

Crystal and Thermal Response of Cellulose Isolation from Bamboo by Two Different Chemical Treatments

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Cellulose is a biodegradable, renewable material, and its chemical, crystalline, and thermal properties place limits on how it can be used in industrial applications. In this study cellulosic preparations made from bamboo were fractionated using a one-step process (EtOH-HNO₃) or a two-step process (NaClO₂ delignification and KOH treatment). Partial degradation of cellulose was observed in alkaline conditions. Although the crystal configuration was maintained, it was observed that the cellulose structure after delignification and alkaline post-treatment exhibited unique exothermic pyrolysis properties. By comparison, the acidic EtOH-HNO₃ process efficiently removed hemicellulose and lignin from bamboo, achieving the highest purity of cellulose (96.8%) with the highest degree of polymerization (DP) value (815).

Keywords: Bamboo; Holocellulose; Cellulose extraction; Nitric acid-ethanol treatment

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INTRODUCTION

One of the greatest challenges for society in the 21st century is to meet the fast-growing energy needs for transportation, heating, industrial processes, and sustainable raw materials (Hahn-Hägerdal *et al.* 2006). Although early attempts to derive bioethanol from sugar or starch has contributed to food insecurity, more recent attempts to produce bioethanol from lignocellulosic feedstocks have gained increasing attention (Wang *et al.* 2009; Chen and Fu 2016; Dominguez *et al.* 2017; Zabed *et al.* 2017; Zhang *et al.* 2018). Because of the more favorable CO₂ balance compared to that of fossil fuels, bioethanol derived from lignocellulosic feedstock could be an attractive long-term option.

As a kind of abundant natural resource with extensive uses, bamboo can contribute as structural material and raw material for construction and papermaking (Tsubota *et al.* 2016; Chong *et al.* 2017; Dixon *et al.* 2018; Jiang *et al.* 2018; Salvati *et al.* 2018). With growing concerns regarding global warming, it is worthy to consider bamboo as a material to produce renewable fuels, taking advantage of its fast growth rate, short renovation, and easy propagation. Compared to other lignocellulosic biomass, bamboo has unique characteristics in chemical composition and anatomical structure. Cellulose, a constituent of most green plants, is the most abundant organic polymer on earth, making up 40 to 50% of the mass in bamboo (Sathitsuksanoh *et al.* 2010; Yamashita *et al.* 2010; Li *et al.* 2014; Zhang *et al.* 2018). Recent studies about cellulose have focused on small-scale applications, such as nanocomposites (Garcia *et al.* 2016; Nypelo *et al.* 2018; Rajinipriya *et al.* 2018; Shanmugam *et al.* 2018; Sogut and Seydim 2018).

Nanocellulose can be classified into two categories based on physical and chemical properties: cellulose nanocrystals (CNC), which are obtained from treatment under acidic conditions, and cellulose nanofibers (CNF), arising from enzymatic and/or mechanical disintegration (Abitbol *et al.* 2016). To obtain nanocellulose, pure cellulose pulp is subjected to various treatments. Although many studies have reported the physicochemical properties of woody biomass, there have been few works concerning on the structural and thermal differences in bamboo biomass.

This work aimed to produce and characterize cellulosic preparations from bamboo through two different methods: a one-step process (EtOH-HNO₃) and a two-step process (NaClO₂ delignification and KOH treatment). Examining the chemical, crystalline, and thermal properties of raw bamboo, holocellulose, and cellulose preparations provided a basis for further comprehensive development and utilization of bamboo cellulose in potential commercial applications.

EXPERIMENTAL

Materials

Moso bamboo (*Phyllostachys heterocycla*) culms were obtained from the central area of Anhui Province, China. The culms were air-dried, ground, and screened through a 40-mesh screen, and bamboo powder was collected for further chemical analysis. The main chemical compositions were glucose (44.1%), xylose (22.4%), lignin (26.0%), extractives (9.9%), and ash (1.2%), according to the standard National Renewable Energy Laboratory (NREL) protocol (Sluiter *et al.* 2006). The raw bamboo and substrates were prehydrolyzed in 75% sulfuric acid for 2 h at room temperature, and then diluted to 3% sulfuric acid for 1 h at 121 °C. The acid-soluble lignin was evaluated at 205 nm on a UV-Visible spectrophotometer using 3% sulfuric acid as the control blank. The acid-insoluble lignin contents were determined by drying the acid-treated samples in a vacuum oven (~5×10³ Pa) at 60 °C for 10 h. The liquid samples were analyzed by ion chromatography using an amperometric detector (Metrohm Corporation; Herisau, Switzerland). Detection of the sugars (glucose and xylos) in the hydrolysates were performed at 32 °C with a Hamilton RCX-30 column and Metrosep RP2 guard column. The deviations of these values from their respective means were all less than 6%. All material samples were stored in plastic bags at room temperature until further processing. The chemical reagents used in our study were purchased and were used as received from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China).

Methods

Preparation of holocellulose

Prior to separation, bamboo powder was extracted in a Soxhlet extractor with a 1:2 mixture of ethanol and toluene (v/v) for 6 h, followed by the extraction with pure ethanol for four hours. The extracted bamboo powder was oven-dried at 60 °C overnight to reach a constant weight. The delignification process was performed with acidified sodium chlorite (NaClO₂) based on a modified method (Ishizawa *et al.* 2009). Briefly, 50 g of extractive-free bamboo powder was immersed in 1 L of 7.5% NaClO₂ solution (w/v) and incubated at 80 °C for 2 h under acidic conditions (pH 3.8 to 4.0), adjusted with concentrated acetic acid. The process was repeated to ensure maximum lignin removal, and the solid residue was thoroughly washed with distilled water and then oven-dried.

Preparation of the cellulosic sample

Hemicellulose in the holocellulose was completely removed by use of 10 wt% potassium hydroxide (KOH) aqueous solution with a solid to liquid ratio of 1:20 (g/mL) at room temperature for 15 h (Sun and Tomkinson 2002). The cellulosic residue was thoroughly washed to a neutral pH with distilled water and oven-dried. The cellulose preparation was kept in a desiccator at room temperature before further analysis.

Preparation of EtOH-HNO₃ cellulose

A nitric acid and ethanol mixture (20:80, v/v) was used to treat the bamboo powder. Lignin was converted into nitrated lignin and dissolved in ethanol for removal (Shi and He 2008). Upon removing the extractives, 5 g of bamboo powder was added to a flask with one liter of nitric acid-ethanol solution. The flask was fitted with a reflux condenser tube and the mixture was refluxed while being shaken in a water bath at 100 °C for 60 min. After being filtered to remove the liquid, the solid was repeatedly treated with the same solution until the liquid became colorless. Finally, the solid residue was recovered by filtration and washed several times with hot water and ethanol until the filtrate reached a pH value of 7.0. Lastly, the EtOH-HNO₃ cellulose was dried in an oven at 60 °C until the weight was constant.

Chemical and physical characterization

The average degree of polymerization (DP) and molecular weights of the cellulosic sample by removing hemicelluloses and EtOH-HNO₃ cellulose were determined using the cupri-ethylenediamine (CED) method (Wang *et al.* 2009) and calculated according to Eq. 1 (Evans and Wallis 1989),

$$DP^{0.9} = 1.65[\eta] / \text{mL g}^{-1} \quad (1)$$

where η is determined by British standard methods for determination of limiting viscosity number in dilute solution (Part 1, Cupri-ethylenediamine(CED) method). The molecular weights were calculated by multiplying their DP values by 162, the molecular weight of an anhydroglucose unit.

Analysis of the crystalline structures of the cellulosic samples was performed through X-ray diffraction (XRD-6000; Shimadzu, Tokyo, Japan), scanning from 5° to 45° 2θ at a scanning speed of 2 ° per min with a Cu K α radiation source ($\lambda = 0.154$ nm) at 40 kV and 30 mA. To determine the crystallinity, the total diffracted area and the area under the crystalline peaks were integrated after correcting the data for absorption. The ratio of the crystalline area to that of total diffracted area was taken as the relative crystallinity (Segal *et al.* 1959). Each sample was measured twice.

Thermal stability

Thermal analysis of all samples was performed using thermo-gravimetric analysis (TGA) and differential thermal analysis (DTA) on a simultaneous thermal analyzer (DTG-60, Shimadzu, Japan). The apparatus was continually flushed with a nitrogen flow of 30 mL/min. Weight loss of the sample was measured across a temperature range from ambient to 600 °C. All samples were heated in a platinum crucible from 50 °C to 600 °C at a rate of 10 °C/min.

RESULTS AND DISCUSSION

Chemical and Crystal Structure Analysis

The degree of crystallinity of cellulose has been considered as an important factor in resisting enzymatic degradation and chemical modification. Therefore, the crystalline characteristics of the two cellulose preparations were obtained through delignification followed by alkaline treatment or nitric acid-ethanol treatment. They were examined by powder X-ray diffraction (XRD) and compared with the raw bamboo and holocellulose (Fig. 1). This measurement is a reliable indicator of the transformation of the cellulose structure. The patterns were typical of semicrystalline cellulose with an amorphous broad band and defined crystalline peaks. The differences in the crystal peaks were largely ascribed to the chemicals and treatment processes used. It was observed that the raw bamboo had typical cellulose I diffraction angles at 22.5° and 15.4° , corresponding to the diffraction planes 020 and $1\bar{1}0$, respectively. The diffraction peaks of the 110 (diffraction angles at 16.7°) and $1\bar{1}0$ planes were always merged together. By fitting a strong peak at 22.5° , a small one at 20.6° emerged, likely relating to the lignin components or lignin-carbohydrate complex (LCC), since it disappeared after the delignification process. Further treatment with alkaline solution at room temperature could not alter the crystalline structure of cellulose, and the color rendering index (CRI) value increased, corresponding with the higher cellulose content, as can be seen in Table 1. The peeling and degradation reactions in cellulose under alkaline conditions removed some of the less ordered cellulose fraction, which could have led to the increase in crystallinity. In comparison, EtOH-HNO₃ cellulose exhibited the highest XRD intensity by reason of its high cellulose purity. The small peak at 20.6° remained in the EtOH-HNO₃ cellulose, suggesting that the small amount of residual lignin could have been activated during the EtOH-HNO₃ process, forming linkages with cellulose. In addition, a new peak at 17.5° emerged by multiplex fitting, indicating that the partial degradation of cellulose occurred during the EtOH-HNO₃ process.

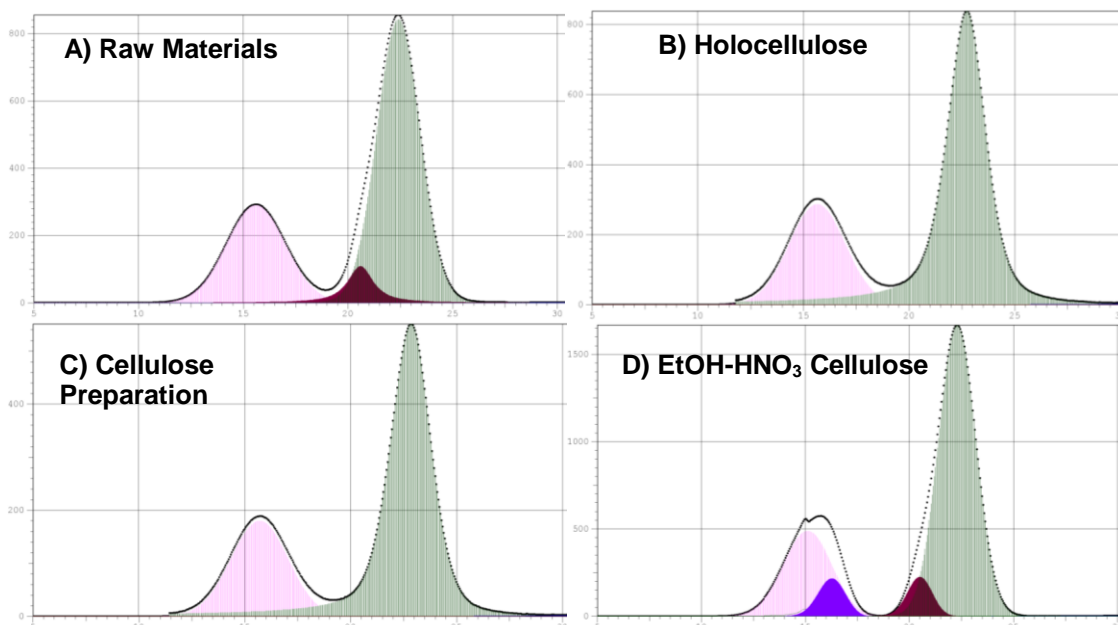


Fig. 1. The XRD patterns for a) raw materials, b) holocellulose, c) cellulose preparation, and d) EtOH-HNO₃ cellulose

Table 1. Properties of Cellulosic Samples

Sample	Glucose (wt%)	Xylose (wt%)	AIL ^a (wt%)	ASL ^b (wt%)	η^c	DP ^d	Mw ^e
Raw Material	48.4	21.6	25.8	1.2	214	679	1.1×10^5
Holocellulose	62.4	32.8	2.6	1.2	235	754	1.2×10^5
Cellulose Preparation	92.8	4.6	1.2	0.7	179	556	0.9×10^5
EtOH-HNO ₃ Cellulose	96.8	1.3	0.9	0.2	252	815	1.3×10^5

Notes: ^aAIL: acid-insoluble lignin; ^bASL: acid-soluble lignin. ^c η : determined by British standard methods for determination of limiting viscosity number in dilute solution Part 1. Cupri-ethylenediamine (CED) method. ^dDP: Degree of polymerization, calculated by $DP^{0.9} = 1.65 [\eta]$. ^eCalculated by $DP \times 162$

The viscosity average DP of cellulose is conveniently assayed from the intrinsic viscosity of its solution in 0.5 M cupriethylenediamine hydroxide (cuene). The molecular weight of the cellulose was estimated multiplying the DP by 162, the molar mass of anhydroglucose. The values of all cellulosic samples are listed in Table 1. The increased values of η , DP, and M_w coincided with the increased content of cellulose, as cellulose obtained the highest DP value among the three main components in biomass.

The delignification process with NaClO₂ significantly decreased the lignin content from 27% to 3.8%, and post alkaline treatment mainly removed the hemicellulose component, leaving only 4.6% hemicellulose and 1.9% lignin in the cellulose preparation. Meanwhile, the partial degradation of cellulose was also observed in the alkaline condition since the DP value reduced from 754 to 556. In contrast, the highest purity of cellulose (96.8%) accompanied with the highest DP value (815) was gained by the acidic EtOH-HNO₃ process.

Thermal Stability

Thermal decomposition parameters were determined from the TGA/DTA curves against temperature for all cellulosic samples (Fig. 2). The mass loss resulted from volatilization or charring process of the degradation products. The thermal destruction of cellulose led to the formation of cavities inside the microfibrils. Hydrogen bonds in cellulose begin to collapse and the microfibrils soften following temperature increases. The mass loss is induced by the aggregation of cavities and the removal of decomposition gases from the cellulose matrix. The carbonaceous residues, as the end-products from the decomposition of cellulose under an inert atmosphere were mainly consist of polycyclic aromatic compounds (Van De Velde and Kiekens 2002). Thermal degradation of cellulose proceeds through two types of reaction in the range of 200 to 500 °C: a gradual degradation, decomposition, and charring by heating at lower temperatures, and a rapid volatilization accompanied by the formation of levoglucosan on pyrolysis at higher temperatures (Balat 2008). By comparison, hemicellulose is easily degraded, which can be attributed to its random and amorphous structure of rich branches. Additionally, the characteristics of difficult decomposition in lignin caused a wide temperature range (200 to 900 °C).

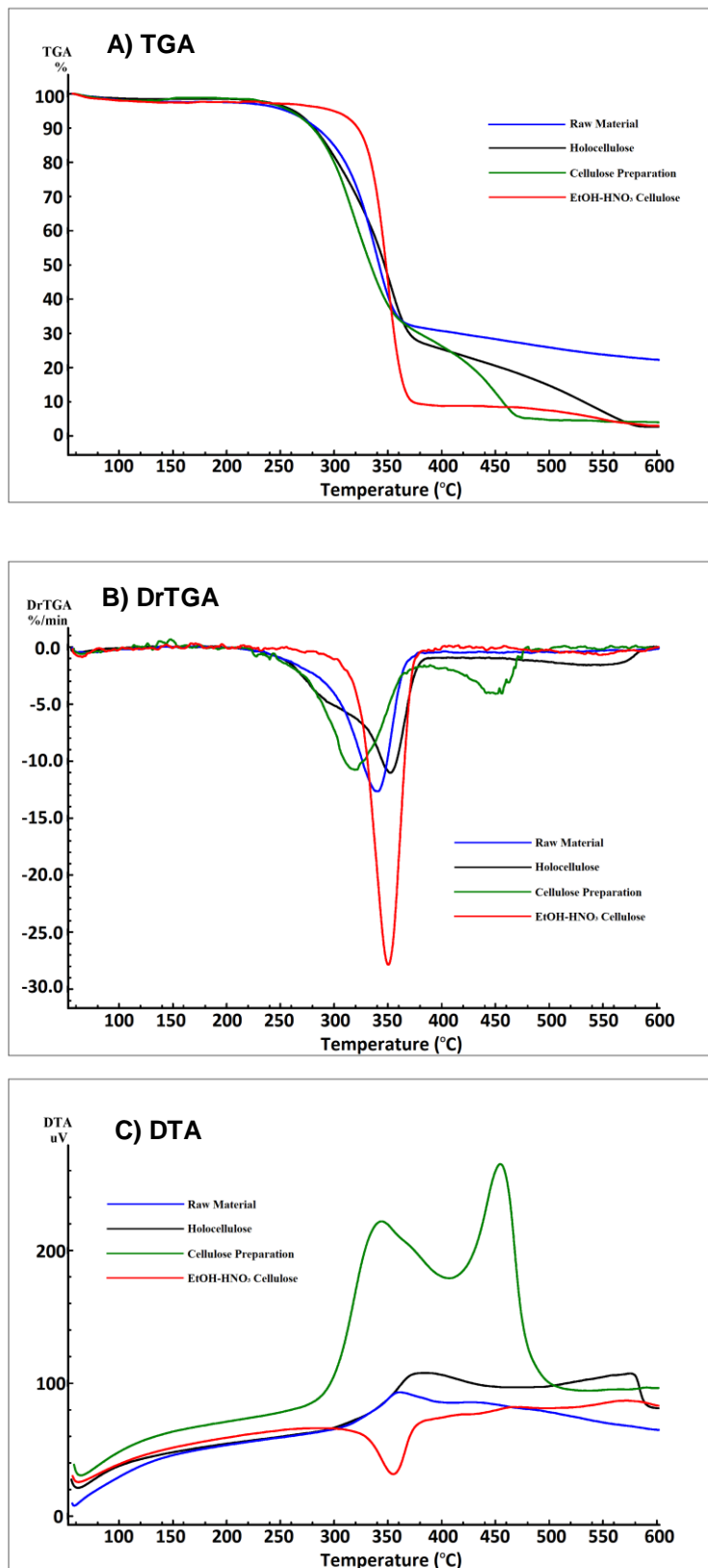


Fig. 2. Thermal analysis results for raw bamboo (blue), holocellulose (black), the prepared cellulose (green), and the ethanol-nitric acid cellulose (red): Thermogravimetric analysis (A); derivative curves for TGA (B); and differential thermal analysis (C)

For the raw bamboo, a typical TGA/Derivative TGA (DrTGA) curve of lignocellulosic biomass was observed, in which a strong peak was observed at around 340 °C, likely having resulted from the simultaneous degradation of cellulose and hemicelluloses. The raw bamboo also experienced the lowest mass loss among all samples due to its high lignin content. After the delignification process, the beginning degradation temperature of holocellulose was slightly increased, and the above peak was split into two peaks. The first one, observed at 270 °C, is ascribed to the degradation of the hemicellulosic component, and the second one at 360 °C is assigned to the thermal degradation of cellulose at 360 °C. Along with the further removal of hemicellulose by the alkaline treatment, the shoulder peak from hemicellulose degradation disappeared in the TGA curve of cellulose preparation. Meanwhile, the degradation peak of cellulose shifted to the lower temperature (~320 °C), which is likely attributable to the partial degradation of cellulose in the alkaline condition. This is further confirmed by the decreased DP value.

For the EtOH-HNO₃ cellulose, a classic cellulose degradation curve was observed (Yang *et al.* 2007). Cellulose pyrolysis was focused in a narrow temperature range (315 to 400 °C), and almost all cellulose was thermally degraded with a very low solid residue at temperatures higher than 400 °C. Furthermore, DTA curves were measured to reflect the energy consumption properties in pyrolysis. The pyrolysis process of cellulose was basically endothermic, but hemicellulose and lignin underwent an exothermic process according to the previous literature (Yang *et al.* 2007). The DTA curve of EtOH-HNO₃ cellulose underwent an endothermic process, and the exothermic degradation of raw bamboo and holocellulose was likely attributable to the high amount of non-cellulosic components in these two samples. However, it is surprising to note that the pyrolysis of cellulose preparation was exothermic. A likely reason for heat liberation could be the DP variation, but further work is in progress.

CONCLUSIONS

1. Two cellulosic preparations were fractionated and fully characterized by chemical, crystalline and thermal properties. The cellulose obtained from the one-step EtOH-HNO₃ process exhibited the highest purity and typical endothermic behavior, although partial degradation of cellulose and linkages with residual lignin were observed by XRD analysis.
2. By comparison, the delignification process with NaClO₂ efficiently removed the lignin components and maintained the DP and thermal characteristics of cellulose. However, the alkaline post treatment led to almost the complete removal of hemicelluloses along with the degradation of cellulose. Meanwhile, the cellulose preparation from two-step fractionation showed unique exothermic behavior, which was closely related to the DP variation. The chemical, crystalline, and thermal behavior of cellulose preparations reported in this work offer a foundation for future studies of cellulose-based polymers.

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