Accelerated hydrolysis of substituted cellulose for potential biofuel production: kinetic study and modeling

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17 **Abstract**

18 In this **work**, kinetics of substitution accelerated cellulose hydrolysis with multiple
19 reaction stages was investigated to lay foundation for mechanism study and molecular
20 design of substituting compounds. High-efficiency hydrolysis of cellulose is critical for
21 cellulose-based bioethanol production. It is known that, substitution could substantially
22 decrease activation energy and increase reaction rate of acidic hydrolysis of glycosidic
23 bonds in cellulose. However, reaction kinetics and mechanism of the accelerated hydrolysis
24 were not fully revealed. In this research, it was proved that hydrolysis only occurred in
25 amorphous regions of cellulose fibers, and was a process with multiple reaction stages.
26 With molar ratio of substitution less than 1%, the overall hydrolysis rate could be increased
27 for around 10 times. We also quantified the relationship between the hydrolysis rate of
28 individual reaction stage and its major influences, including molar ratio of substitution,
29 activation energy of acidic hydrolysis, pH and temperature.

30
31 **Keywords:** Cellulose Pretreatment; Accelerated Hydrolysis; Substitution Effect; Kinetics;
32 Bioethanol.

1. Introduction

As the most abundant natural macromolecular resources, cellulose has great potential for production of bioethanol, targeting replacement of fossil fuels (Alvira et al., 2010; Fockink et al., 2015; Mosier et al., 2005; Singhania et al., 2014). To enhance economic feasibility of bioethanol production, glucose needs to be easily obtained from cellulose with high efficiency. However, acidic hydrolysis, one of the most widely used degradation approaches, has critical drawbacks that restricted its applications in bioethanol production. If the processing temperature is low, high concentrations (10-30%) of strong acid must be used, leading to corrosion of equipment, generation of large quantities of waste acid, difficulty in glucose separation and severe environmental pollution. If acid concentration is low, high temperature (160 °C) and high pressure (10 atm) must be used (Iranmahboob et al., 2002), resulting in low yield (<30%) of glucose (Nguyen et al., 1999), and generation of 5-hydroxymethyl-furfural (HMF), which is detrimental to microbes for fermentation (Verardi et al., 2011). In general, low conversion efficiency of cellulosic hydrolysis remains a critical problem in cellulose-based bioethanol production (Lange et al., 2007).

Efforts have been devoted to increase the conversion rate of cellulose in hydrolysis. For example, incorporation of aluminum nitrite decreased crystallinity of cotton cellulose and increased its degradation rate in enzymatic hydrolysis (Zhang et al., 2015). Dissolution using ionic liquid also decreased crystallinity of cellulose in switch grass (Zhang et al., 2014). However, these approaches did not effectively increase the hydrolysis efficiency or used solvents that were high in cost.

Multiple examples proved that cellulose grafted or substituted by compounds with certain functional groups could accelerate breakage of glycosidic bonds. Higher degree of

carboxymethyl substitution led to faster hydrolysis of carboxymethyl cellulose (Borsa et al., 1990). Cellulose sulfate could be hydrolyzed twice as fast as unmodified cellulose (Ranby et al., 1959). Besides, cellulose substituted by reactive dyes could be hydrolyzed more easily than unsubstituted cellulose (Yang and Hughes, 1997). This phenomenon could provide new approaches to expedite acidic hydrolysis for production of cellulose-based bioethanol. In order to effectively utilize chemical modifications for high-efficiency cellulose hydrolysis, mechanism of substitution accelerated cellulose hydrolysis should be fully understood.

Chemical substitution is a mature approach for cellulose modification, as substituted cellulose products are widely used in different industrial fields for decades. For example, carboxymethylcellulose is used as thickener, binder and stabilizer in food, textile, paper and pharmaceutical industries (Liimatainen et al., 2009), and cellulose esters are important thermoplastic materials (Crepy et al., 2011). Cotton gin dust was treated with alkaline for removal of lignin and enlargement of surface area of left cellulose (Fockink et al., 2015). Waste cotton textiles, mostly are substituted cellulose, might also be readily used as the feedstocks for bioethanol production in the future.

Controversy exists regarding degradation mechanism of substituted cellulose in acidic hydrolysis. Jiang et al. proposed that cellulose crystals in cotton fibers could be disrupted by reaction with cyanuric chloride and transformed into amorphous regions (Jiang et al., 2012). However, even hydrogen ions with much smaller size than cyanuric chloride could not penetrate into cellulose crystals (Nelson & Tripp, 1953). Thus, the possibility for cyanuric chloride to penetrate into cellulose crystals is questionable.

Mechanistic study to elucidate the accelerated hydrolysis needs to be conducted.

Kinetic information is the key to the fully understanding of mechanisms. Determination of hydrolysis rates of glycosidic bonds in cellobioses could be used to evaluate the influence of substituting groups, and thus, provide necessary information for reaction mechanism study.

In this research, a reactive dye with proven capability in accelerating hydrolysis of cellulose was used to substitute cotton cellulose fibers. Degree of polymerization, the main parameter used to characterize hydrolysis rate, was calculated based on the molecular weight of hydrolyzed cellulose measured via gel permeation chromatography. Subsequently, kinetics of the multiple-stage hydrolysis of substituted cellulose was studied and the relationship between hydrolysis rates of different steps and their major influences, including ratio of substitution, activation energy of hydrolysis, pH and temperature was investigated.

2. Experimental

2.1. Materials

Cotton yarns (45s) purchased from Sunray Textile Company (Henan, China) were made of fine staple cotton fibers with length ranged from 22 to 33 mm, and diameter ranged from 15-19 μm . Cotton fibers are composed of about 95% cellulose and 5% impurities, including wax, polysaccharides, ash, etc. Reactive Red 120 (RR120) with purity of 80% was purchased from Sigma (Shanghai, China). Hydrochloric acid, sulfuric acid, potassium biphthalate, N, N-dimethylacetamide (DMAc), lithium chloride, sodium chloride and sodium carbonate were purchased from Sinopharm Company (Shanghai, China). All

the chemicals were analytical grade, while the DMAc used as mobile phase in gel permeation chromatography (GPC) was HPLC grade.

2.2. Substitution

Cotton was dried at 105 °C under vacuum for 24 h. About 3.0 g of cotton was immersed in 60 mL of water with 5-12 g L⁻¹ of RR120 and 80-160 g L⁻¹ of sodium chloride in one canister. The dyeing bath was heated up to 65 °C and held for 45 min with stir. After adding about 20 g L⁻¹ of sodium carbonate, the bath was heated to 95 °C and held for 1 h with stir. Cotton was removed from the bath after the canister was cooled to room temperature, followed with washing in cold water, hot water (40-50 °C), soap, hot water (70 °C) and cold water, subsequently. The substituted cotton was dried at 105 °C under vacuum to constant weight.

2.3. Acidic hydrolysis

The substituted cotton yarns were placed in HCl-potassium biphthalate buffer (mixed 50 mL 0.2 mol L⁻¹ of potassium biphthalate solution and 0.2 mol L⁻¹ of HCl solution with different volumes, added distilled water to a final volume of 200 mL) at pH 2.5, 3.0 and 3.5. The hydrolysis was conducted under 70-100 °C for 0.5 to 170 h. To study the effect of degree of substitution, acidic hydrolysis of substituted and unsubstituted cotton was carried out at pH 3.0 under 100 °C. To study the reaction kinetics, the acidic hydrolysis was carried out at pH 3.0 under 70 °C, 80 °C, 90 °C and 100 °C. To study the effect of pH, the acidic hydrolysis was carried out at pH 2.5, 3.0 and 3.5 under 100 °C. Each experiment was performed in duplicate. The cotton residue was collected from the bath, neutralized and dried at 105 °C under vacuum for further analysis.

2.4. Calculation of molar ratio of substitution

Molar ratio substitution, the ratio of the mole of substituted cellobioses over the mole of all the cellobioses of cotton, was calculated based on the method developed by Murtagh et al (Murtagh and Taylor, 2004). RR 120 was dissolved in cold 70% sulfuric acid and then diluted for 2, 4 and 8 times. The concentration of RR 120 in cotton was measured at a wavelength of 535 nm based on Beer-Lambert law using a Shimadzu UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The calibration curve had $r^2 > 0.999$.

2.5. Crystallinity of cotton

After dried at 105 °C under vacuum for 24 h, crystallinity of cotton before and after substitution was tested on a Rigaku D/max-2550 PC system (Rigaku, Tokyo, Japan) with Cu K α radiation ($\lambda=0.15406$ nm, 8 kW, 40 kV $\times$ 200 mA). A scanning speed of 4 ° min⁻¹ for 2 θ ranging from 5° to 60° was utilized. The crystallinity index, CI, was calculated by the Segal method (Eq. 1) using height of the 200 peaks (I_{200} , 2 θ = 22.7°) and minimum intensity between the 200 and 110 peaks (I_{am} , 2 θ = 18°).

$$CI = (1 - \frac{I_{am}}{I_{200}}) \quad (1)$$

Wherein, CI is the crystal index, I_{am} is the height of amorphous reflection at 2 θ of 18°, and I_{200} is the height of reflection at 2 θ of 22.7°.

2.6. Degree of polymerization (DP) of cotton

About 100 mg of cotton was meshed, and then dispersed in 10 mL of DMAc with stir (Timpa 1991). The containers were sealed, heated at 150 °C for 2 h, and then cooled to 100 °C, added with 0.8 g of dried LiCl, and consequently cooled to 50 °C until all the cotton was dissolved. Pure DMAc was added into the solution to make the final concentration 1 mg mL⁻¹. The solution was filtered through 0.22 μ m PTFE filters.

Weight-average degree of polymerization (DP_w) of cotton before and after hydrolysis was measured on a Malvern GPC (Malvern, Malvern, England). The columns were CLM1013 (I-MBHMW-3078) and CLM1014 (I-OLIGO-3078) (Viscotek, Houston, USA), with temperature set at 60 °C. The mobile phase was 0.05% LiCl/DMAc. A 270 dual detector system with a dual-angle (7° and 90°) laser scattering detector and a refractive index detector with increment at 0.077 mL g⁻¹ and temperature set at 40 °C were used.

Light scattering detector was used to determine the molecular weight and subsequently degree of polymerization of cellulose. Before measurement, polystyrene with narrow distribution ($M_w = 99,000$, $DPI = 1.05$) and wide distribution ($M_w = 105,000$, $DPI = 1.50$) was used as standards for calibration. The molecular weight of cellulose was then measured using the GPC system. Degree of polymerization was calculated by dividing the molecular weight of cellulose by the molecular weight of glucose. The unit glucose was not changed significantly as the highest molar substitution percentage was less than 1%.

2.7. FTIR measurement

IR spectra of the substituted and unsubstituted cellulose yarns were recorded using Nicolet iS10 FTIR Spectrometer (Thermo Scientific). A background scan of clean Zn–Se diamond crystal was processed before the sample scanning procedures. A bundle of yarns were placed on the ATR crystal. All FTIR spectra were collected with a spectrum resolution of 4 cm⁻¹, with 128 co-added scans in the range 650–4000 cm⁻¹. Three replications were performed for each sample.

2.8. Kinetic study

Kinetic parameters were calculated based on the DP of cotton after hydrolysis for different time periods. The hydrolysis rate, k , was calculated based on assumption of pseudo zero-order reaction using Ekenstam equation (Eq. 2) (Ekenstam, 1936).

$$1/DP_t - 1/DP_0 = kt \quad (2)$$

Wherein, DP_t is the DP at hydrolysis time t , DP_0 is the initial DP. K is the hydrolysis rate, t is the hydrolysis time.

Calvini converted Eq. 2 into Eq. 3 using the calculation for first order reaction (Calvini et al., 2012):

$$1/DP_t - 1/DP_0 = (1 - 1/DP_0)(1 - \exp(-kt)) \approx 1 - \exp(-kt) \approx kt \quad (3)$$

When DP_0 is high and kt is negligible, Eq. 2 is equivalent to Eq. 3. When kt is very small (<0.003), $1 - \exp(-kt)$ could approximate kt , based on Taylor's Expansion. Using Arrhenius Equation, k could be correlated with activation energy E_a , pH and temperature, as shown in Eq. 4 (Zou et al., 1996):

$$k = A(a[H^+])\exp(-E_a/(RT)) \quad (4)$$

Wherein, A is the frequency factor, a is the coefficient of hydrogen ion effect, $[H^+]$ is the concentration of hydrogen ion, E_a is the activation energy of hydrolysis, R is the ideal gas constant, T is the hydrolysis temperature.

Calvini et al. provided model more suitable for characterizing hydrolysis of substituted cellulose, in which hydrolysis rates of substituted and unsubstituted cellobiose might be different. In their model for hydrolysis of unsubstituted cellulose, glycosidic bonds in amorphous regions were categorized into "oxidized cellobiose" and "unoxidized cellobiose". Oxidized cellobiose degraded faster than unoxidized cellobiose (Calvini et al.,

2008). A modified version of the model could be used to represent the relationship between DP and hydrolysis rates of substituted and unsubstituted cellobioses, as shown in Eq. 5.

$$1/DP_t - 1/DP_0 = n_{sub}(1 - \exp(-k_{sub}t)) + n_{unsub}(1 - \exp(-k_{unsub}t)) \quad (5)$$

Wherein, k_{sub} and k_{unsub} are the hydrolysis rates of substituted and unsubstituted cellobioses, respectively. K_{sub} represents the effect of substituent on the hydrolysis process. N_{sub} and n_{unsub} are the molar ratio of substituted and unsubstituted cellobioses in the amorphous regions of cellulose, respectively. N_{unsub} plus n_{sub} equals 1.

K_{sub} was calculated based on the GPC measurement of number-average DP (DP_n). However, it was widely accepted that, DP_w should be used in the model when the cellulose was degraded into segments with constant dispersity (Calvini and Gorassini, 2012). To eliminate the effect of unstable dispersity of DP, the number of scissions per chain was utilized in this research (Calvini and Gorassini, 2012). Based on the method proposed by Calvini et al., Eq. 6 was developed to directly calculate the number of scission per cellulose chain. The method could reduce errors due to DP measurement, and eliminate the effect of unstable dispersity due to uneven cleavage of cellulose chains.

$$S = DP_0/DP_t - 1 \quad (6)$$

Wherein, S is the number of scission.

Combining Eq. 5 and Eq. 6, Eq. 7 could be obtained as follows.

$$\begin{aligned} DP_{w0}/DP_{wt} - 1 &= DP_{w0}[n_{sub}(1 - \exp(-k_{sub}t)) + (1 - n_{sub})(1 - \exp(-k_{unsub}t))] \\ &= DP_{w0}(1 - \exp(-k_{apparent}t)) \end{aligned} \quad (7)$$

Wherein, DP_{w0} and DP_{wt} represent DP_w at time 0 and time t, $k_{apparent}$ is the hydrolysis reaction rate of substituted cotton. After removal of DP_{w0} on both sides of Eq. 7 and subsequent transformation, resultant Eq. 8 is shown as below.

$$1 - \exp(-k_{sub}t) = (1/DP_{wt} - 1/DP_{w0})/n_{sub} - (1 - \exp(-k_{unsub}t))(1 - n_{sub})/n_{sub} \quad (8)$$

k_{sub} could be calculated via conversion of Eq. 7.

$$1 - \exp(-k_{\text{sub}}t) = [1 - \exp(-k_{\text{apparent}}t) - (1 - n_{\text{sub}})(1 - \exp(-k_{\text{unsub}}t))]/n_{\text{sub}} \quad (9)$$

The relationship between $(1/DP_{\text{wt}} - 1/DP_{\text{w0}})/n_{\text{sub}} - (1 - \exp(-k_{\text{unsub}}t))(1 - n_{\text{sub}})/n_{\text{sub}}$ and hydrolysis time t could be established to obtain k_{sub} . N_{sub} could be obtained by dividing molar ratio of RR120 in fibers by molar ratio of amorphous region in fibers, which could be calculated based on the CI measured on XRD.

2.9. Statistics

Power analysis in SAS software ((SAS Institute Inc., NC) was employed to analyze the data. The confidence interval was set at 95% and a p value less than 0.05 was considered to be a statistically significant difference.

3. Results and Discussion

Substitution of cellulose in amorphous region

FTIR spectra of substituted cellulose and RR 120 showed peaks at around 1500, 1533, 1551 and 1581 cm^{-1} due to the vibration of aromatic rings in RR 120. These peaks did not exist in the spectra of cellulose, indicating successful substitution of RR 120 in the cellulose.

XRD analysis showed that cotton before substitution had CI of 0.75, close to 0.76, CI of cotton after substitution, inferring that the crystalline regions were not destroyed by substitution. The XRD curves of cotton before and after substitution had peaks at 14.0, 16.0, 22.7 and 34.0 degrees with similar shapes, representing crystal planes of 200, 400, 110 and $\bar{1}\bar{1}0$, respectively. Theoretically, hydrogen ions with sizes much smaller than RR120, could not penetrate into the crystals of cellulose. Thus, it should be more difficult for RR120 to penetrate into the crystals of cotton.

Accelerated cellulose hydrolysis after substitution

Fig. 1

As shown in Fig. 1, the retention volume of unsubstituted and substituted cotton before hydrolysis was 12.2 mL, representing DP_w of 3600; the retention volumes of unsubstituted and substituted cotton after 35 h of hydrolysis were 13.1 and 13.5 mL, representing DP_w of 696 and 328, respectively. Thus, substitution did accelerate degradation of cotton under acidic condition.

3.2. Hydrolysis processes of substituted cotton

Fig. 2

To obtain k based on Eq. 3, $1/DP_t - 1/DP_0 = 1 - \exp(-kt) \approx kt$, the relationship between $1/DP_t - 1/DP_0$ of substituted and unsubstituted cotton and different hydrolysis time t and their linear regression lines is shown in Fig. 2.

Hydrolysis of unsubstituted cotton, as shown by line I in Fig. 2, was a one-step reaction, lasting for 120 h with reaction rate of $2.6 \times 10^{-5} \text{ h}^{-1}$. Afterwards, hydrolysis approached leveling-off, at which stage all the amorphous regions in cellulose backbones were hydrolyzed, leaving only crystalline regions intact.

Hydrolysis of substituted cellulose fibers was a two-step reaction, as shown in linear regression lines a and b in Fig. 2. The first “fast reaction” stage from 0 to 6 h had reaction rate of $2.3 \times 10^{-4} \text{ h}^{-1}$. The apparent hydrolysis rate of substituted cotton in the first stage was defined as k_{apparent} , approximated the slope of line a in Fig. 2. The second “slow reaction” stage from 6 to 60 h had reaction rate of $2.6 \times 10^{-5} \text{ h}^{-1}$. The hydrolysis reached leveling-off after 60 h, 50% shorter than that took unsubstituted cotton.

Based on Eq. 3, the reaction rates of unsubstituted cellulose and second “slow reaction” of substituted cotton were the same. The results were consistent with the similar slopes of line b and line I in Fig. 2. Also, the leveling-off lines via linear regression overlaid with each other, indicating close final DP of substituted and unsubstituted cotton. It also could be inferred that the crystalline regions were intact during substitution, and the accelerated hydrolysis only occurred in amorphous regions.

3.4. Determination of activation energy of hydrolysis via reaction rate calculation

Table 1

Existence of both fast and slow reactions in hydrolysis could be attributed to presence of both substituted and unsubstituted cellobioses in the amorphous regions of cotton. In the first “fast reaction” stage, hydrolysis of substituted cellobioses dominated, though hydrolysis of unsubstituted cellobioses also existed. The first stage ended when all the substituted cellobioses were cleaved. In the second “slow reaction” stage, hydrolysis of remaining unreacted unsubstituted cellobioses predominated, with reaction rate similar to that of hydrolysis of unsubstituted cotton.

K_{apparent} , the apparent hydrolysis rate of substituted cotton in the stage when both of substituted and unsubstituted cellobiose were hydrolyzed, was affected by k_{unsub} , k_{sub} and n_{sub} . For one type of cotton substituted by one type of compound, k_{unsub} and k_{sub} were constant, and thus k_{apparent} was only affected by n_{sub} .

Thus, k_{sub} could better represent the accelerating effect of substitution on hydrolysis of cellobiose. Comparing k_{sub} and k_{unsub} could quantify the acceleration effects. K_{sub} could be calculated using Eq. 9.

Table 1 illustrates the hydrolysis parameters of substituted cotton under different n_{subs} . k_{sub} differed with different substituting compounds, k_{unsub} representing hydrolysis rate of unsubstituted cellobioses in amorphous regions, was a constant. K_{sub} of RR120 substituted cotton with different n_{subs} ranged from 6.0×10^{-3} to 3.9×10^{-2} with no statistically significant differences among each other. The average k_{sub} of $5.0 \times 10^{-3} \text{ h}^{-1}$ was 190 times higher than the k_{unsub} of $2.6 \times 10^{-5} \text{ h}^{-1}$.

Table 2

Decreasing activation energy E_a could accelerate hydrolysis of substituted cotton.

Table 2 shows k_{unsub} and k_{sub} under different temperatures. The linear relationship, $\ln k_{\text{unsub}} = -15878.0/T + 33.7$, was established based on Arrhenius Equation. E_a of hydrolysis of unsubstituted cotton was 132 kJ mol^{-1} , which was in accordance with reported 102-147 kJ mol^{-1} (Higgins et al., 1958). Similarly, E_a of hydrolysis of RR120 substituted cotton was 122 kJ mol^{-1} , 10 kJ mol^{-1} lower than that of the hydrolysis of unsubstituted cotton.

3.5. pH effect

Fig. 3

Fig. 3 illustrates that k_{sub} increased much faster than k_{unsub} as the concentration of hydrogen ion increased. Slope of the line, representing k of RR120 substituted cotton, was 3.46, while the slope of the line representing hydrolysis of unsubstituted cotton was 0.02. Combining with Eq. 4, $k = A_0(a[\text{H}^+] + b) \exp(-E_a/RT)$, with known hydrolysis rate k_{sub} and k_{unsub} , and activation energy E_a , the hydrogen ion coefficient a , could be obtained for unsubstituted cotton and RR120 substituted cotton.

3.6. Modeling of accelerated hydrolysis induced by substitution

Fig. 4

In Fig. 4, experimental data fit simulated relationship between $(1/DP_t - 1/DP_0)$ and hydrolysis time t for substituted cotton with different n_{sub} . In Fig. 4a, hydrolysis of unsubstituted cotton ($n_{\text{sub}} = 0$) and substituted cotton with all cellobioses substituted with RR120 in the amorphous regions ($n_{\text{sub}} = 1$) are indicated. The slopes, indicating hydrolysis rates of these two dot lines ($n_{\text{sub}} = 0$) and dash line ($n_{\text{sub}} = 1$) were k_{unsub} and k_{sub} , representing the lowest and highest hydrolysis rates of cotton under the current hydrolysis condition, respectively.

When $0 < n_{\text{sub}} < 1$, the hydrolysis rate of partially substituted cotton, which contained both substituted and unsubstituted cellobioses in the amorphous regions, was designated as k_{apparent} , affected by both k_{unsub} and k_{sub} , and the ratio of substituted and unsubstituted cellobioses. There was a critical n_{sub} (n_{subc}), at which, the time to complete hydrolysis of both substituted and unsubstituted cellulose was the same. And thus, only one hydrolysis rate for the substituted cotton with n_{subc} existed as indicated by the straight solid line ($n_{\text{subc}} = 0.075$) in Fig. 4a. Except n_{subc} , all the other n_{sub} would result in two hydrolysis rates. When there were more unsubstituted cellobioses in the cotton, namely, $0 < n_{\text{sub}} < n_{\text{subc}}$, the rate of first-stage hydrolysis, k_{apparent} , was higher than k_{unsub} . All the substituted cellobioses were degraded during the first stage, and all the remaining unsubstituted cellobioses were hydrolyzed during the second stage. On the other hand, when there was more substituted cellulose in the cotton, namely, $n_{\text{subc}} < n_{\text{sub}} < 1$, the rate of first stage, k_{apparent} , was lower than the rate of second stage, k_{sub} . All the unsubstituted cellobioses were degraded during the first stage, and all the remaining substituted cellobioses were hydrolyzed during the second stage. The magnified view of hydrolysis process of cotton when $n_{\text{subc}} < n_{\text{sub}} < 1$ is shown in Fig. 4b. Calculation of the hydrolysis rates will be discussed later.

The relationship between n_{sub} and hydrolysis time of substituted cotton is shown in Fig. 4c. The hydrolysis time decreased from 129 h to 8 h, as n_{sub} increased from 0 to 0.075, while it only decreased from 8 h to 0.7 h, as n_{sub} increased from 0.075 to 1. When $n_{\text{sub}} < 0.075$, the whole process was dominated by hydrolysis of unsubstituted cellobioses, the rate of which was 190 times smaller, while the molar ratio was much higher than that of substituted cellobioses. Thus, when the ratio of substituted cellobioses increased, the overall hydrolysis rate increased in a fast manner. However, when $n_{\text{sub}} > 0.075$, the whole

process was dominated by hydrolysis of substituted cellobioses. Due to the much lower reaction rate and ratio, hydrolysis of unsubstituted cellobioses had a minor impact, which further decreased as n_{sub} increased. In general, even with a very low ratio of substitution, hydrolysis of cellulose fibers could be remarkably accelerated.

All the parameters involved in estimation of the hydrolysis parameters were calculated as follows. To obtain the lines of simulated hydrolysis rates of cotton with different n_{sub} , the hydrolysis rates and the time for transition of different hydrolysis stages should be calculated.

The hydrolysis rates could be obtained via Eq. 10.

$$k = A_0 \left(\frac{1}{\text{LODP}} - \frac{1}{\text{DP}_0} \right) [n_{\text{sub}} a_1 [\text{H}^+] \exp \left(-\frac{E_{a1}}{RT} \right) + n_{\text{unsub}} a_2 [\text{H}^+] \exp \left(-\frac{E_{a2}}{RT} \right)] \quad (10)$$

Wherein, LODP is the DP of cellulose when the hydrolysis reaches leveling-off, namely, all the amorphous regions disassemble. DP_0 is the DP before hydrolysis. Thus, $(1/\text{LODP} - 1/\text{DP}_0)$ could be used to represent the accessibility of cellobioses, which could be degraded only when they were in amorphous regions. a_1 and a_2 represent the effects of hydrogen ions or pH effect. A_0 is a frequency factor independent of $(1/\text{LODP} - 1/\text{DP}_0)$, $[\text{H}^+]$, and n_{sub} . E_{a1} and E_{a2} , activation energy of substituted and unsubstituted cellulose, were affected by the types of substituting compounds. The more efficient the acceleration of hydrolysis is, the lower the E_a is. Eq. 10 could be used to obtain k_{sub} , k_{unsub} and k_{apparent} .

Considering cotton was substituted with RR120, a_1 was 7.6, a_2 was 1 and $1/\text{LODP} - 1/\text{DP}_0$ was 0.0035, calculated using Eq. 4. Based on k and E_a , A_0 was $2.3 \times 10^{19} \text{ h}^{-1}$. Using all these parameters, Eq. 11 representing hydrolysis rate of RR120 substituted cotton could be converted from Eq. 10.

$$k = 8.1 \times 10^{16} [7.6 [\text{H}^+] n_{\text{sub}} \exp \left(-\frac{E_{a1}}{RT} \right) + [\text{H}^+] (1 - n_{\text{sub}}) \exp \left(-\frac{E_{a2}}{RT} \right)] \quad (11)$$

All the k depicted by slopes of lines in Fig. 4a calculated using Eq. 11 were in consistence with the k obtained from simulation of experimental data. For example, when $n_{\text{sub}} = 0.039$, the rates of first stage and second stage of hydrolysis were $2.2 \times 10^{-4} \text{ h}^{-1}$ and $2.6 \times 10^{-5} \text{ h}^{-1}$ ($n_{\text{sub}} = 0$), respectively.

Regarding the two-stage hydrolysis of substituted cellulose, the time of transition from first stage to second stage and the overall time for hydrolysis should be calculated.

t_1 , the time to complete the first stage of a two-stage hydrolysis of substituted cellulose, could be determined via Eq. 12. When $0 < n_{\text{sub}} < n_{\text{subc}}$, DP_{t1} , the DP of cellulose at time t_1 , could be obtained via simulation of experimental data when $n_{\text{sub}} = 0.006, 0.011$ and 0.039 . S_1 , the number of scission needed to complete first stage of hydrolysis, could be obtained from Eq. 12-I. When $n_{\text{subc}} < n_{\text{sub}} < 1$, S_2 , the number of scission needed to cleave remaining substituted cellobioses to complete second stage of hydrolysis in Eq. 12-II could be calculated from S_1 (The detailed calculation will be presented in supplementary information.). Subsequently, the corresponding t_1 ($n_{\text{subc}} < n_{\text{sub}} < 1$), which could not be obtained directly due to lack of experimental data, could be calculated based on Eq. 12-II.

$$t_1 = \frac{\frac{1}{DP_{t1}} - \frac{1}{DP_0}}{k_{\text{apparent}}} \begin{cases} = \frac{S_1}{k_{\text{apparent}} DP_0} & (0 < n_{\text{sub}} < n_{\text{subc}}) \quad \text{(I)} \\ = \frac{1}{\frac{LODP(S_2 + 1)}{k_{\text{apparent}}} - \frac{1}{DP_0}} & (n_{\text{subc}} < n_{\text{sub}} < 1) \quad \text{(II)} \end{cases} \quad (12)$$

t , the time to complete hydrolysis of substituted cellulose with certain n_{sub} , is the x value of intersection between the hydrolysis line and the top line of $1/LODP - 1/DP_0$ in Fig. 4, and could be calculated from Eq. 13. When $n_{\text{sub}} = 0, 0.075$ or 1 , there is only one step of hydrolysis and one hydrolysis rate. Given the starting point and end point of the line are $(0, 0)$ and $(t, 1/LODP - 1/DP_0)$, slopes are k_{unsub} , k_{apparent} and k_{sub} , which could be obtained

from Eq. 11, t could be calculated using Eq. 13-I, 13-III and 13-V. When $0 < n_{\text{sub}} < n_{\text{subc}}$ or $n_{\text{subc}} < n_{\text{sub}} < 1$, there are two steps in hydrolysis. Regarding each n_{sub} , the line representing second step of hydrolysis has starting point of $(t_1, 1/DP_t - 1/DP_0)$ and end point of $(t, 1/LODP - 1/DP_0)$, slope of k_{unsub} and k_{sub} . Thus, t could be calculated using Eq. 13-II and 13-IV.

$$t = \begin{cases} \frac{\frac{1}{LODP} - \frac{1}{DP_0}}{k_{\text{unsub}}} (n_{\text{sub}} = 0) & \text{(I)} \\ \frac{\frac{1}{LODP} - \frac{1}{DP_0} + (k_{\text{unsub}} - k_{\text{apparent}})t_1}{k_{\text{unsub}}} (0 < n_{\text{sub}} < n_{\text{subc}}) & \text{(II)} \\ \frac{\frac{1}{LODP} - \frac{1}{DP_0}}{k_{\text{apparent}}} (n_{\text{sub}} = n_{\text{subc}}) & \text{(III)} \\ \frac{\frac{1}{LODP} - \frac{1}{DP_0} + (k_{\text{sub}} - k_{\text{apparent}})t_1}{k_{\text{sub}}} (n_{\text{subc}} < n_{\text{sub}} < 1) & \text{(IV)} \\ \frac{\frac{1}{LODP} - \frac{1}{DP_0}}{k_{\text{sub}}} (n_{\text{sub}} = 1) & \text{(V)} \end{cases} \quad (13)$$

The time to complete the first step and the whole process of hydrolysis obtained from Eq. 12 and 13, matched the experimental data. For example, when $n_{\text{sub}} = 0.039$, the hydrolysis was a two-step reaction, with the rate of first step smaller than that of second step. t_1 , the time to complete the first step of hydrolysis, calculated from Eq. 12, was 8 h, very similar to 7.5 h, the time simulated from experimental data. t , the time to complete the whole hydrolysis, calculated from Eq. 13, was 59 h, close to 60 h, the time simulated from experimental data.

To verify validity of the model, DP_t was obtained using n_{sub} of 0.022 with b of 146 and c of 2 calculated based on Eq. S1. Using the obtained DP_t , t_1 of 10.5 h could be calculated using Eq. 12-I. A red dot line shown in Fig. 4 represents the hydrolysis process simulated

using the t_1' of 10.5 h, obtained from Eq. 12 and 14. A black dot line, linear regression of experimental data of hydrolysis of substituted cellulose with n_{sub} of 0.22 (hollow triangle in Fig. 4a), was also shown in Fig. 4a. t_1 of 12 h, the x value of the intersection of two sections of this black dot line, could be obtained. The red dot line obtained from calculation and black dot line obtained from experimental data are very close to each other, indicating the effectiveness of the model.

4. Conclusion

It was found that even a small amount of substitution could substantially decrease the reaction time of acidic hydrolysis. Relationship between hydrolysis rate and its influence: ratio of substitution, activation energy, pH, and temperature was quantified. This work provided an approach to obtain hydrolysis rate and activation energy of cellobiose in different cellulose fibers substituted by different reactive compounds. It was also confirmed that substitution and hydrolysis of cellulose only occurred in the amorphous regions. Kinetic study might facilitate mechanism study on accelerated hydrolysis substituted cellulose. This study could be important for converting cellulosic substrates into bioethanol and other products.

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Table 1
 k_{sub} and k_{unsub} , the hydrolysis rates of unsubstituted cellobiose and cellobiose substituted
with RR120 at different n_{sub} .^a

n_{sub}	$k_{\text{apparent}} (\text{h}^{-1})$	$k_{\text{sub}} (\text{h}^{-1})^b$	$k_{\text{unsub}} (\text{h}^{-1})$
0	-	-	2.6×10^{-5}
6.0×10^{-3}	5.2×10^{-5}	4.9×10^{-3}	-
1.1×10^{-2}	7.7×10^{-5}	5.0×10^{-3}	-
2.2×10^{-2}	1.1×10^{-4}	5.1×10^{-3}	-
3.9×10^{-2}	2.3×10^{-4}	5.1×10^{-3}	-
Mean $k_{\text{sub}} (\text{h}^{-1})$		5.0×10^{-3}	

^a k_{apparent} and k_{unsub} were calculated based on Eq. 8. The CI of cotton was 0.75. (Hydrolysis condition: pH 3.0, 373K, cotton-to-liquid ratio: 1:20, the substrates were cotton yarns)
^b After power analysis of values of k_{sub} from each n_{sub} , no significant difference was found among them.

Table 2
 k_{sub} and k_{unsub} , the hydrolysis rates of substituted and unsubstituted cellobioses at different temperatures.^a

Temperature (K)	k_{sub} (h^{-1})	k_{unsub} (h^{-1})
343	1.6×10^{-4}	5.8×10^{-7}
353	5.6×10^{-4}	2.8×10^{-6}
363	1.6×10^{-3}	8.2×10^{-6}
373	5.0×10^{-3}	2.6×10^{-5}
E_a (kJ mol^{-1})	122	132

^a Hydrolysis condition: pH 3.0, 373 K, cotton-to-liquid ratio: 1:20, n_{sub} of RR120: 0.039

Figure Caption

Fig.1. Characterizations of unsubstituted and substituted cotton cellulose before and after hydrolysis. GPC elution patterns. (Hydrolysis condition: pH 3.0, 373 K, cotton-to-liquid ratio: 1:20, n_{sub} of RR120: 0.039; The cotton was hydrolyzed for 35 h)

Fig. 2. The relationship between $1/DP_t - 1/DP_0$ of unsubstituted and substituted cotton and hydrolysis time. ■ represents the hydrolysis process of cotton substituted by RR120. a, fast reaction, hydrolysis of substituted cellobioses dominated, while hydrolysis of unsubstituted cellobioses also existed; b, slow reaction, hydrolysis of remaining unsubstituted cellobioses; c, leveling off. □ represents the hydrolysis process of unsubstituted cotton. I, hydrolysis of unsubstituted cellobioses in amorphous regions; II, leveling off. (Hydrolysis condition: pH 3.0, 373 K, cotton-to-liquid ratio: 1:20, n_{sub} of RR120: 0.039)

Fig. 3. Effect of pH on hydrolysis rates of substituted and unsubstituted cotton. The magnified view represents the small slope of unsubstituted cotton. (Hydrolysis condition: pH 3.0, 373 K, cotton-to-liquid ratio: 1:20, n_{sub} of RR120: 0.039)

Fig. 4. a) Comparison of experimental data and simulated relationship between $(1/DP_t - 1/DP_0)$ and hydrolysis time t for substituted cotton with different n_{sub} , which was the molar ratio of substituted cellobioses in the amorphous regions of cotton. The markers, ■, ▲, ◆, △, ● represented experimental data points of substituted cotton with different n_{sub} . The dot lines, solid line and dash lines represented hydrolysis processes when $n_{\text{sub}} < n_{\text{subc}}$, $n_{\text{sub}} = n_{\text{subc}}$, and $n_{\text{sub}} > n_{\text{subc}}$, respectively. The thin solid line on the top represented $1/LODP - 1/DP_0$. Red line is simulated based on Eq. 12. **b)** Magnified view of lines representing hydrolysis of substituted cotton with $n_{\text{sub}} > n_{\text{subc}}$; **c)** Estimated time for hydrolysis of

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517 substituted cotton with different n_{sub} according to Eq. 12. (Hydrolysis condition: pH 3.0,
518 373 K, cotton-to-liquid ratio: 1:20, n_{sub} of RR120: 0.039)

Figure 1

Figure 2

Figure 3

Figure 4