

The properties of enzyme-hydrolyzed cellulose in aqueous sodium hydroxide

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Abstract

Pure natural cellulose (softwood pulp) modified with cellulase is allowed to react with sodium hydroxide in a muller, and changes in structure and properties are investigated by FTIR and DSC. The reactivity of cellulose for some dissolving and derivatization processes is shown to be improved by an enzymatic hydrolysis and admixture with sodium hydroxide. The modified cellulose dissolved at 9% (wt) sodium hydroxide at -10°C at 6% pulp consistency, while the \overline{DP} of cellulose is > 350 . © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Cellulose is the most abundant and renewable biopolymer on Earth. It is in demand for regenerated celluloses that are produced by environmentally friendly processes. Acidic and high-temperature degradation are unsatisfactory in that waste cellulose contains impurities that generate unwanted byproducts under these harsh conditions. The enzymatic modification of cellulose is a challenge in all applications using cellulose-based fibers due to the potential benefits.¹

Despite the high density of hydroxyl groups in the molecule, cellulose is not soluble either in water or in aqueous alkaline solutions.² This is due to the existence of intra- and intermolecular hydrogen bonds in solid-phase cellulose that hinder the dissolution of cellulose into a solvent.^{3,4} Dissolution of cellulose in a solvent can be realized only by an appropriate combination of specific modifications of the structure of cellulose and solvent. Cellulose can be directly dissolved in organic solvents, such as *N*-methylmorpholine *N*-oxide.⁵ Activation of the cellulose, which is defined as opening up the cellulose structure to the solvent, is necessary for

dissolution. Cellulose can be activated by swelling and degradation processes, by mechanical treatment and by drying or thermal treatment.⁶

The question of the dissolution of cellulose directly in an aqueous solution of sodium hydroxide has remained pressing over the long history of the viscose method of production of regenerated cellulose fibers. Studies in this direction are aimed toward selecting an effective method of modifying the cellulose in order to prepare it for dissolution.

The aim of the present study is to investigate the physicochemical properties of a mixture of cellulose with solid sodium hydroxide and the improvement of its alkali solubility.

2. Results and discussion

Effects of mixture on the \overline{DP} and alkaline solubility of the pulp.—The \overline{DP} of cellulose after activation decreased by no more than 10% in comparison to the \overline{DP} of the initial cellulose, as seen in Table 1. More important changes in \overline{DP} were observed after dissolution and precipitation. Table 1 shows the \overline{DP} of cellulose in solution. All of the solutions prepared contained different amounts of incompletely dissolved particles, and for the most part, the solubilities of the cellulose samples

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was about 95%, which was required for acceptable filterability of the solutions. Urea promotes dissolution of cellulose, which may be due to the formation of cellulose carbamate according to one report.⁷

As was expected, enzymatic treatment with cellulase was found to be efficient in hydrolyzing cellulose, decreasing the \overline{DP} and improving the alkaline solubility. This is in accordance with the results of two previous studies.^{8,9}

Effect of the activation on the structural properties of cellulose.—The FTIR spectroscopy data in Fig. 1 all indicate that enzymatic treatment and activation have some effects on the structure of cellulose. The FTIR spectra show characteristic cellulose peaks around 1000–1200 cm^{-1} .¹⁰ The band near 1160 cm^{-1} is representative of the antisymmetric bridge stretching of C–O–C groups in cellulose and hemicellulose, and the band near 1318 cm^{-1} could be ascribed to CH_2 -wagging vibrations in cellulose and hemicellulose. The 895 cm^{-1} band, which is characteristic for β -linkages, especially in hemicelluloses,¹¹ was reduced after enzyme treatment. There was also a substantial reduction in the band in the 1635–1640 cm^{-1} region that has been attributed to HOH deformation vibrations.

The bands near 3400 cm^{-1} are representative of OH vibrations. The band of OH vibrations is broad and moves to a low wave number (3370 cm^{-1}) because of the presence of intermolecular and intramolecular hydrogen bonds. After the enzymatic hydrolysis with crude cellulase, the bands near 3400 cm^{-1} became narrower and are moved to higher wave numbers (3400 cm^{-1}), which is good evidence that a number of the hydrogen bonds were broken in the enzymatic hydrolysis.

The spectrum of the mixture of cellulose and sodium hydroxide is not additive with respect to the spectrum of the cellulose. In particular, a strong band appears in the region of deformation vibrations of CH at 1450 cm^{-1} , and the character of the absorption differs from the absorption in the region both in the spectrum of sodium hydroxide and in the spectrum of cellulose or cellulose after enzymatic treatment. In comparison to the spectrum of cellulose, the ratio of the absorption bands in the region of skeletal vibrations at 1000–1160 cm^{-1} and strong leveling of absorption in the 1250–1350 cm^{-1} region, which has been attributed to CH and CH_2 deformation vibrations, changes significantly. In addition, the spectra of mixtures of cellulose and

Table 1
Properties of cellulose after treatment

Cellulose sample	Original	Enzyme-hydrolyzed	Treatment of grinding with NaOH		
			Original	Enzyme-hydrolyzed	Additive
\overline{DP}	756	476	706	432	432
S (%)	21	72	30	87	95
\overline{DP} of solute	310	420	340	370	380

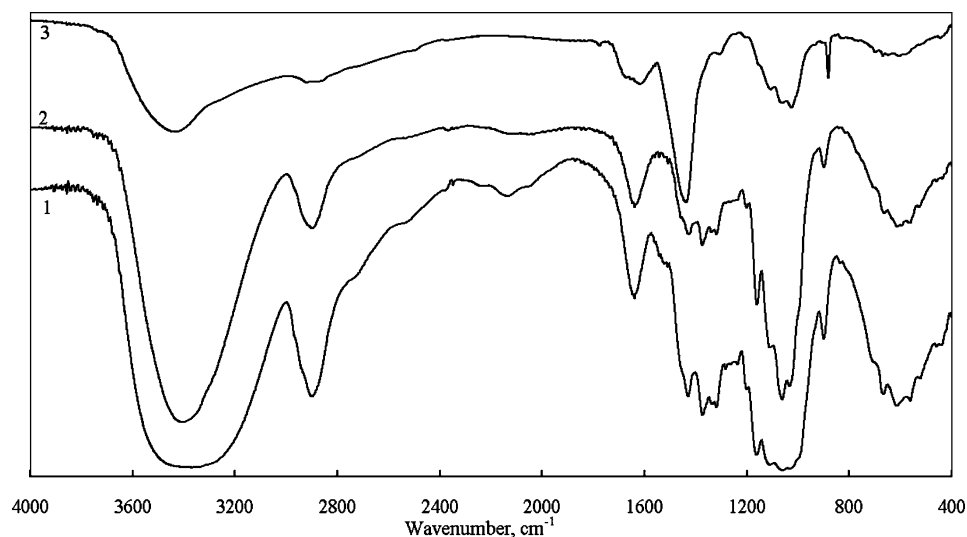


Fig. 1. FTIR of the samples. (1, Original cellulose; 2, enzyme-hydrolyzed cellulose; 3, enzyme-hydrolyzed cellulose ground with sodium hydroxide.)

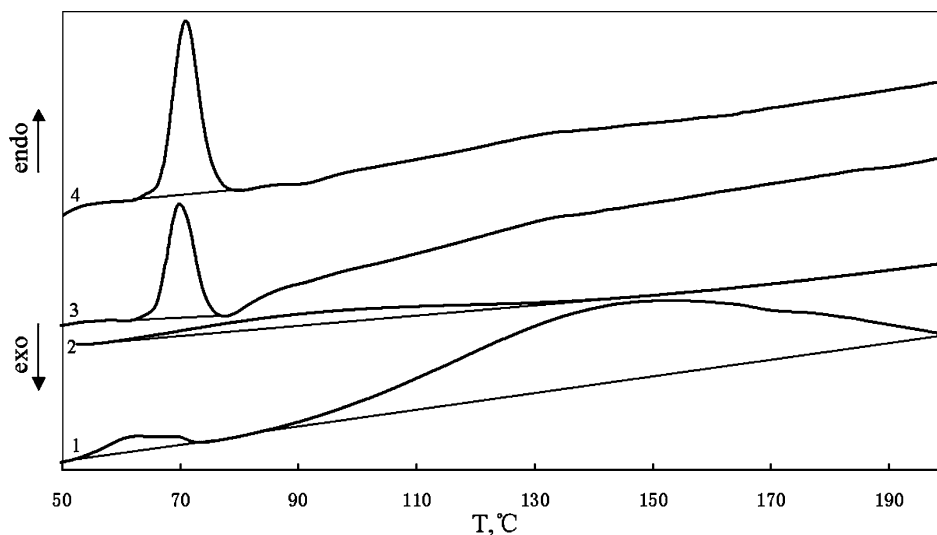


Fig. 2. DSC curves of the samples. (1, Enzyme-hydrolyzed cellulose ground with sodium hydroxide; 2, cellulose; 3, sodium hydroxide; 4, original cellulose ground with sodium hydroxide.)

sodium hydroxide exhibit differences in the region of HOH deformation vibrations at 1640 cm^{-1} and OH stretching vibrations near 3400 cm^{-1} .

The FTIR spectroscopy data showed that very pronounced reactions take place with cellulose after enzymatic treatment and sodium hydroxide treatment under the activation conditions.

The DSC data also show the formation of alkaline cellulose in the reaction of enzymatically treated cellulose with sodium hydroxide in Fig. 2. The DSC curve of the cellulose has one extensive endothermic peak with the maximum $\sim 105^\circ\text{C}$. The endothermic peak is probably caused by separated water. There is an endothermic peak about 70°C on the curve of sodium hydroxide in the $50\text{--}200^\circ\text{C}$ temperature interval that could be assigned to the melting of sodium hydroxide monohydrate. Another endothermic peak caused by melting of sodium hydroxide clearly appears at 296°C . This part of the curve is not shown in Fig. 1, because the experiments were conducted up to a temperature no higher than 200°C .

The DSC curve of the activated cellulose with sodium hydroxide is characterized by the presence of three endothermic peaks. The endothermic peak about 62°C should be assigned to the melting of alkaline cellulose dual hydrate $((\text{C}_6\text{H}_{10}\text{O}_5)_n\text{NaOH}\cdot 2\text{H}_2\text{O})$, and the endothermic peak about 70°C is caused by the melting of sodium hydroxide monohydrate. The results are in accordance with the published data.¹² The endothermic peak at 150°C is most probably correlated with separation of water from alkaline cellulose $((\text{C}_6\text{H}_{10}\text{O}_5)_n\text{NaOH}\cdot\text{H}_2\text{O})$ formed. There is an endothermic peak about 70°C on the curve of the original cellulose mixed with sodium hydroxide. It is caused by melting of sodium hydroxide monohydrate.

A comparison of the DSC curve of cellulose activated by sodium hydroxide with the DSC curve of original cellulose mixed with sodium hydroxide led to the conclusion that alkaline cellulose is formed in the mixture of enzymatically treated and sodium hydroxide under conditions of grinding. The result shows alkaline cellulose obtained in the reaction of cellulose with sodium hydroxide in the solid phase, but the formation of alkaline cellulose only takes place in the reaction of cellulose with an aqueous solution of sodium hydroxide according to one report.¹³ It is one of key factors for improvement on the alkaline solubility of the pulp after grinding.

3. Experimental

Materials

Dissolving pulp. Softwood dissolving pulp was Allicell-Super (Western Pulp, Ltd., Vancouver, BC, Canada) with high α -cellulose content. The characteristics of the pulp are shown in Table 2. Prior to enzymatic and alkaline treatment the pulp was disintegrated. The pulp with enzymatic treatment has low \overline{DP} value of 432.

Chemical reagents. The solid sodium hydroxide and urea used were of analytical purity.

Enzymes. Multicomponent cellulase was derived from *Humicola insolens*. The activities of the enzymes are given as international unites (IU), in which one unit of activity is defined as the amount of enzyme required to liberate $1\text{ }\mu\text{mol}$ of product per minute. The endoglucanase (CMCase), xylanase, and filter paper activities were measured on carboxymethylcellulose (1% CMC, Sigma), xylan (1% birchwood xylan, Sigma) and filter

paper (No. 1 Whatman), respectively, using methods described previously.¹⁴ The amount of reducing sugar released was estimated by the dinitrosalicylic acid method¹⁵ using glucose as the standard. The specific activities of cellulase are presented in Table 3. Cellulase is shown to contain high levels of xylanase activity.

Analysis and treatment

Activation of cellulose with solid sodium hydroxide.

Cellulose was ground into a powder and freeze-dried. It was then mixed with solid sodium hydroxide in a muller (IKA® A11 Basic) for 60 s. The ratio of cellulose and sodium hydroxide was 1:1 in the mixture. Urea was added to the mixture at a dosage of the additive of 0.2 g/g pulp (dw).

Enzymatic treatment of pulp. All enzyme treatments were incubated at 50 °C under continuous agitation at 175 rpm, at 3% pulp consistency in 50 mM phosphate buffer (pH 7.0) for 5 h. The enzyme concentration in the pulp treatments was based on the mass of oven-dry pulp. The dosage for the cellulase was 2 mg/g (dw) pulp. The enzyme reactions were terminated by boiling for 15 min.

Analytical methods. The viscosity and characteristics of the pulp were measured according to the related methods of ISO/TC6 (5351/1, 1762,535)

Determination of \overline{DP} .—The average degree of polymerization (\overline{DP}) was measured viscosimetrically in CuEn (copper ethylene diamine solution), and the intrinsic viscosities obtained converted into the respective values of \overline{DP} by Eq. (1):¹⁶

$$\overline{DP}^{0.905} = 0.75[\eta_{\text{CuEn}} (\text{cm}^3/\text{g})] \quad (1)$$

Cellulose was separated from the activated mixtures by washing with water to remove sodium hydroxide.

Alkaline solubility of the pulp.—The alkaline solubility of the reference and enzyme-treated pulps was determined by a modification of the previously reported method.¹⁷ The pulps were dissolved under vigorous stirring (1500 rpm) in 9% (wt) sodium hydroxide at –10 °C at 6% pulp consistency for 2 h. Thereafter the

solutions were centrifuged at $1000 \times g$ for 10 min. The dissolved part was discarded, and the undissolved part was washed with 9% sodium hydroxide. The washed cellulose was treated with 3% hydrochloric acid and then washed with deionized water in a ceramic sinter. The solubility (S) of cellulose was calculated from the following equation:

$$S = (W - W_U)/W \times 100\%$$

W , weight (dw) of enzymatically treated or control cellulose sample.

W_U , weight (dw) of undissolved cellulose.

FTIR spectroscopy.—Fourier-transform infrared spectra (FTIR) were obtained with a Perkin–Elmer FTIR 2000 system spectrometer. The wave number range scanned was $4000\text{--}350 \text{ cm}^{-1}$. After milling, the powdered samples were compacted into KBr disks and analyzed.

Differential scanning calorimeter (DSC).—The DSC data were obtained with a Perkin–Elmer DSC 7 instrument with a heating rate of 40 °C/min in the 50–200 °C range in a dry nitrogen atmosphere.

4. Conclusions

Enzymatic treatment and activation with solid sodium hydroxide modified the cellulose material. We have examined the effects of the methods on charges in the viscosity-average degree of polymerization (\overline{DP}), solubility towards aqueous alkali solution, the variety of structure and properties of cellulose analyzed by FTIR spectra and DSC curves. The following conclusions can be summarized briefly from this study: (1) Modified cellulose activated with solid sodium hydroxide could be dissolving in 9% (wt) sodium hydroxide at –10 °C at 6% pulp consistency while the \overline{DP} of cellulose is > 350 . (2) The hydrogen bonds of the OH groups were partly broken in the enzyme-hydrolyzed cellulose as shown by FTIR spectroscopy. (3) Alkaline

Table 2
Characteristics of the pulp

\overline{DP}	α -Cellulose (%)	Ash content (%)	Fe (ppm)	Alkali absorption velocity (mm/5min)	Swelling capacity (%)
756	92.8	0.09	7	35×33	408

Table 3
The specific activities of cellulase

Enzymes	CMCase (IU/mg)	Xylanase (IU/mg)	Filter paper (IU/mg)
Cellulase	2.07	63.2	0.21

cellulose is formed in the mixture of enzymatically treated cellulose and sodium hydroxide under the conditions as determined by analyzing the FTIR spectra and DSC data. Such treatment improves the alkaline solubility of cellulose.

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