the chipping thickness was about 8 μm; powder samples obtained by cellulase degradation were ground into thin powers. These thin chips and thin powers (2 mg) were evenly mixed with KBr matrix (200 mg) by grinding adequately, and then a pellet was formed by compressing the mixture at 167 MPa and used for FTIR measurement in a Nicolet Nexus670 FTIR spectrometer. A total of 100 scans were taken for each sample with resolution of 2 cm^{-1}.

^{1}H NMR spectroscopy

The samples (20 mg) were dissolved in 0.8 mL deuterated dimethyl sulfoxide (DMSO) in a 5 mm diameter n.m.r tube. After dissolution, they were run at 25°C in a Bruker AV400 NMR spectrometer.

X-ray diffraction analysis

X-ray diffraction was recorded at room temperature from 5° to 50° at a scanning speed of 0.02°/s with a Rigaku-D/Max-2550PC diffractometer using Ni-filtered Cu K_α radiation of wavelength 0.1542 nm.

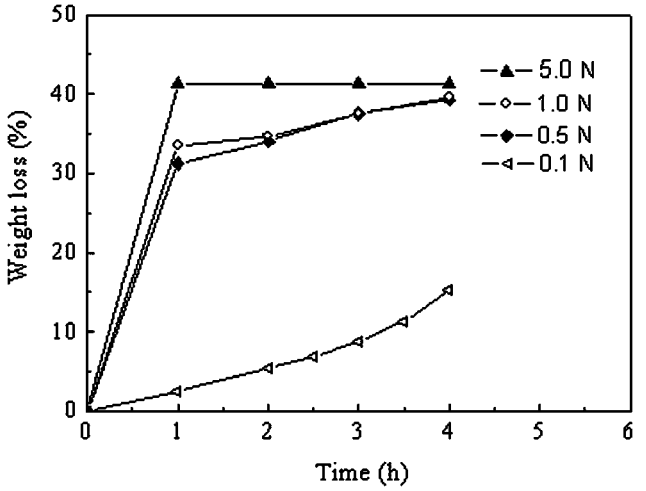

Figure 1 Weight changes of CA fibers immersed in NaOH solutions with various concentrations at room temperature.

The operating voltage and current were 40 kV and 30 mA, respectively.

RESULTS AND DISCUSSION

Degradation behavior

Figure 1 presents weight changes of CA fibers immersed in NaOH solutions with various concentrations at room temperature. In a lower concentration like 0.1*N* NaOH, the weight loss increased gradually with time, however, the weight loss approached the level value within 1 h and there was little variation with respect to time and NaOH concentration in 0.5*N* NaOH or NaOH of a greater concentration. CA has ester bonds and glycosidic linkages in its structure, and these bonds can react to hydrolyze by acid and base catalysis. If CA (DS, 2.5) deacetylated completely, there has been a weight loss of about 39% owing to the removal of acetic acid. It would be interesting that the level values of 38–41% in the concentrations of 0.5*N* and greater accord well with the theoretical value obtained by complete deacetylation. Considering the relatively good stability for glycosidic linkages of CA in acidic and basic solution,[8] it can be speculated that hydrolysis of CA fibers in alkaline solution occurred mainly at acetyl groups (proved by IR analysis, see later).

Dry samples obtained by alkaline immersion in various concentrations were then treated by cellulase under the same condition, and the weight changes (based the dry weight of samples obtained by alkaline immersion) are shown in Figure 2 with respect to alkaline treatment time. It is obvious that alkaline treatment can promote the degradation of CA fibers by cellulase. The weight loss of samples after cellulase degradation increased slightly with alkaline treatment time in 0.1*N* NaOH, while alkaline treatment by 0.5*N* NaOH or a greater concentration resulted in 65–71% of weight loss in cellulase degradation and it was constant after 1 h of alkaline treatment. Under the same condition, the weight loss of absorbent cotton was 62.3% in degradation by cellulase. This demonstrated that cellulose-like structure had come into being after 1 h of alkaline treatment in a concentration of 0.5*N* or greater. It is observed that the weight loss in cellulase degradation for the CA fibers treated by 5*N* NaOH solution was 71%, whereas it was 65% for the fibers treated by 0.5*N* or 1.0*N* for 4 h in despite of complete deacetylation shown by DS analysis.

Changes in weight loss and DS of CA fibers immersed in NaOH solutions of small concentrations (0.1–0.5*N*) for 1 h, and the results of subsequent degradation by cellulase are exhibited in Figure 3. With the increment of alkaline concentrations, weight loss of CA fibers induced by alkaline treatment increased, especially markedly in NaOH concentration increasing from 0.1 to 0.25*N*. The symmetry of two curve expressing weight loss and DS indicates that weight loss of CA fibers in alkaline solution was derived from deacetylation. In agreement with previous reports, biodegradability of CA depends on deacetylation, and the decrease of DS promotes the degradation of CA by cellulase. Original CA fibers degraded only 1.3% in cellulase solution, while CA with DS 1.0 obtained by immersion of alkaline solution showed 23.3% weight loss. Cellulase degradation increased most markedly when the DS of samples obtained by alkaline treatment reduced from 1.0 to 0.8, only decreasing 0.2 of DS, weight loss increased from 23.3 to 46.3%. This token, 0.8 is a critical point of DS for degradability of CA by cellulase.

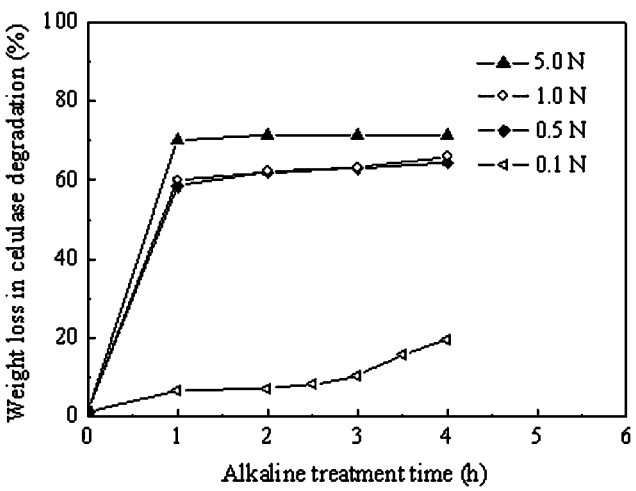

Figure 2 Relationship between weight loss of the samples obtained by immersing in NaOH solutions with various concentrations during cellulase degradation and the times of alkaline immersion.

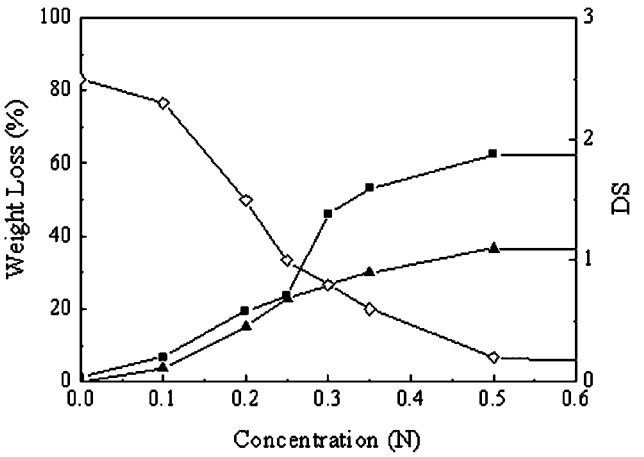

Figure 3 The changes of weight loss (▲) and DS (◇) of CA fibers during alkaline treatments within 1 h, as well as weight loss (■) in subsequent cellulase degradation, with respect to the concentrations of NaOH solutions.

Morphological changes observed by SEM

Yamashita and Endo investigated the morphological change of CA film in 3.0*N* HCl solution by SEM.[10] The CA film was eroded extensively and much fine powder formed on its surface after treated by HCl solution. This was attributed by them to the difference of solubility of CAs with different DS in water because only CAs with DS in the range of 0.6–0.8 was soluble in water.[11] CA molecules can elute into water once this critical DS has reached by deacetylation in acidic solution, then these molecules dissolved in water are deposited as fine cellulose powder by further deacetylation.

However, CA fibers recovered from NaOH solution showed different morphological change from CA films in HCl solution. SEM photographs of CA fibers obtained by immersing in alkaline solutions with different concentrations for 1 h are shown in Figure 4. Being similar to original CA fibers [Fig. 4(a)], the CA fibers immersed at lower concentration such as 0.3*N* NaOH showed a smooth surface but a little thin holes in surfaces [Fig. 4(c)]. In the case of 3.0*N* NaOH, a smooth surface was still kept for the CA fibers but the holes in surfaces increased and enlarged [Fig. 4(d)]. When NaOH concentration increased to 5*N*, it was found in experiment that deacetylation of CA fibers finished instantaneously, coupled with shrinkage of fineness, and microfibers perpendicular to fiber axis distributed over the surfaces of fibers gained, as shown in Figure 4(b). It needs to be emphasized that fine powders observed in HCl solution did not appear in surfaces of the fibers and no deposit was found in alkaline solutions in these concentrations.

It is known that cellulose can be converted into alkaline cellulose through NaOH treatment, which is insoluble in water.[12] Obviously CA deacetylated by NaOH treatment became alkaline cellulose, thereby CA molecules (DS, 0.6–0.8) obtained by NaOH treatment, which were soluble in water in the case of deacetylation by HCl solution, could not be eliminated from the fibers. In contrast to acidic solution, there were no deposits and erosion in the surfaces of CA fibers in the case of alkaline solution and the thin holes in the surfaces served as the passages of releasing acetic acid formed during alkaline treatment.

In 5.0*N* NaOH, a high concentration of OH^- enough to initiate deacetylation reaction immediately after the solution entering into the interior from any situation of CA fiber surfaces, the reaction proceeded with linearity instantaneously, at the same time intramolecular and intermolecular hydrogen bonds also established owing to the elimination of acetyl groups, resulting in regular microfibrillar structure and fissures from the exterior to the interior of the fibers. Consequently, the specific surface area of the fibers obtained by alkaline treatment in 5.0*N* NaOH was greater than obtained in other lower concentration. Under the same condition of DS and crystallinity, the increase of specific surface is in favor of cellulase degradation because of the increase of contactability with the enzyme solution.[13] Therefore, weight loss of cellulase degradation for CA fibers treated by 5.0*N* NaOH solution was more than that for CA fibers treated by 0.5*N* and 1.0*N* NaOH solution for 4 h about 6%, in spite of entire deacetylation shown by DS analysis in the two concentration. Moreover, the fineness of the fibers was not further reduced with the increase of the concentration of NaOH, and this was consistent with the change of weight, suggesting degradation of CA in alkaline solution was due to deacetylation.

The roughening of the surface texture for CA fibers treated by 0.3*N* NaOH solution for 1 h was observed by SEM after degraded by cellulase solution for 2 h, indicating the attack of cellulase on the fibers [Fig. 4 (e)]. When cellulase degradation carried out for 5 h, exfoliation in fiber surfaces could be explicitly recognized and the fineness of fibers decreased, as shown in Figure 4(f). After degradation in cellulase solution for 24 h, only a small amount of fine powder remained in the solution. There was no change after cellulase degradation in the surfaces of original CA fibers and the fibers obtained by immersion in 0.1*N* NaOH solution for 1 h (not shown).

^{1}H-NMR analysis

The signal assignment of ^{1}H NMR spectroscopy of CA was first reported by Goodlett et al.[14] and has been confirmed by other workers.[15] Figure 5 presents ^{1}H NMR spectra of CA (DS, 0.8) obtained by alkaline

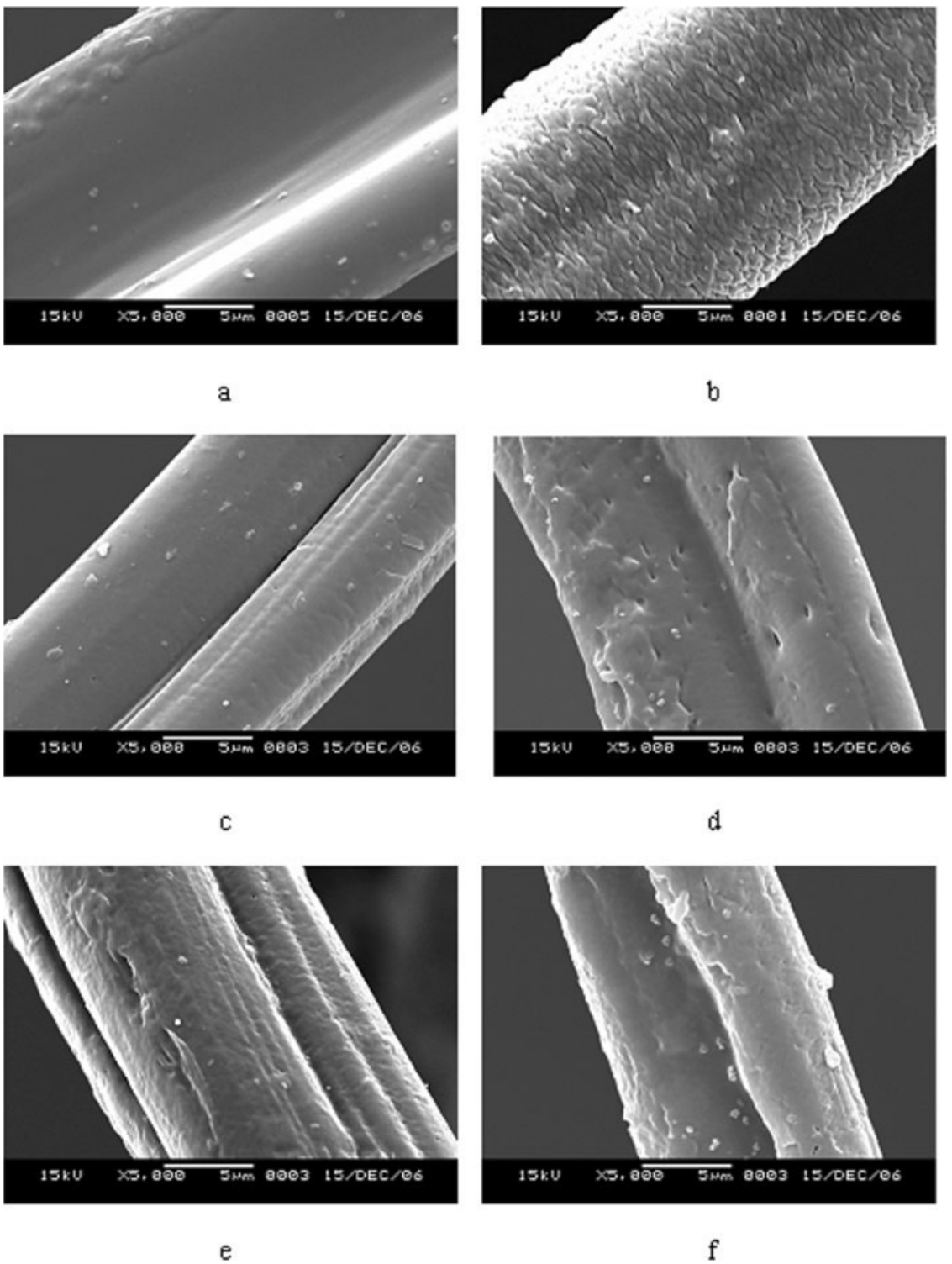

Figure 4 SEM photographs of the original CA fiber (a); the fibers immersed in NaOH solutions of 0.3*N* (c), 3.0*N* (d), and 5.0*N* (b) for 1 h; the fiber obtained by cellulase degradation of the fiber (c) for 2 h (e) and 5 h (f).

treatment in 0.3*N* NaOH solution for 1 h and its product of cellulase degradation, and the assignment of the peaks is also shown in Figure 5(a). The DS values of samples were calculated by means of the integration ratio of proton signals at the region of glucose ring (3.6–5.1 ppm) and the region corresponding to the methyl protons of acetate group (1.7–2.2 ppm). The individual DS (IDS) at 2, 3, and 6 positions of AUG was calculated by the integration ratio of the corresponding peaks in the methyl region. DS and IDS of CA obtained by alkaline treatment and sequent cellulase degradation are shown in Table I.

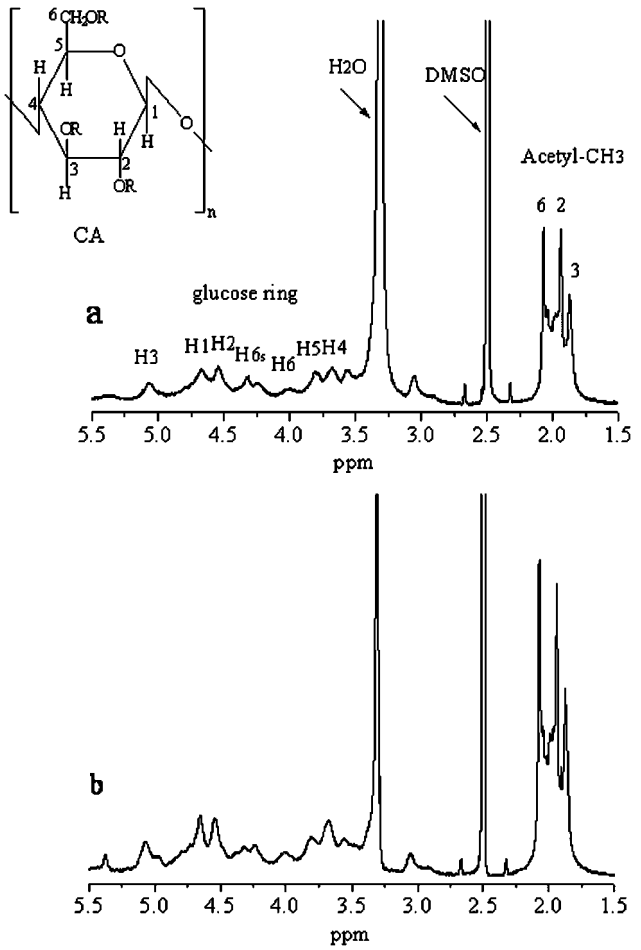

Figure 5 ^{1}NMR spectra of the sample (a) with a DS of 0.8 obtained by alkaline treatment and its product (b) of cellulase degradation. (R=$COCH_3$ or H in CA structure).

A discovery was that DS determined by NMR for CA obtained by alkaline immersion in lower concentration was larger than that by chemical method, whereas there was no difference between the two methods for original CA. DMSO is a preferred solvent for the investigation of CA, which dissolves CA with DS greater than 0.5.

Doyle et al. studied the ^{13}C NMR spectra of CA with various DS in DMSO solution, which was synthesized by heterogeneous acetylation of cotton linters.[16] They found that for CA with a DS greater than 0.5, irrespective of the DS, the spectrum obtained was that of triacetate, and there was no evidence for partially substituted or unsubstituted cellulose, despite the fact that chemical analysis clearly showed acetylation was far from complete. They considered that the rate of acetylation was not controlled by the reactivity of particular sites but rather by accessibility. The initial acetylation in cotton linters occurred in disordered accessible regions of cellulose, once this had been completed, further acetylation occurred in ordered regions (crystalline region) without losing their integrity. Therefore, the solution subsequently obtained contained dispersions of these ordered regions and hence the spectrum corresponded to the solubilized part acetylated completely.

Being different from the acetylation of cotton linters, for the heterogeneous deacetylation of CA fibers in alkaline solution, there were no obvious crystalline regions in original fibers, which were amorphous, and the structures of the fibers were accessible to solution. However, there was a gradient of the amount of OH^- in fibers from surface to core, and accessibility was still an important factor of affecting reaction. This would result in a dispersivity of the DS in CA molecules and the acetyl groups of polymers in the surfaces of samples were eliminated preferentially because of their immediate contact with NaOH solution. DS measured by NMR was the average value of CA molecules dissolved in DMSO with a DS greater than 0.5, while DS determined by chemical method was the average value in whole DS range. The dispersivity of the DS of CA molecules after deacetylation by alkaline treatment, differing from the duality of the DS of CA molecules obtained by heterogeneous acetylation (i.e. unsubstituted or substituted completely), can be proved by sequent degradation experiment by cellulase because the DS of their cellulase degradation product determined by NMR increased (Table I), indicating the degradation of CA molecules with a DS greater than 0.5 in cellulase solution.

A statement can be made about the reactivity of the three ester groups in CA molecule. In theory, the ester group at 6-position should be attacked first. The ester group at 2-position should be attacked second and the 3-position last, as the position 2 ester would be closer to the (O)-linkage, and also exposed than the 3-position. The structures of polymers not

TABLE I
DS and IDS of the Samples Obtained by Alkaline Treatment in Various Concentrations for 1h and Subsequent Cellulase Degradation

Conditions				IDS[b]		
Alkaline treatment	Cellulase degradation	$DS_{chemical}$[a]	DS_{NMR}[b]	6	2	3
Control[c]	Control[c]	2.5	2.44	0.85	0.89	0.70
0.2*N* NaOH	Nd	1.5	1.92	0.74	0.68	0.50
0.2*N* NaOH	√[d]	nd	2.12	0.75	0.76	0.61
0.25*N* NaOH	Nd	1.0	1.76	0.64	0.64	0.48
0.3*N* NaOH	Nd	0.8	1.71	0.60	0.60	0.51
0.3*N* NaOH	√[d]	Nd	1.94	0.71	0.71	0.52

[a] Measured by chemical method.
[b] Determined by ^{1}NMR.
[c] The original CA fibers.
[d] Subsequent cellulase degradation after alkaline treatment.

dissolved in DMSO should be accessible to that of cellulose because of their DS lower than 0.5. Supposing that these polymers would not be considered, as they formed quite likely by deacetylation of CA molecules in surfaces, which could contact with NaOH solution directly and the deacetylation reactions depended on OH^- rather than on the reactivity of ester groups at the three positions, by looking at the IDS at the three positions (Table I), it can be seen that the picture is more complicated and does not follow the theoretical trend.

In a lower NaOH concentration (0.2*N*), the deacetylation rates at position 2 and 3 were more marked than at position 6 because of the IDS values at position 2 and 3 decreased more within the same time. When the concentration of NaOH increased to 0.25*N*, the deacetylation at position 6 began to accelerate, whereas there was no change for the IDS at position 3. The similar IDS values at the three positions could be observed when deacetylation of CA fibers proceeded in 0.3*N* NaOH solution, demonstrating that the reaction of deacetylation would not depend on the reactivity of the three ester groups owing to a larger reactive capability of solution, i.e. a higher concentration of OH^-.

FTIR analysis

Deacetylation of CA fibers in alkaline solutions was proved by FTIR spectra, as shown in Figure 6(A). After 1 h of immersion in 0.3*N* NaOH solution, the evidence of deacetylation in CA fibers are provided by the lowing of the intensity of the bands related to acetyl group at 1754 cm^{-1}, attributed to C=O stretching; 1245 cm^{-1}, attributed to C—O stretching of acetyl group; and 1375 cm^{-1}, attributed to C—H blending in the methyl of acetyl group. Furthermore, the increase of the absorbance of OH stretching band at 3470 cm^{-1} also presents further evidence of deacetylation. Figure 6(B) shows the relationship of the ratios of H1754/H1050 and H1245/H1050, indicating the acetyl contents, and weight loss of CA fibers immersed in various NaOH concentrations for 1 h. The contents of acetyl groups declined as the weight loss increased, and when the weight loss arrived at about 38%, the acetyl groups disappeared completely. This was coincident with the theoretical value of 39%, suggesting that the weight loss during alkaline treatment was the result of deacetylation.

The area of carbonyl peak at 1754 cm^{-1} and the height of OH peak at 3470 cm^{-1} were recorded for the samples obtained by alkaline treatment in various concentrations for 1 h. A graph of the ratio of the height of the OH peak to the area of carbonyl peak (OH/C=O) versus DS measured by chemical method could be plotted, as shown in Figure 7. The ratio of OH/C=O (*Y*) decreases linearly with DS (*X*), and the equation $Y = 44.8011 - 15.5332X$ ($r^2 = 0.9920$) is obtained from the linear graph, which can be used as a calibration curve determining the DS of sample as long as its spectrum is available.

Samios et al. studied the biodegradation of CA with given DS using *aspergillus fumigatus*.[17] They found the enhancement of the biodegradability in *aspergillus fumigatus* with the decrease of DS, according to our observation in cellulase degradation experiments. However, they also observed the decrease of DS of the product obtained by degrading CA using *aspergillus fumigatus*. To the contrary, for CAs with various DS obtained by alkaline treatment, after cellulase degradation, the augment of DS of the degradation products could be observed. Figure 6(A) pres-

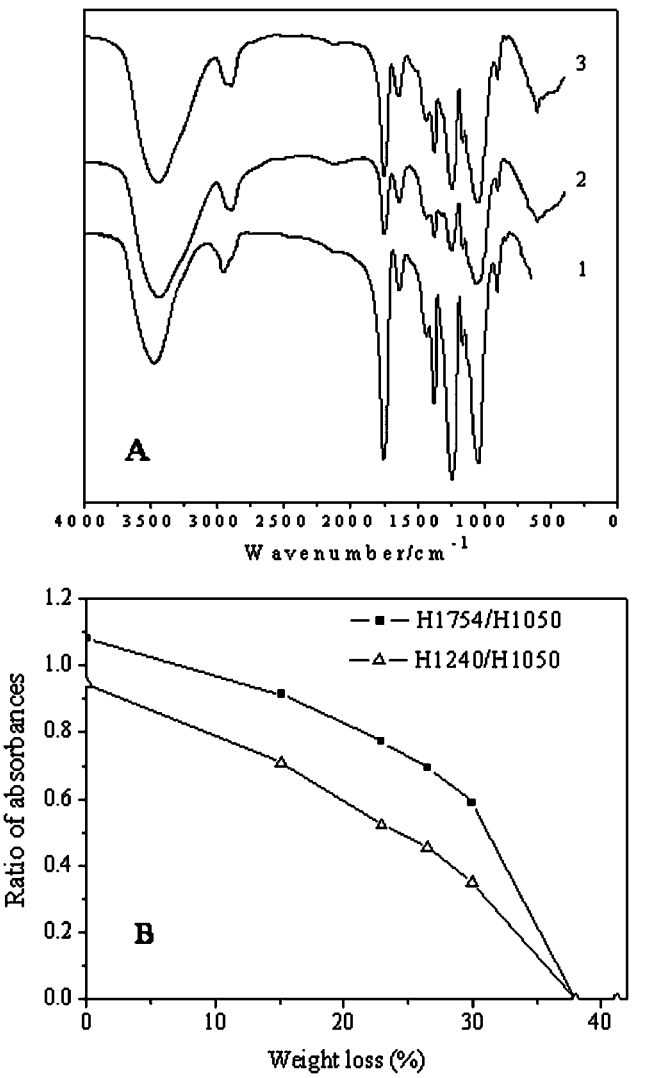

Figure 6 (A) IR spectra of the origin CA fibers (spectrum 1), the fibers (spectrum 2) with a DS of 0.8 obtained by immersing in 0.3*N* NaOH solution for 1 h and its product (spectrum 3) of cellulase degradation; (B) The changes of the absorbance ratios of H1754/H1050 and H1240/H1050 with respect to weight loss of CA fibers immersed in NaOH solutions of various concentration for 1 h.

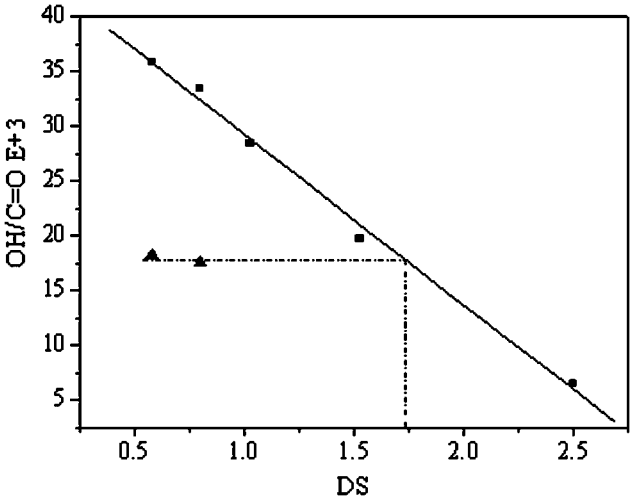

Figure 7 Graph of OH/C=O versus DS of CA fibers treated in NaOH solutions with various concentrations for 1 h. (▲) indicates the OH/C=O values of the products after cellulase degradation for CA fibers with a DS of 0.5 and 0.8 obtained by immersing in NaOH solution of 0.3*N* and 0.35*N* for 1 h.

ent the IR spectra before and after cellulase degradation of CA with DS 0.8 obtained by alkaline immersion in 0.3*N* NaOH for 1 h. It is revealed that the absorbance of the bands relative to acetyl group at 1754 cm^{-1}, 1240 cm^{-1}, and 1375 cm^{-1} increased after cellulase degradation.

As illustrated in Figure 7, CAs that had been degraded in cellulase solution and with a starting DS of 0.8 and 0.6 obtained by immersion of CA fibers in 0.3*N* and 0.35*N* NaOH solutions for 1 h, were analyzed by the above IR method, and their final DS increased to about 1.75. As mentioned earlier, as far as the product of deacetylation of CA fibers in alkaline solution is concerned, the DS values of the polymer molecules are inhomogeneous and the DS determined by chemical method is an average DS value of the molecules in sample. As distinguished from *aspergillus fumigatus*, cellulase, a special enzyme for degrading cellulose by cleaving cellulose chain, is unavailable to deacetylation, and the existence of acetyl group in CA inhibits the attack of cellulase by reason of steric hindrance. Therefore, the lower-DS polymers with a more cellulose-like structure was preferentially depolymerized into oligomer by cellulase, at the same time the higher-DS polymers remained because of their poor biodegradability.

Previous spectroscopic studies indicated that the bands at 1160, 1122, and 1047 cm^{-1}, which attributed to asymmetric stretching of COC (glycosidic linkage); C—C stretching in ring and C—O stretching at C3, respectively, reflected the crystalline fraction of cellulose and could be therefore be used to monitor changes in crystallinity.[18] Thus, the ratio of A1160/A1047 was used as crystallinity index (Cr.I) to estimate the changes of crystallinities in CA fibers during alkaline immersion and subsequent cellulase degradation. Figure 8 demonstrates that the Cr.I shows an increase with the decrease of DS for CA fibers during alkaline treatment, indicating that deacetylation in alkaline solution enhances the crystallinity of sample. Furthermore, it can be found that the Cr.I of CA with a DS of 0.8 obtained by alkaline immersion decreases from 0.60 to 0.49 after cellulase degradation, while the Cr.I of CA with a DS of 1.5 is 0.52, suggesting that the actual DS of the product of cellulase degradation for CA with a DS of 0.8 is slightly greater than 1.5. This accords with the result of DS = 1.75, proving the dispersivity of DS of polymer molecules after alkaline treatment for CA in heterogeneous condition, and the prior biodegradation for lower-DS polymers.

X-ray diffraction analysis

In contrast with original CA fibers which display the two wide peaks at 2θ angles of 9.8 and 17.6° in X-ray diffractogram [Fig. 9(a)] , expressing a dominant amorphism in structure, the fibers obtained by complete deacetylation in 5.0*N* NaOH solution show a crystalline pattern of cellulose II [Fig. 9(e)]. The study has revealed that CA with a DS up to 1.5 obtained by homogeneous deacetylation for commercial CDA showed a typical spectrum of CDA like Figure 9(a).[17] However, the X-ray diffractogram of CA (DS = 1.5) obtained by immersion in NaOH solution for 1 h exhibits another profile. A unconspicuous reflect peak at 2θ angle of 19.8° attributed to $10\bar{1}$ plane of cellulose II appears in its diffractogram [Fig. 9(c)], indicating the existence of cellulose

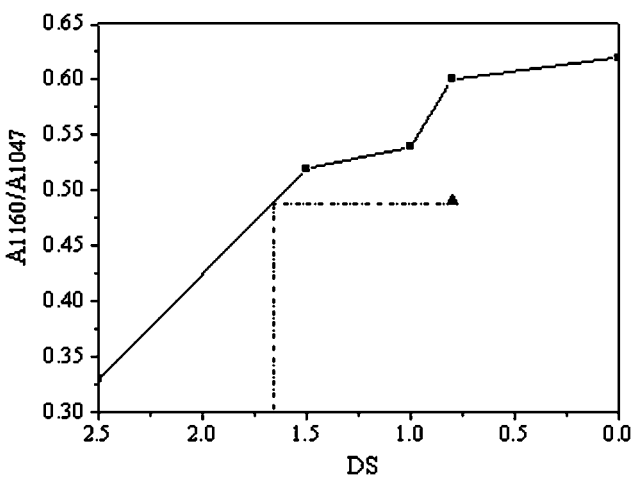

Figure 8 The changes of the absorbance ratio of A1160/A1047 with respect to the DS during alkaline treatment in various NaOH concentrations. (▲) indicates the A1160/A1047 values of the products after cellulase degradation for CA fibers with a DS of 0.8 obtained by immersing in NaOH solution of 0.3*N* for 1 h.

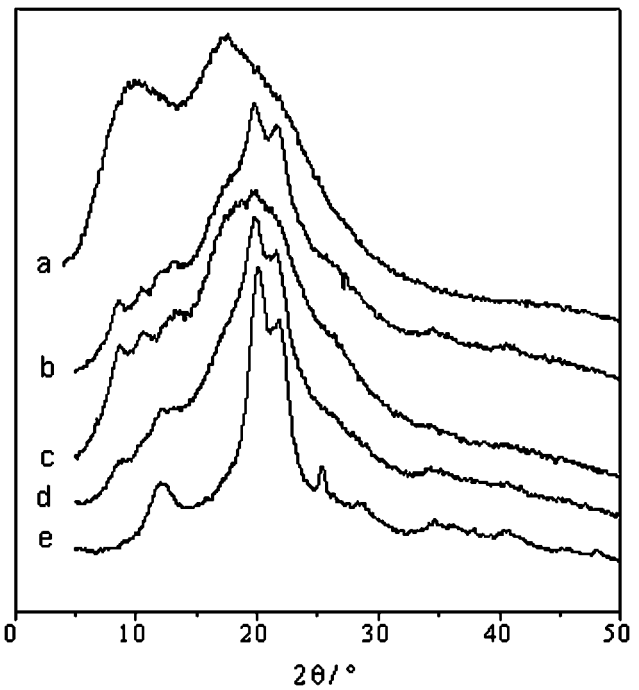

Figure 9 X-ray diffractograms of the original CA fibers (a), the fibers with a DS of 1.5 (c), 0.8 (d), and the fibers deacetylated completely (e) obtained by immersion in NaOH solutions of 0.2*N*, 0.3*N*, and 5.0*N* for 1 h, and the product (b) of cellulase degradation for the fibers (d).

molecules or lower-DS polymers with cellulose-like structure. In addition, three small reflect peaks at 2θ angles between 8° and 14°, which usually appear in the diffractograms of the CTAII or the CDA obtained by recrystallization in acetone,[19] demonstrate that recrystallization of CA molecules occurred because incursion of OH^- led to the broken and rebuild of hydrogen bond and the rearrange of molecule chains through alkaline immersion, in other words, OH^- not only could catalyze the hydrolysis of acetyl group, but also could affect the hydrogen bond that had existed. When the DS of CA immersed in NaOH solution is deduced to 0.8, the characteristic reflect peaks of cellulose II such as $10\bar{1}$, 002 planes can be clearly resolved in its diffractogram [Fig. 9(d)], and only an unconspicuous peak appears at 2θ angle of 8.9°, however, the three peak at 2θ angles between 8° and 14° assigned to recrystallized CDA are clearly observed again after cellulase degradation [Fig. 9(b)]. This also discloses that the fraction of the higher-DS polymers increased with the lower-DS polymers with cellulose-like structure the degradation of cellulase and also proves our previous analysis about the deacetylation mechanism of CA fibers in alkaline solution.

CONCLUSIONS

The work studied the degradation behaviors and structure changes of CA fibers in NaOH solutions, as well as in subsequent cellulase solution. Degradation of CA fibers in alkaline treatment was due to deacetylation. Deacetylation of CA fibers in alkaline solutions promoted their cellulase degradation, and cellulase degradation increased most markedly when the DS of samples reduced to 0.8. There was no erosion in fiber surfaces except some thin holes in a lower NaOH concentration because of the formation of alkaline cellulose, but in 5.0*N* NaOH solution regular microfibrillar structure could be observed by SEM. The attack of cellulase on the surfaces of CA fibers obtained by immersion in 0.3*N* NaOH solution for 1 h could be explicitly recognized, while this could not be observed in the original or the fibers treated by 0.1*N* NaOH solution for 1 h. ^{1}NMR analysis showed the dispersivity of CA molecules in sample after alkaline treatment and the increase of DS of sample after subsequent cellulase treatment because of the removal of lower-DS polymer molecules. This was proved by the analysis of IR and X-ray. Only in a lower NaOH concentration ($\leq$0.25*N*), deacetylation reaction of CA in alkaline solution was related to the reactivity of ester groups at the three positions of AUG, but not follows the theoretic trend in the three positions. X-ray analysis demonstrated OH^- not only could catalyze the hydrolysis of acetyl group, but also could affect the hydrogen bond that had existed, resulting in recrystallization of CA molecules.

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