# EFFECTS OF DILUTE ACID HYDROLYSIS ON COMPOSITION AND STRUCTURE OF CELLULOSE IN *EULALIOPSIS BINATA*

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Dilute sulfuric acid hydrolysis was performed before the isolation of cellulose from *Eulaliopsis binata*. And then, the effects of dilute acid hydrolysis on composition and structure of the cellulose was studied in detail. The results indicated that hemicellulose was dissolved mostly and that the lignin-hemicellulose-cellulose interactions were also partially disrupted during the dilute acid hydrolysis. Cellulose in *Eulaliopsis binata* was identified as the cellulose I allomorph with low crystallinity. What's more, hydrolysis with dilute acid at high temperature increased the degree of cellulose crystallinity and relatively reduced the proportions of less ordered cellulose allomorphs. This was attributed to a preferential degradation of amorphous cellulose and less ordered crystalline forms during the hydrolysis. The cellulose preparation from *Eulaliopsis binata* after dilute acid hydrolysis had a higher thermal stability than the cellulose preparation from untreated *Eulaliopsis binata*.

*Keywords: Eulaliopsis binata; Dilute acid hydrolysis; Cellulose; Composition; Structure*

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## INTRODUCTION

All lignocellulosic materials, including woody biomass from hardwood and softwood trees and agricultural plants, are composed of cellulose, hemicellulose, and lignin, which are closely associated with each other at the plant cell level (Zhang et al. 2004). *Eulaliopsis binata* is a perennial grass belonging to the subtribe Apocopidinae in Gramineae, which is widely distributed in southern and central China (Liu 1988), where it has been used in the conservation of water and soil (Zhou 1990). *Eulaliopsis binata* is an excellent natural cellulose material, noted for its long fibers that have good strength and toughness (Zhang et al. 1996). The unique physical properties of *Eulaliopsis binata* make it an excellent fiber resource for the pulp and papermaking industry (Ma 1996).

Over the last 50 years, the kraft process has become established as the most versatile and economical chemical pulping process. During pulping, most of the hemicelluloses and lignin are dissolved in the waste pulping liquor, leaving cellulosic fibers as the solid phase. What's more, the loss in solids is up to 50% or more. Although the waste liquor is combusted to regenerate pulping chemicals, alkalinity, and $Na_2S$ (van Heiningen 2006), the hemicellulose content has not been fully utilized during the kraft process. The reasons are that the hemicellulose not only has a low heating value, but also it results in a viscosity increment of the spent liquor that tends to interfere with the alkali

recovery process (van Heiningen 2008). In view of this situation, van Heiningen (2006, 2008) initiated a concept of converting chemical pulp and paper mills into integrated forest biorefineries (IFBRs), which produce bioenergy or new biomaterials in addition to traditional paper products. An important part of this concept is that hemicellulose should be extracted from the materials before pulping and then can be converted into new bioproducts such as ethanol, acetic acid, or chemical intermediates (van Heiningen 2006).

In many studies (Mark 1985; Schell 1991; Esteghlalian et al.1997; Garrote 2002; Nabarlatz et al. 2007; Jin et al. 2010), dilute acid hydrolysis has been found to be an effective method to dissolve hemicellulose. However, all these studies focused on the hydrolysis conditions and hydrolyzed solution. As for the effect of the hydrolysis on the cellulose in the residuals, it was not analyzed. To address this point, the composition and structure of cellulose isolated from untreated *Eulaliopsis binata* and dilute sulfuric acid hydrolyzed *Eulaliopsis binata* were thoroughly investigated in this study.

## EXPERIMENTAL

### Materials

*Untreated Eulaliopsis binata*

The *Eulaliopsis binata* was kindly provided by a pulp and papermaking mill located in central China. The material was cut into about 30-mm-long pieces and washed with water to remove any adhering soil particles. After that, the washed *Eulaliopsis binata* pieces were dewatered, air dried, and stored in a self-sealed plastic bag.

*Acid-hydrolyzed Eulaliopsis binata*

The *Eulaliopsis binata* was hydrolyzed in a rotary digester with 0.5% dilute sulfuric acid solution. The ratio of volume of solution to the mass of *Eulaliopsis binata* chips was 10:1. Hydrolysis temperature was ramped from a room temperature of 30 $^{o}$C to the desired terminal temperature of 160 $^{o}$C in 65 minutes, and the hydrolysis time was 30 minutes. After hydrolysis, the *Eulaliopsis binata* pieces were washed with distilled water and then stored in a self-sealed plastic bag after air drying.

### Isolation of Cellulose from the Untreated and Acid hydrolyzed *Eulaliopsis binata*

The isolation of cellulose from the untreated or acid hydrolyzed *Eulaliopsis binata* was carried out according to the following procedure. First, the air-dried sample was immersed in chloroform–ethanol solution (1:1, v/v) overnight. Then the sample was transferred into acetone to replace the residual chloroform and ethanol. After that, the sample was washed with distilled water thoroughly. Second, the sample was extracted with water for 6 h at 90 $^{o}$C. Then the sample was treated with 0.5% ammonium oxalate at 70 $^{o}$C for another 6 h. Subsequently, the samples were extracted twice with 0.3% dodecyl sulfate for 12 h and with an aqueous solution of 50% urea for another 12h at room temperature. After that, the sample was bleached in a 0.6% sodium chlorite solution buffered with acetic acid (pH=4.9) for 4 h at 80 $^{o}$C. Next, they were immersed in 5% KOH solution overnight at room temperature. Finally, the sample was washed thoroughly

with distilled water and freeze-dried with tert-butyl alcohol. At each step, the solutions with samples were gently shaken during treatment (Liu et al. 2005).

## Chemical Composition Determination

The ash and Klason lignin content were determined using TAPPI standard methods T211 and T222, respectively. The carbohydrates, including glucose, xylose, arabinose, galactose, mannose, glucuronide, and galacturonic acid, were determined by ion chromatography (IC). Before determination, the samples needed to be pulverized and treated by sulfuric acid twice according to the methods formulated by the US National Renewable Energy Laboratory (NREL).

## Intrinsic Viscosity, Degree of polymerization, and Molecular weight

The viscosity of the cellulose preparations was determined by British Standard Methods for determination of limiting viscosity number of cellulose in dilute solutions, using the cupri-ethylene-diamine (CED) method (BS 6306: Part 1: 1982). The average degree of polymerization (DP) of the cellulose preparations was estimated according to the formula of $DP^{0.90} = 1.65[\eta]$ /ml·g$^{-1}$(Evans et al. 1989), where the $[\eta]$ is the intrinsic viscosity of sample dissolved in CED hydroxide solution. The molecular weight ($M_w$) of the cellulose preparations was calculated based on the formula of $Mw = 162*DP$, where the 162 Daltons is the molecular weight of an anhydroglucose unit.

## SEM Analysis

Scanning electron microscopy (SEM) analysis of untreated and acid hydrolyzed *Eulaliopsis binata* was performed using a Hitachi S-570 SEM operated at 25 kV. The samples were coated with gold with an Eiko IB-3 Incoater before testing.

## Characterization of the Cellulose Preparations

Fourier transform infrared (FTIR) analysis of cellulose preparations was carried out on a Bruker IFS-113V FTIR spectrometer with a resolution of 2 cm$^{-1}$.

X-ray diffraction (XRD) spectra of cellulose preparations were recorded by Ni-filtered CuKα radiation from a Rigaku D/Max-rB X-ray diffractometer. The operating voltage and current were 40 kV and 40 mA, respectively. The crystallinity was calculated according to the diffraction intensity data. A two-phase structure (crystalline–amorphous) was assumed, and an arbitrary background to the diffraction trace was obtained by a line between the intensity minima, thus separating an arbitrary crystalline phase from an arbitrary amorphous phase. The crystallinity Xc (%) was calculated by,

$$X_C = \frac{A_{cr}}{A_{cr} + A_{am}} \times 100 \quad (1)$$

where $A_{cr}$ and $A_{am}$ are the integrated area of the crystalline and amorphous phases, respectively (Alexander 1969).

The thermal properties of the cellulose preparations were analyzed using a thermogravimetric analysis (TGA) device. The apparatus was continually flushed with nitrogen, and the sample was weighed, being between 9 and 11 mg. Each sample was heated from room temperature to 600 $^{o}$C at a rate of 10 $^{o}$C per minute.

## RESULTS AND DISCUSSION

### Chemical Composition

The composition of the untreated and acid hydrolyzed *Eulaliopsis binata* is presented in Table 1. *Eulaliopsis binata* exhibited the typical chemical composition of the grass family, containing more ash and hemicellulose but less lignin than wood materials. The carbohydrate fraction was predominantly composed of glucose and xylose. The glucose content was 49.9% and the xylose content was 19.8%. Other carbohydrate fractions, such as mannose, glucuronide, and galacturonic acid, were relatively minor components.

**Table 1.** Comparison of the Chemical Composition

| Component | *Eulaliopsis binata* contents [a] | | Cellulose preparations contents [b] | |
|---|---|---|---|---|
| | Untreated | Acid hydrolyzed | Untreated | Acid hydrolyzed |
| Ash | 5.77 | 0.76 | 0.38 | 0.12 |
| Klason lignin | 18.2 | 17.8 | 0.21 | Tr① |
| Glucose | 49.9 | 45.7 | 96.1 | 98.9 |
| Xylose | 19.8 | 1.91 | 1.15 | 0.36 |
| Arabinose | 2.78 | 0.43 | 0.73 | 0 |
| Galactose | 0.94 | 0.11 | 0.34 | Tr① |
| Mannose | 0.28 | 0.22 | 0.19 | 0.09 |
| Glucuronide | 0.15 | Tr① | Tr① | Tr① |
| Galacturonic acid | 0.24 | Tr① | 0 | 0 |
| Weight [c] | 100% | 68.6% | 48.7% | 45.2% |

[a] Content reported as % (w/w) based on the dry weight of untreated *Eulaliopsis binata*
[b] Content reported as % (w/w) based on the dry weight of cellulose preparations
[c] weight reported as % (w/w) based on the dry weight of untreated *Eulaliopsis binata*
① Tr = Trace

The solid material recovered after the dilute acid hydrolysis mainly contained Klason lignin and glucose. The hemicellulosic sugars comprised a relatively lower proportion of the carbohydrates in the acid hydrolyzed *Eulaliopsis binata* as compared to the untreated material. The ash content decreased following the hydrolysis process.

As shown in Table 1, the cellulose preparations isolated by the method used had a higher content of glucose and a lower content of lignin. The glucose content of the cellulose preparation from acid hydrolyzed *Eulaliopsis binata* reached 98.9%, which was higher than the cellulose preparation from untreated *Eulaliopsis binata*. It has been shown that hemicellulose is almost completely dissolved during the process of extraction, and lignin-hemicellulose-cellulose interactions are also disrupted (Ladisch 1989; Day 1989).

### Intrinsic Viscosity, Degree of Polymerization, and Molecular weight

Table 2 lists the intrinsic viscosity **($\eta$)**, degree of polymerization (*DP*), and molecular weight ($M_w$) of the cellulose preparations from the untreated and acid hydrolyzed *Eulaliopsis binata*.

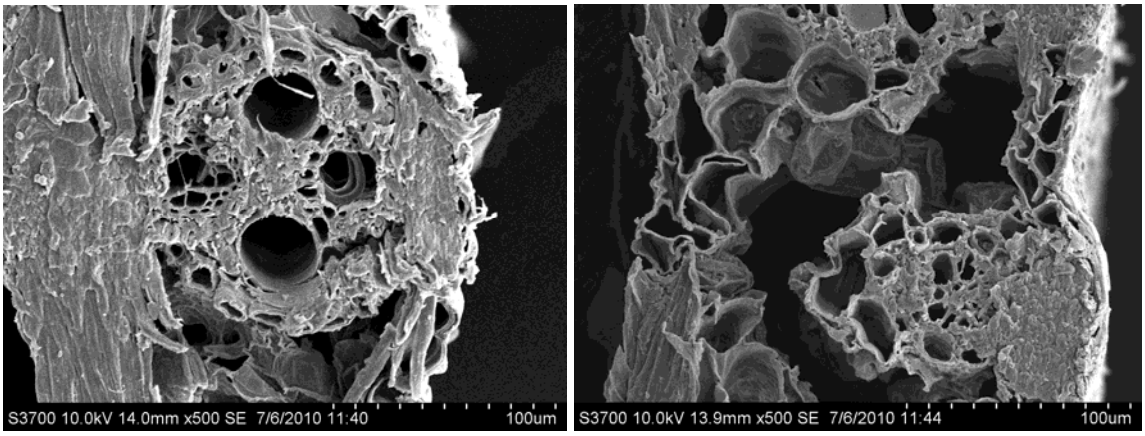
**Table 2.** Intrinsic Viscosity ($\eta$), Degree of Polymerization (*DP*) and Molecular Weight ($M_w$) of Cellulose Preparations

| Cellulose preparations | $\eta$ /ml·g$^{-1}$ | *DP* | $M_w$ /g·mol$^{-1}$ |
|---|---|---|---|
| Untreated *Eulaliopsis binata* | 703 | 2521 | 408,359 |
| Acid hydrolyzed *Eulaliopsis binata* | 618 | 2185 | 353,932 |

As can be seen, the two cellulose preparations both had a high degree of polymerization and molecular weight, which indicated that *Eulaliopsis binata* is a potential resource for producing high-intensity fibre products or biocomposites. The intrinsic viscosity, degree of polymerization, and molecular weight of the cellulose preparation from acid hydrolyzed *Eulaliopsis binata* were a bit less than the cellulose from acid hydrolyzed *Eulaliopsis binata*. The results showed that the dilute acid hydrolysis had little effect on the cellulose degradation.

## SEM Analysis

Hydrolysis with dilute acid may affect the structure of biomass by solubilizing or altering hemicelluloses, altering lignin structure, and increasing the available surface area and pore volume of the substrate (Esteghlalian et al. 1997). The effects of acid hydrolysis on the structure of *Eulaliopsis binata* are shown in Fig. 1.




(a) untreated Eulaliopsis binata (b) acid hydrolyzed Eulaliopsis binata

**Fig. 1.** SEM photographs of the cross section

From the SEM photograph of cross section of untreated *Eulaliopsis binata* (Fig.1(a)), it can be seen that the cells are connected very tightly. But after acid hydrolysis (Fig.1(b)), it is clear that some cell tissues have been destroyed during the hydrolysis process, and the connections between cells are looser than in the case of the untreated *Eulaliopsis binata*. This could be attributed to the preferential degradation of the labile components, such as acid-soluble lignin and hemicellulose. All these processes are helpful for the isolation of cellulose and lead to less hemicellulose and lignin content in the cellulose preparation from acid hydrolyzed *Eulaliopsis binata* (as shown in Table 1).

## FI-IR Spectra

The FTIR spectra of celluloses extracted from the untreated and acid hydrolyzed *Eulaliopsis binata* samples are shown in Fig. 2. It can be distinctly seen that the structures of the two cellulose preparations were nearly the same. This implies that most of the crystalline cellulose in the *Eulaliopsis binata* was not disrupted by the acid-catalyzed hydrolysis at 160 $^{o}$C. The results were in agreement with a previous study, which reported that the crystalline cellulose in corn stover (Kumar et al. 2009) and rice straw (Hsu et al. 2010) also could not be disrupted by acid pretreatment. The characteristic band for hemicelluloses at 1735 cm$^{-1}$ (Chen et al. 1997; Gastaldi et al. 1998) in the FTIR spectrum was not discernible, which indicated that hemicelluloses can be mostly extracted by the applied procedure. Meanwhile, the characteristic absorbances of lignin at 1595 cm$^{-1}$ (Revol 1982; Stewart et al. 1995) and at 1510 cm$^{-1}$ (Revol 1982; Stewart et al. 1995) were absent in the FTIR spectrum. This indicated that the cellulose preparations were relatively free of residual lignin. This was also proved by the chemical composition analysis (as shown in Table 1).

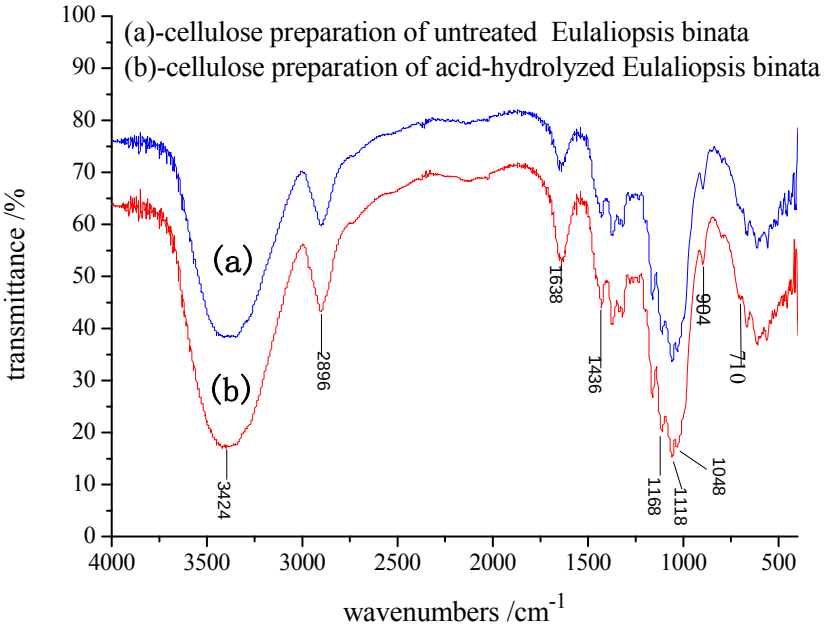



**Fig. 2.** FTIR spectra of cellulose preparations

The absorption band at 1638 cm$^{-1}$ was attributed to absorbed water in the cellulose (Chen et al. 1997; Gastaldi et al. 1998). The absorption at 3424 cm$^{-1}$ was assigned to stretching of –OH groups, and that at 2896 cm$^{-1}$ was assigned to the C–H stretching. A noticeable peak at 1436 cm$^{-1}$ was due to $CH_2$ bending. The peak at 1168 cm$^{-1}$ arose from C–O anti-symmetric bridge stretching. The absorption band at 1118 cm$^{-1}$ was attributed to C–OH skeletal vibration. The C–O–C pyranose ring skeletal vibration gave a prominent band at 1048 cm$^{-1}$. A small sharp band at 904 cm$^{-1}$ represented the glycosidic $C_1$–H deformation with ring vibration contribution and OH bending, which is characteristic of β-glycosidic linkages between glucoses in cellulose.

## X-ray Diffraction Spectra

Figure 4 shows the XRD curves of the cellulose preparations of untreated and acid hydrolyzed *Eulaliopsis binata*. Correspondingly, the crystallinity values calculated according to the diffraction intensity data are listed in Table 3.

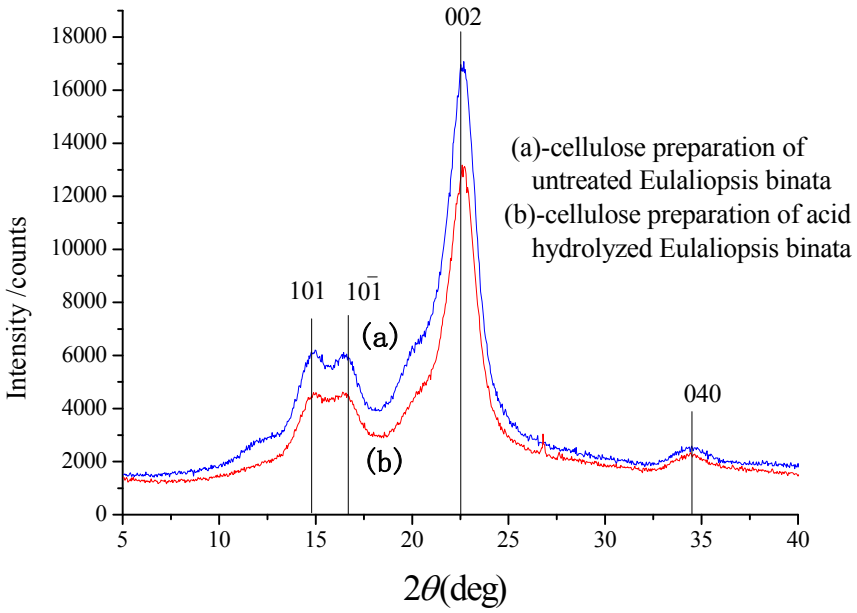



**Fig.4.** X-ray diffraction of cellulose preparations

**Table 3.** Crystallinity Index of Cellulose Preparations

| Cellulose preparations | Crystallinity (%) |
|---|---|
| Untreated Eulaliopsis binata | 56.7 |
| Acid hydrolyzed Eulaliopsis binata | 65.9 |

The XRD curves show that the two cellulose preparations both had the typical cellulose Ⅰ allomorph characteristics with low crystallinity. The diffraction peaks of the (101) and ($10\bar{1}$) planes merged together, while the diffraction peak (040) was very weak. The crystallinity of the cellulose preparation from untreated *Eulaliopsis binata* was fairly low, about 56.7% (Table 3). The structure of cellulose in *Eulaliopsis binata* was found to be similar to that of the native crystalline cellulose in the cell wall of *Oomycota* (fungal-like protista that have motile cells, cellulose cell walls, and chromosome structure that distinguish them from the kingdom of fungi) (Helbert et al. 1997; Liu et al. 2005) and those from *Crambe abyssinica* (crucifer family, an annual herbaceous plant) hull (Gastaldi et al. 1998; Liu et al. 2005). The results indicated that the native cellulose crystalline polymorphism was not destroyed during the isolating process.

When the *Eulaliopsis binata* was hydrolyzed by the dilute sulfuric acid, the crystallinity increased to 65.9%. This was attributed to the degradation of the less stable amorphous regions and less ordered crystalline forms of cellulose during the dilute acid hydrolysis (Sannigrahi et al. 2008).

## Thermogravimetric Analysis

Thermal analysis is one of the most commonly used methods to study the properties of the polymers (Ouajai et al. 2005). Figure 5 shows the thermal properties of the cellulose preparations of untreated and acid hydrolyzed *Eulaliopsis binata*.

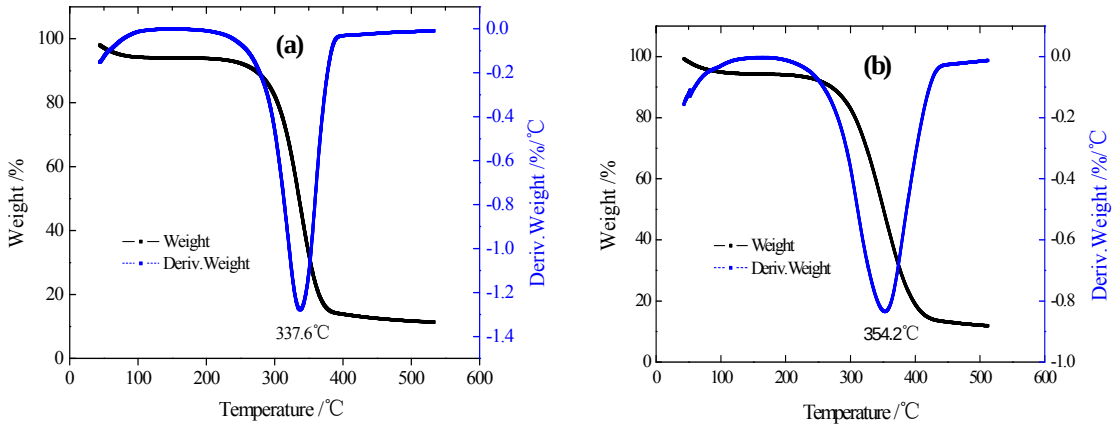



**Fig. 5.** Thermal properties of the cellulose preparations of untreated (a) and acid hydrolyzed (b) *Eulaliopsis binata*

The weight losses of samples in a nitrogen atmosphere that occurred within the range from room temperature (30 °C) to 200 °C were less than 10%. Within this stage, the weight losses were due to the elimination of absorbed or combined water (Tomczak et al. 2007). The greatest decomposition of cellulose preparation occurred from 234.9 °C to 392.4 °C for the cellulose preparation isolated from untreated *Eulaliopsis binata*. As for the cellulose preparation isolated from acid hydrolyzed *Eulaliopsis binata*, the greatest decomposition of cellulose preparation occurred from 240.1 °C to 426.9 °C. The gross weight loss for this stage was about 80%. From the curves of derivative of weight vs. temperature shown in the Fig. 5, it could be seen that the maximum rates of thermal decomposition were observed at 337.6 °C and 354.2 °C for the cellulose preparations of untreated and acid hydrolyzed *Eulaliopsis binata*, respectively. The results confirmed that the cellulose preparation isolated from acid hydrolyzed *Eulaliopsis binata* had a higher thermal stability than the cellulose preparation from untreated *Eulaliopsis binata*. This was attributed to its crystallinity being higher compared to untreated *Eulaliopsis binata*.

## CONCLUSIONS

1. During dilute acid hydrolysis, hemicellulose in *Eulaliopsis binata* was mostly dissolved and lignin-hemicellulose-cellulose interactions were also disrupted. Dilute acid hydrolysis was helpful to obtain high-purity cellulose without notable cellulose degradation.
2. Dilute acid hydrolysis of *Eulaliopsis binata* didn't change the crystal structure of the cellulose, and *Eulaliopsis binata* had the typical cellulose I allomorph with low crystallinity.

3. Hydrolysis with dilute acid increased the cellulose crystallinity of *Eulaliopsis binata*. This was attributed to a preferential degradation of amorphous cellulose and less ordered crystalline forms during the high temperature hydrolysis.
4. The cellulose preparation isolated from acid hydrolyzed *Eulaliopsis binata* had a higher thermal stability than the cellulose preparation from untreated *Eulaliopsis binata*.

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