# Comparative Study of Cellulose Isolated by Totally Chlorine-Free Method from Wood and Cereal Straw

**J. X. Sun,[1] F. Xu,[2] Z. C. Geng,[1] X. F. Sun,[1] R. C. Sun[2,3]**

[1]*College of Forestry, North-Western Sciences and Technology University of Agriculture and Forestry, Yangling 712100, China*
[2]*State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510641, China*
[3]*BioComposites Centre, University of Wales, Bangor, Gwynedd LL57 2UW, United Kingdom*

Received 15 June 2004; accepted 28 October 2004
DOI 10.1002/app.21728
Published online in Wiley InterScience (www.interscience.wiley.com).

**ABSTRACT:** Highly purified cellulose preparations were obtained by pretreatment of dewaxed barley straw, oil palm frond fiber, poplar wood, maize stems, wheat straw, rice straw, and rye straw with 2.0% $H_2O_2$ at 45°C and pH 11.6 for 16 h, and sequential purification with 80% acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min. The purified cellulose obtained was relatively free of bound hemicelluloses (2.3–3.2%) and lignin (0.4–0.6%) and had a yield of 35.5% from barley straw, 39.6% from oil palm frond fiber, 40.8% from poplar wood, 36.0% from maize stems, 34.1% from wheat straw, 23.4% from rice straw, and 35.8% from rye straw. The weight-average molecular weights of the purified cellulose ranged from 39,030 to 48,380 g/mol. The thermal stability of the purified cellulose was higher than that of the corresponding crude cellulose. In comparison, the isolated crude and purified cellulose samples were also studied by Fourier transform IR and cross-polarization/magic-angle spinning $^{13}$C-NMR spectroscopy. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 97: 322–335, 2005

**Key words:** cellulose; chlorine-free method; peroxide; acetic acid; cross-polarization/magic-angle spinning $^{13}$C-NMR

## INTRODUCTION

Cellulose, the most abundant component of plant biomass, is found in nature almost exclusively in plant cell walls, such as wood and cereal straws, which contain approximately 35–50% cellulose.[1] In a few cases (notably cotton bolls), cellulose is present in a nearly pure state. In most cases, however, the cellulose fibers are embedded in a matrix of other structural biopolymers, primarily hemicelluloses and lignin, which comprise 20–35 and 5–30% of plant dry weights, respectively.[2] These cellulosic materials are particularly attractive because of their relatively low cost and plentiful supply. In particular, the overall worldwide production of cereal straw, which contains 30–40% cellulose and constitutes an alternative to wood as a raw material for making pulp because of its high growth rate and adaptability to various soil types, is estimated to exceed 2900 million tons each year. Most of this residue is burned, thereby losing energy and causing significant pollution, which is illegal in some countries. This raw material therefore possesses a high economic and environmental potential.[3]

Structurally, cellulose is a polydisperse polymer of high molecular weight comprising long chains of D-glucose units jointed together by $\beta$-1,4-glucosidic bonds.[4] It is synthesized in nature as individual molecules, which undergo self-assembly at the site of biosynthesis.[5] Approximately 30 individual cellulose molecules are assembled into larger units known as elementary fibrils (protofibrils), which are packed into larger units called microfibrils, and these are in turn assembled into the familiar cellulose fibers. An important feature of cellulose, which is relatively unusual in the polysaccharide world, is its crystalline structure. The highly ordered cellulose in the interior of crystallites is considered to be crystalline cellulose; but the less ordered cellulose, including the fibril surfaces, is considered to be amorphous.[6] The polymorphism of ordered cellulose was established relatively early[7,8] and apart from the native cellulose I, three other crystalline forms (II, III, and IV) are now well known as distinguished by X-ray diffraction.[9,10] In addition, cellulose I was found to be a mixture of two crystalline modifications, cellulose $I_\alpha$ and $I_\beta$. The existence of these two crystalline phases was confirmed by the

*Correspondence to:* R. C. Sun (bcs00a@bangor.ac.uk).
Contract grant sponsor: National Natural Science Foundation of China; Contract grant number: 30271061, 30430550.
Contract grant sponsor: Shaanxi and Guangdong Natural Science Foundation; Contract grant number: 2003k08-G18, 013034.
Contract grant sponsor: Ministry of Education China.

Journal of Applied Polymer Science, Vol. 97, 322–335 (2005)
© 2005 Wiley Periodicals, Inc.

methods such as IR spectroscopy,[11] electron microdiffraction,[12] and $^{13}$C solid-state NMR spectroscopy.[6] The $I_\alpha$ form corresponds to a single-chain triclinic crystallographic symmetry, whereas $I_\beta$ is monoclinic and characterized by two parallel chains. Native cellulose is a mixture of these two forms, and the ratio between $I_\alpha$ and $I_\beta$ depends on the source. It was found that the $I_\alpha$ phase was metastable and could be converted into the $I_\beta$ form by a hydrothermal treatment at 260°C.[13] Meanwhile, the crystalline transformation of cellulose I to II is observed in the mercerization–regeneration treatment of native cellulose by the use of an aqueous sodium hydroxide solution with a concentration of more than 11 wt %.[14] Ago and coworkers[15] also reported that a mechanochemical treatment of native cellulose with a specific amount of water (~30 wt %) present in the cellulose solid state caused the crystalline transformation from cellulose I into cellulose II polymorph. Furthermore, cellulose I is composed of parallel cellulose chains and cellulose II is composed of antiparallel cellulose chains in the unit cell.[16] Celluloses I and II may also have differences in the conformation of the cellobiose units.[17]

Purified celluloses used for studies of hydrolysis and microbial utilization vary considerably in fine structural features, and the choice of substrate for such studies undoubtedly affects the results obtained. Microcrystalline celluloses (e.g., Avicel and Sigmacell) are nearly pure cellulose, and the dilute-acid treatment used in their preparation removes both hemicelluloses and the more extensive amorphous regions of the cellulose fibers. Commercial microcrystalline celluloses differ primarily in particle size distribution, which has significant implications for the rate of hydrolysis and utilization.[2] Thus, cellulose represents a vast potential feedstock for a number of industries and has created a great deal of research interest. Cellulose can be used for the production of liquid fuels (bioethanol), pharmaceuticals, food, and chemical feedstocks, apart from papermaking.[18] In addition, the use of cellulose and its derivatives in a diverse array of other applications, such as films, plastics, coatings, suspension agents, and composites, continues to grow on a worldwide basis.[19]

Utilization of cellulosic biomass is more complex than that of pure cellulose because of the former's complex composition (i.e., the presence of hemicelluloses and lignin). In particular, wood and straw have thick cell walls and highly lignified middle lamella separating the cells from one another. These cell walls must be attacked from the inside (luminal) surface out through the secondary wall (as opposed to particles of pure cellulose, which are degraded from the outside inward). Thus, in addition to the constraints imposed by the structure of cellulose itself, additional limitations are imposed by diffusion and transport of the cellulolytic agent to the site of attack. These constraints may severely limit utilization in some habitats.[2,20] Moreover, the central technological impediment to more widespread utilization of this important resource is the general absence of low-cost technology for overcoming the recalcitrance of cellulosic biomass. A protocol using acidified sodium chlorite is frequently applied to delignify wood as an initial step in the isolation of cellulose.[21] However, the environmental impact of isolation effluents is one of the main concerns of the cellulose or pulping industry. The environmental risks associated with the traditional pretreatment using elemental chlorine fostered the development of new isolation sequences free from elemental chlorine or totally chlorine free (TCF).[22] More importantly, the increasing use of cellulosic polymers in many different applications has also led to a fundamental understanding of the chemical nature of cellulose. In this article we describe a sequential TCF procedure for cellulose isolation from dewaxed barley straw, oil palm frond fiber, poplar wood, maize stems, wheat straw, rice straw, and rye straw, based on the simultaneous fractionation of hemicelluloses and lignin by using an alkaline peroxide pretreatment followed by purification with an acetic acid–nitric acid mixture. The cellulosic preparations isolated under TCF conditions were subject to analyses of their content of associated hemicelluloses and lignin, viscosity, molecular weight, and thermal stability. The structural changes were revealed by using Fourier transform IR (FTIR) and solid-state cross-polarization/magic-angle spinning C$^{13}$-NMR (CP/MAS $^{13}$C-NMR) spectroscopy.

## EXPERIMENTAL

### Materials

Barley, wheat, rice, and rye straws and maize stems were obtained from the experiment farm of North-Western Sciences and Technology University of Agriculture and Forestry (Yangling, China). These raw materials were dried in sunlight and then cut into small pieces. The cut straw was ground to pass a 1-mm size screen. An 11-year-old poplar tree was harvested in December of 2001 at the above university's forest. After the outer and inner barks were peeled off, the remainder was chipped and dried, and the chips were ground to pass a 1.0-mm screen. Oil palm frond fiber supplied by the South China University of Agriculture was cut by a hammer mill into lengths of 0.8–1.2 mm and screened to remove fines and dusts. All of the ground samples were further dried at 60°C for 16 h and dewaxed with toluene/ethanol (2:1, v/v) in a Soxhlet apparatus for 6 h.

### Isolation of crude and purified cellulose

Crude cellulose was obtained by pretreatment of the dewaxed barley straw, oil palm frond fiber, poplar

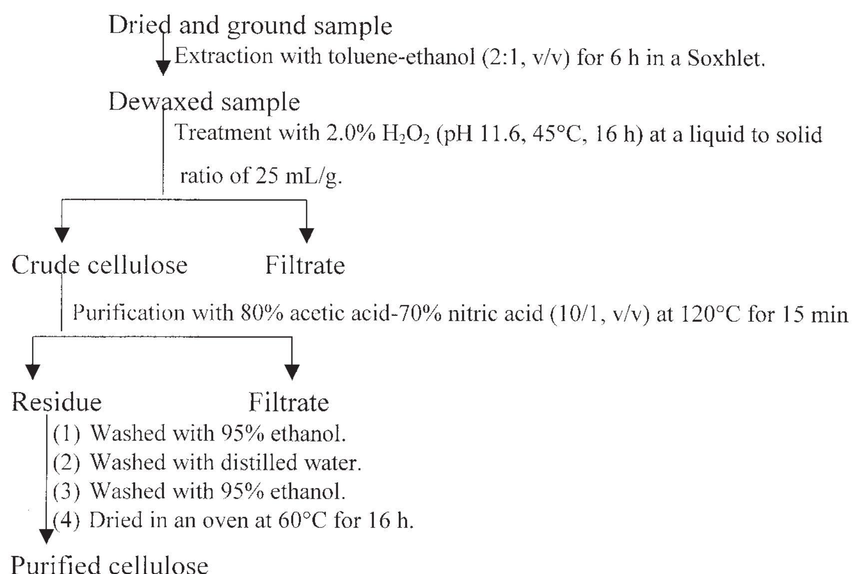


**Figure 1** The scheme for isolation of crude and purified cellulose from dewaxed barley straw, oil palm frond fiber, poplar wood, maize stems, wheat straw, rice straw, and rye straw.

wood, maize stems, wheat straw, rice straw, and rye straw (9.80 g) with 2.0% $H_2O_2$ at 45°C under pH 11.6 for 16 h with a solid to extractant ratio of 1:25 (g/mL) in a 500-mL glass reactor at atmospheric pressure. Afterward it was filtered off and washed thoroughly with water and ethanol until the filtrate was neutral. The residue was then dried in an oven at 60°C for 16 h. Note that the residues obtained from dewaxed barley straw, oil palm frond fiber, poplar wood, maize stems, wheat straw, rice straw, and rye straw were labeled as crude cellulose preparations $C_{1a}$, $C_{2a}$, $C_{3a}$, $C_{4a}$, $C_{5a}$, $C_{6a}$, and $C_{7a}$, respectively. To purify the cellulose, the crude cellulose preparation of $C_{1a}$, $C_{2a}$, $C_{3a}$, $C_{4a}$, $C_{5a}$, $C_{6a}$, or $C_{7a}$ (~250 mg) was weighed into a 30-mL Pyrex tube. Subsequently, 5.0 mL of 80% (v/v) acetic acid and 0.5 mL of concentrated nitric acid (70%, v/v) were added. The tubes were sealed using screw caps fitted with Teflon liners and placed into a preheated oil bath at 120°C for 15 min. Once cooled, the supernatant was then carefully decanted and pellets were washed sequentially with 95% ethanol (20 mL), distilled water (30 mL), and 95% ethanol (20 mL) to remove extraction breakdown products and traces of nitric acid. Finally, the purified cellulosic preparations were dried in an oven at 60°C for 16 h. Purified cellulose preparations $C_{1b}$, $C_{2b}$, $C_{3b}$, $C_{4b}$, $C_{5b}$, $C_{6b}$, and $C_{7b}$ were obtained from crude cellulose samples $C_{1a}$, $C_{2a}$, $C_{3a}$, $C_{4a}$, $C_{5a}$, $C_{6a}$, and $C_{7a}$, respectively. The scheme for the isolation of crude and purified cellulose from wood and straw is illustrated in Figure 1. All experiments were performed at least in duplicate. Yields of the crude and purified celluloses are given on a dry weight basis relating to the starting material. Standard errors or standard derivations were observed to be lower than 5%.

### Analysis of crude and purified cellulosic preparations

The neutral sugar composition of the isolated crude and purified cellulosic preparations was determined by gas chromatography analysis of the corresponding alditol acetates. In more detail, the sample (10 mg) was treated with 72% $H_2SO_4$ (0.125 mL) for 45 min at room temperature by agitation on a vortex mixture. The solution was then diluted to 1.475 mL, heated at 100°C for 2.5 h, cooled, and neutralized with 0.32 mL of 15*M* ammonia. After reduction, the resulting alditols were acetylated for gas chromatography analysis as described by Blakeney et al.[23] Methods for the determination of phenolic acids and aldehydes in nitrobenzene oxidation mixtures of lignins associated in the crude cellulosic preparations with high performance liquid chromatography and thermal analysis of the crude and purified cellulose samples were described in a previous article.[24] The klason lignin content in crude and purified cellulosic preparations was determined according to TAPPI method T 249 cm-85.

The viscosity of the crude cellulosic preparations was determined by British standard methods for the determination of the limiting viscosity number of cellulose in dilute solutions, according to the cupri-ethylene-diamine method (BS 6306, Part 1). The viscosity-average degree of polarization ($P$) of the crude cellulose samples was estimated by $P^{0.90} = 1.65\ [\eta]$.[25] The molecular weight of the crude cellulosic preparations was then calculated from their $P$ value and multiplying by 162, the molecular weight of an anhydroglucose.

To determine the molecular weight of the purified cellulosic preparation by gel permeation chromatog-

**TABLE I**
**Yield of Crude and Purified Cellulosic Preparations (% Dry Matter) Isolated by Treatment of Various Cellulosic Materials**

| | Barley straw | Oil palm frond fiber | Poplar wood | Maize stems | Wheat straw | Rice straw | Rye straw |
|---|---|---|---|---|---|---|---|
| CC | 58.4 | 58.2 | 78.3 | 57.5 | 47.3 | 38.4 | 52.4 |
| Content of hemicelluloses in CC | 12.3 | 7.5 | 17.5 | 10.6 | 10.6 | 6.8 | 14.5 |
| Content of lignin in CC | 7.8 | 8.6 | 21.8 | 3.4 | 4.9 | 3.6 | 3.5 |
| Purified cellulose | 35.5 | 39.6 | 40.8 | 36.0 | 34.1 | 23.4 | 35.8 |

The materials were treated with 2.0% $H_2O_2$ at 45°C and pH 11.6 for 16 h, and sequentially purified with 80% acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min. CC, crude cellulose.

raphy (GPC), the purified cellulose sample (~7 mg) was first suspended in 3.5 mL of *N*,*N*-dimethylacetamide (DMAc)/4% lithium chloride (LiCl), followed by sequential heating to 120°C for 2 h and 80°C for 5 h under stirring and cooling to room temperature. The mobile-phase solvent for GPC was DMAc/0.5% LiCl prepared by raising the temperature of 1000 mL of DMAc to 100°C and then adding 5 g of LiCl (dried). After the salt was stirred until it dissolved, the solvent was filtered through a Teflon filter (0.5 $\mu$m, Millipore) with a glass filter apparatus. Filtered samples were analyzed on a Knauer GPC system (Berlin). Molecular weight determination was performed on a PL mixed A column (10 $\mu$m, 0.75 (i.d.) × 30 cm, Polymer Laboratories Ltd., Shropshire, UK). The column was placed inside a high temperature column oven (model 60C, Knauer, Berlin) set at 80°C to reduce the solvent viscosity for greater mass transfer and to enhance the column efficiency. The mobile phase of DMAc/0.5% LiCl was pumped through at 1.0 mL/min. The injection volume was 200 $\mu$L. Five pullulans (Polymer Laboratories) were used to calibrate the molecular weight.

The FTIR spectra of both crude and purified celluloses were recorded from KBr pellets containing 1% finely ground samples on a Nicolet-510 FTIR spectrometer. CP/MAS $^{13}$C-NMR spectra were recorded at 75.5 MHz on a Bruker MSL300 spectrometer employing both CP and MAS, and each experiment was recorded at ambient temperature (293 ± 1 K). The speed of rotation was 5 kHz, the proton 90° pulse was 6 $\mu$s, the contact pulse was 800 $\mu$s, and the delay between repetitions was at least 0.8 s.

## RESULTS AND DISCUSSION

### Yield of crude and purified cellulose

Currently, with the development of TCF isolating and bleaching technologies, there has been a growing interest in the use of hydrogen peroxide as one of the oxidants to replace chlorine-based reagents, because the by-products are environmentally benign.[26] It is generally accepted that the hydroperoxide anion ($HOO^-$) formed in alkaline media is the primary species responsible for bleaching and it is a product of the reaction between $H_2O_2$ and NaOH in aqueous solution. However, hydrogen peroxide is unstable in alkaline conditions and readily decomposes into hydroxyl radicals ($HO\cdot$) and superoxide anion radicals ($O_2^-\cdot$). This is particularly true in the presence of certain transition metals such as manganese, iron, and copper. These radicals are thought to cause the oxidation of lignin structures, which lead to the introduction of hydrophilic (carboxyl) groups, cleavage of some interunit bonds, and eventually the dissolution of lignin and hemicelluloses.[27] In addition, Taher and Cates[28] proposed a mechanism where the hydroxyl radical $HO\cdot$ and the perhydroxyl radical $OH_2\cdot$ form a chain reaction, resulting in the decomposition of $H_2O_2$ into oxygen and water. Bleaching occurs when the fabric reacts with these radicals and thereby acts in a chain termination role.[29] As can be seen from Table I, pretreatment of dewaxed barley straw, oil palm frond fiber, poplar wood, maize stems, wheat straw, rice straw, and rye straw with 2.0% $H_2O_2$ at pH 11.6 for 16 h at 45°C resulted in the release of 79.7, 85.7, 49.5, 78.2, 87.1, 92.7, and 79.4% of the original hemicelluloses and 70.9, 72.1, 24.9, 87.3, 85.7, 88.6, and 89.8% of the original lignin removal, respectively. This high solubility of hemicelluloses and lignin from cereal straws and oil palm frond fiber was probably due to the significant cleavage of ester bonds between hydroxycinnamic acids such as *p*-coumaric and ferulic acids and hemicelluloses or lignin, and the $\alpha$-benzyl ether linkages between lignin and hemicelluloses from the cell walls of oil palm frond fiber and cereal straws. In comparison, a much lower yield of released hemicelluloses and lignin was observed from poplar wood sample. Similar results were reported by Gould[30] using alkaline peroxide treatment of various agricultural by-products. However, it should be noted that the pretreatment of rice straw under the conditions given also solubilized small amounts of the original cellulose.[31] The depolymerization of cellulose resulted mainly from the hydroxyl radicals, which were generated from the decomposition of peroxide under the

**TABLE II**
**Content of Neutral Sugars (Relative % Dry Residues) in Crude Cellulosic Preparations**

| Neutral sugar | Crude cellulosic preparation | | | | | | |
|---|---|---|---|---|---|---|---|
| | $C_{1a}$ | $C_{2a}$ | $C_{3a}$ | $C_{4a}$ | $C_{5a}$ | $C_{6a}$ | $C_{7a}$ |
| Arabinose | 2.0 | 0.6 | 0.5 | 2.3 | 1.4 | 2.0 | 2.3 |
| Xylose | 11.2 | 7.5 | 16.0 | 9.6 | 9.0 | 7.6 | 11.8 |
| Mannose | Tr | 2.2 | 2.4 | Tr | 0.3 | ND | 0.4 |
| Glucose | 86.7 | 89.7 | 81.0 | 88.1 | 89.3 | 90.3 | 85.3 |
| Galactose | Tr | Tr | ND | ND | Tr | Tr | ND |

$C_{1a}$, $C_{2a}$, $C_{3a}$, $C_{4a}$, $C_{5a}$, $C_{6a}$, and $C_{7a}$, the crude cellulosic preparations obtained by pretreatment of dewaxed barley straw, oil palm frond fiber, poplar wood, maize stems, wheat straw, rice straw, and rye straw, respectively, with 2.0% $H_2O_2$ at 45°C and pH 11.6 for 16 h. Tr, trace; ND, not detectable.

alkaline solution, particularly in the presence of transition metals.[32] The data in Table I also show that the yield of crude cellulose was 58.4% from barley straw, 58.2% from oil palm frond fiber, 78.3% from poplar wood, 57.5% from maize stems, 47.5% from wheat straw, 38.4% from rice straw, and 52.4% from rye straw, which contained 12.3, 7.5, 17.5, 10.6, 10.6, 6.8, and 14.5% hemicelluloses and 7.8, 8.6, 21.8, 3.4, 4.9, 3.6, and 3.5% lignin, respectively. The difficulty of removing the residual hemicelluloses and lignin by the alkaline peroxide pretreatment suggested that the sorption is not limited at the outer fiber surface, and some amounts of hemicelluloses or lignin may be distributed near the outer fiber surface and on, or near, the lumen surfaces and pores.[33] This is particularly true for the samples of poplar wood.

The effective removal of residual lignin and hemicelluloses from the crude cellulosic preparations during the purifying or bleaching processes and the stability of whiteness are essential from the commercial point of view. In this study we used a modified protocol for cellulose purification based on the simultaneous degradation of bound lignins and removal of residual noncellulosic polysaccharides by using an acetic acid–nitric acid mixture, which is a TCF technology. In this case, nitric acid was used as a catalyst for the purification of crude cellulose. At the initial stage of the carbon oxidation, the oxidizing agent is a nitric acid solution [$HNO_3$, $(OH)_2NO^+$, $NO_2^+$, $NO_3^-$, $H_3O^+$], which, during reduction, forms, among others, nitrogen oxides.[34] During purifying or bleaching, the various substitutions, side chain cleavage, and oxidation reactions observed with nitric acid are all likely to occur with nitrogen dioxide. In addition, free-radical initiated reactions involving the addition of nitrogen dioxide to the aromatic ring (in lignins), hydrogen abstraction (from cellulose and lignin), and electron transfer (mainly from phenolic groups) should also take place. Interestingly, the reactions of nitric acid with lignocellulosic materials can be largely limited to lignin and the damage to cellulose can be minimized. As shown in Table I, the yield of purified cellulose, obtained by further treatment of the corresponding crude cellulose samples with 80% acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min, was 35.5% from barley straw, 39.6% from oil palm frond fiber, 40.8% from poplar wood, 36.0% from maize stems, 34.1% from wheat straw, 23.4% from rice straw, and 35.8% from rye straw, which were almost consistent with the values of cellulose in barley straw (37.6%), oil palm frond fiber (41.2%), poplar wood (43.8%), maize stems (38.1%), wheat straw (38.9%), rice straw (29.5%), and rye straw (38.5%). However, it should be noted that minor quantities of cellulose were degraded or oxidized into acid-soluble substances during the post-treatment with an acetic acid–nitric acid mixture under the given conditions because of the slightly lower value of purified cellulose obtained in this experiment.

### Content of hemicelluloses and their neutral sugar composition

Hemicelluloses are polysaccharides that are biosynthesized in the cell wall in the majority of plants and trees. The major components of the secondary layers of the cell wall in wood fibers are cellulose and hemicelluloses.[35] Hemicelluloses can associate strongly with cellulose microfibrils and modify the aggregation behavior of celluloses.[36] The strong interactions between hemicelluloses and cellulose also play an important role during wood pulping or other isolating processes. Hemicelluloses dissolved during pulping can be adsorbed to a considerable extent on cellulose fibers.[37] This phenomenon has been explained by cocrystallization of hemicellulosic segments with cellulose and by the formation of strong hemicelluloses–cellulose hydrogen bonds.[38,39] Table II lists the neutral sugar composition of the crude cellulosic preparations, determined by acid hydrolysates. Apparently, glucose was the predominant sugar component in all of the seven crude cellulosic preparations, comprising 81.0–90.3% of the total sugars, indicating a relatively high content of cellulose. A small amount of xylose (7.5–16.0%) and minor quantities of arabinose (0.5–2.3%) as well as traces of mannose and galactose indicated that the crude cellulose contained small

**TABLE III**
**Yield (% Dry Sample, w/w) of Phenolic Acids and Aldehydes from Alkaline Nitrobenzene Oxidation of Associated Lignin in Crude Cellulosic Preparations**

| Phenolic acids and aldehydes | Crude cellulosic preparations | | | | | | |
|---|---|---|---|---|---|---|---|
| | $C_{1a}$ | $C_{2a}$ | $C_{3a}$ | $C_{4a}$ | $C_{5a}$ | $C_{6a}$ | $C_{7a}$ |
| *p*-Hydroxybenzoic acid | 0.29 | 0.50 | 0.38 | 0.33 | 0.42 | 0.61 | 0.83 |
| *p*-Hydroxybenzaldehyde | 0.064 | 0.070 | 0.035 | 0.066 | 0.052 | 0.075 | 0.031 |
| Vanillic acid | 0.031 | 0.094 | 0.11 | 0.021 | 0.041 | 0.027 | 0.012 |
| Syringic acid | 0.18 | 0.22 | 0.30 | 0.44 | 0.13 | 0.10 | 0.11 |
| Vanillin | 0.66 | 0.70 | 2.60 | 0.32 | 0.44 | 0.30 | 0.89 |
| Syringaldehyde | 0.54 | 1.98 | 3.62 | 0.48 | 0.62 | 0.28 | 0.84 |
| Acetovanillone | 0.13 | 0.17 | 0.12 | 0.052 | 0.11 | 0.21 | 0.057 |
| Acetosyringone | 0.048 | 0.11 | 0.18 | 0.053 | 0.10 | 0.069 | 0.016 |
| *p*-Coumaric acid | 0.047 | 0.048 | 0.010 | 0.026 | 0.073 | 0.065 | 0.036 |
| Ferulic acid | 0.053 | 0.10 | 0.010 | 0.036 | 0.062 | 0.068 | 0.057 |
| Total | 2.04 | 3.99 | 7.37 | 1.82 | 2.05 | 1.80 | 2.88 |
| Content of k lason lignin | 7.8 | 8.6 | 21.80 | 3.4 | 4.9 | 3.6 | 3.5 |

See Table II for the crude cellulosic preparations.

amounts of associated hemicelluloses. The difficulty of removing all of the hemicelluloses by an alkaline peroxide pretreatment under the given conditions suggested that the sorption is not limited at the outer fiber surface. Similarly, in a study about the rates of alkaline extraction of hemicelluloses from pulp fibers, Scott[33] came to the conclusion that extraction is highly dependent on the hemicellulose distribution near outer fiber surfaces and on or near the lumen surfaces and pores. Further, electron microscopy of the microfibrils revealed that native hemicelluloses, which had not been previously extracted from microfibrillar material, showed a more even repartition as a thin layer on the microfibrils by strong hydrogen bondings.[38] It is interest that posttreatment of the crude cellulose with 80% acetic acid–70% nitric acid at a volume ratio of 10/1 resulted in a substantial removal of the residual hemicelluloses as shown by the glucose content between 96.2 and 97.3% (data not shown) in purified cellulosic preparations $C_{1b}$–$C_{7b}$, implying higher purity of the cellulose.

### Content of bound lignin and its composition of phenolic compounds

The interactions through hydrogen bonds between hemicelluloses and cellulose are the main reason for not thoroughly removing the noncellulosic polysaccharides during the alkaline peroxide pretreatment, but it is also possible that hydrophobic substituents (e.g., lignin residues) are involved in the aggregating phenomenon.[40] Evidence for the existence of chemical bonds between lignin and polysaccharides in wood and straw, that is, lignin–carbohydrate complexes, has been accumulated over a period of several years and the concept is now largely accepted.[41] The restriction to removing almost all of the hemicelluloses during the alkaline peroxide pretreatment is undoubtedly attributable to the presence of lignin–carbohydrate complexes. Table III lists the content of lignin and its phenolic composition in seven crude cellulosic preparations. The data showed that the six crude cellulosic preparations ($C_{1a}$, $C_{2a}$, $C_{4a}$, $C_{5a}$, $C_{6a}$, and $C_{7a}$) obtained from cereal straws and oil palm frond fiber contained small amounts of bound lignin (3.5–8.6%), whereas the crude cellulosic sample ($C_{3a}$) obtained from poplar wood contained a substantial amount of lignin (21.8%). This low content of associated lignin in crude cellulose obtained from cereal straws and oil palm frond fiber revealed that the $\alpha$-benzyl ether linkages between lignin and hemicelluloses in the cell walls of straws and oil palm frond fiber were substantially cleaved during the pretreatment with alkaline peroxide under the conditions used. In contrast, the occurrence of a substantial amount of bound lignin in crude cellulose sample $C_{3a}$ obtained from poplar wood strongly implied that lignin in the cell wall of wood is tightly linked to hemicelluloses or cellulose and is more alkali resistant. The major products obtained from alkaline nitrobenzene oxidation were detected to be syringaldehyde (0.28–3.62%) and vanillin (0.30–2.60%). This demonstrated that the associated residual lignin in the crude cellulosic preparations was mainly composed of noncondensed syringyl and guaiacyl units. In addition, small amounts of *p*-hydroxybenzoic acid (0.29–0.83%), syringic acid (0.10–0.44%), and acetovanillone (0.052–0.21%) and traces of *p*-hydroxybenzaldehyde, vanillic acid, acetosyringone, ferulic acid, and *p*-coumaric acid were also identified in the products of alkaline nitrobenzene oxidation. More importantly, the relative absence of associated lignin in all purified cellulosic preparations $C_{1b}$–$C_{7b}$ (0.4–0.6%, data not shown) verified that an acetic acid–nitric acid mixture is a powerful agent for removal of residual lignins from both straw and wood.

**TABLE IV**
**Intrinsic Viscosity ($\eta$), Viscosity-Average Degree of Polymerization ($P$), and Weight-Average Molecular Weight ($M_w$) of Isolated Crude Cellulosic Preparations**

| | Crude cellulosic fractions | | | | | | |
|---|---|---|---|---|---|---|---|
| | $C_{1a}$ | $C_{2a}$ | $C_{3a}$ | $C_{4a}$ | $C_{5a}$ | $C_{6a}$ | $C_{7a}$ |
| $\eta$ (mL/g)[a] | 592.6 | 383.0 | 322.5 | 662.3 | 453.0 | 487.7 | 438.5 |
| $P$[b] | 2085.3 | 1284.6 | 1061.4 | 2359.3 | 1547.7 | 1679.8 | 1492.8 |
| $M_w$[c] | 337.820 | 208.100 | 171.950 | 382.210 | 250.720 | 272.130 | 241.830 |

See Table II for the crude cellulosic fractions.
[a] Determined by British standard methods for the determination of the limiting viscosity number of cellulose in dilute solutions, Part 1. Cupri-ethylene-diamine method.
[b] Calculated by $P^{0.9} = 1.65[\eta]$.
[c] Calculated by $P \times 162$.

### Intrinsic viscosity, viscosity-average degree of polymerization, and weight-average molecular weight

The intrinsic viscosity ($\eta$), viscosity-average degree of polymerization ($P$), and weight-average molecular weight ($M_w$) of the seven crude cellulosic preparations ($C_{1a}$–$C_{7a}$) are shown in Table IV. Obviously, crude cellulose preparations $C_{1a}$ and $C_{4a}$, obtained from barley straw and maize stems, respectively, gave the highest $\eta$ (592.6 and 662.3 mL/g), $P$ (2085.3 and 2359.3), and $M_w$ (337,820 and 382,210 g/mol), but the two crude cellulosic samples $C_{2a}$ and $C_{3a}$, isolated from oil palm frond fiber and poplar wood, respectively, exhibited the lowest $\eta$ (383.0 and 322.5 mL/g), $P$ (1284.6 and 1061.4), and $M_w$ (208,100 and 171,950 g/mol). The reason for the lowest $P$ and $M_w$ values of $C_{2a}$ and $C_{3a}$ is presumably less removal of the low molecular weight of lignin during the alkaline peroxide posttreatment of oil palm frond fiber and poplar wood under the given conditions, thereby decreasing the viscosity and molecular weight.

A solution of lithium chloride in DMAc represents a solvent system that is very common in cellulose chemistry. bBcause of the fact that the mixture is able to effect dissolution of cellulose within a certain concentration range of LiCl and cellulose, it is widely used for analytical purposes, for example, GPC measurements of cellulose.[42] To verify the degradation of cellulose during the posttreatment with an acetic acid–nitric acid mixture at 120°C for 15 min, the molecular weight of seven purified cellulose preparations was determined by GPC and the results are given in Table V. Clearly, the seven purified cellulosic preparations ($C_{1b}$–$C_{7b}$) showed no significant difference in their $M_w$ values, which ranged from 39,030 to 48,380 g/mol. However, they gave much lower $M_w$ values than that of the purified cellulose fraction $C_{8b}$ ($M_w$ = 123,970 g/mol) obtained by posttreatment of crude cellulosic sample $C_{5a}$ with 10% NaOH at 25°C for 6 h, indicating that the posttreatment of the crude cellulose with 80% acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min degraded the macromolecular structure of cellulose to a noticeable extent, except for removal of residual lignin and hemicelluloses. In addition, purified cellulosic polymers $C_{1b}$–$C_{7b}$ (obtained by an acetic acid–nitric acid mixture) exhibited lower polydispersity ($M_w/M_n$ = 1.50–1.83) than purified cellulosic sample $C_{8a}$ (isolated by posttreatment of the crude cellulose $C_{5a}$ with 10% NaOH under the conditions used), as shown by a $M_w/M_n$ value of 2.1.

### FTIR spectra

Besides solid-state NMR spectroscopy, FTIR spectroscopy has played a key role in the investigation of the structure of cellulose fibers, being a complementary

**TABLE V**
**Weight-Average ($M_w$) and Number-Average ($M_n$) Molecular Weights and Polydispersity ($M_w/M_n$) of Purified Cellulosic Preparations**

| | Purified cellulosic fractions | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $C_{1b}$ | $C_{2b}$ | $C_{3b}$ | $C_{4b}$ | $C_{5b}$ | $C_{6b}$ | $C_{7b}$ | $C_{8b}$ |
| $M_w$ | 48,380 | 43,670 | 46,410 | 47,030 | 41,820 | 42,150 | 39,030 | 123,970 |
| $M_n$ | 27,450 | 23,860 | 25,640 | 31,010 | 27,510 | 28,110 | 24,390 | 59,030 |
| $M_w/M_n$ | 1.76 | 1.83 | 1.81 | 1.52 | 1.52 | 1.50 | 1.60 | 2.1 |

$C_{1b}$, $C_{2b}$, $C_{3b}$, $C_{4b}$, $C_{5b}$, $C_{6b}$, and $C_{7b}$, the purified cellulose preparations isolated with 80% acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min from corresponding crude cellulosic samples $C_{1a}$, $C_{2a}$, $C_{3a}$, $C_{4a}$, $C_{5a}$, $C_{6a}$, and $C_{7a}$, respectively; $C_{8b}$ the purified cellulose fraction obtained by posttreatment of crude cellulosic sample $C_{5a}$ with 10% NaOH at 25°C for 6 h.

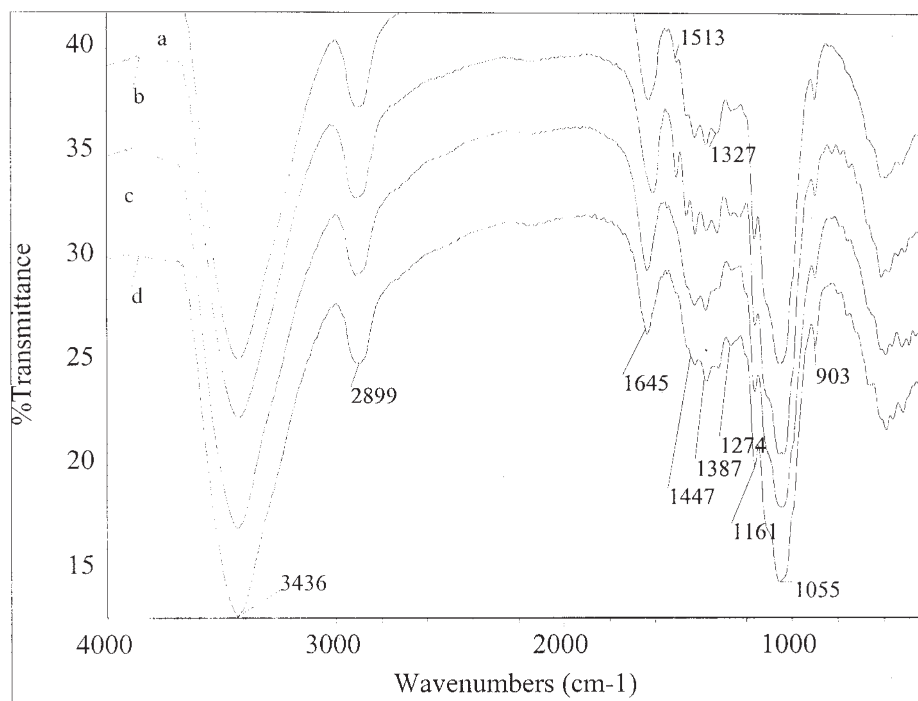


**Figure 2** FTIR spectra of the crude cellulosic preparations obtained by treatment with 2.0% $H_2O_2$ at pH 11.6 for 16 h at 45°C from dewaxed oil palm frond fiber ($C_{2a}$, spectrum a), poplar wood ($C_{3a}$, spectrum b), rice straw ($C_{6a}$, spectrum c), and rye straw ($C_{7a}$, spectrum d).

method, yielding information about the chemical composition, molecular conformation, and hydrogen bonding patterns of the cellulose allomorphs.[43] In this study the FTIR spectra were recorded to find the characteristic groups in both crude and purified celluloses. Figure 2 illustrates FTIR spectra of the crude cellulosic preparations obtained by pretreatment with 2.0% $H_2O_2$ at pH 11.6 for 16 h at 45°C from dewaxed oil palm frond fiber ($C_{2a}$, spectrum a), poplar wood ($C_{3a}$, spectrum b), rice straw ($C_{6a}$, spectrum c), and rye straw ($C_{7a}$, spectrum d). Clearly, all the spectra give very typical peaks for a number of special groups. The band at 670 $cm^{-1}$ is due to OH out of plane bending. An absorption band at 903 $cm^{-1}$ arises from the $\beta$-glycosidic linkages. The prominent band at 1055 $cm^{-1}$ is attributable to C—O and C—O—C stretching.[24] The band at 1161 $cm^{-1}$ relates to C—O antisymmetric bridge stretching. There are C—H bending vibration bands at 1274 and 1387 $cm^{-1}$.[44] The band at 1327 $cm^{-1}$ represents OH in-plane bending, CH bending, or C—C and CO skeletal vibrations. The peak at 1447 $cm^{-1}$ is assigned to $CH_2$ bending. The characteristic peak at 1645 $cm^{-1}$ originates from the —O— tensile vibration band neighboring the H group[44] and the bending mode of the absorbed water.[31] The peak at 2899 $cm^{-1}$ corresponds to the C—H asymmetric and symmetric tensile vibration band. The —OH stretching gives an ample peak at 3436 $cm^{-1}$. It is very likely that a small sharp band at 1520 $cm^{-1}$ in spectrum b in Figure 2 or a shoulder in spectrum a is indicative of aromatic skeletal vibrations in bound lignin, which correspond to their klason lignin contents.

Figure 3 shows three FTIR spectra of the purified cellulosic preparations ($C_{2b}$, spectrum a), poplar wood ($C_{3b}$, spectrum b), and rye straw ($C_{7b}$, spectrum c) obtained by posttreatment with 80% acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min from corresponding crude cellulosic samples $C_{2a}$, $C_{3a}$, and $C_{7a}$, respectively. In comparison with the spectra of the corresponding crude cellulose samples, the spectra of the purified cellulose samples show three important ester bands at 1739 (C═O ester), 1387 (—C—$CH_3$), and 1255 $cm^{-1}$ (—C—O— stretching). This indicated that acetylation of hydroxyl groups in cellulose occurred during the purification of crude cellulose with 80% acetic acid–70% nitric acid under the given conditions. This is also confirmed in spectrum b of Figure 4, showing sample $C_{8b}$ obtained by posttreatment of crude cellulosic fraction $C_{5a}$ with 10% NaOH at 25°C for 6 h, because there were no ester peaks in the spectrum of $C_{8b}$. However, the posttreatment with 10% NaOH at 25°C for 6 h did not completely remove the associated lignin from the crude cellulose preparation as shown by a shoulder at 1520 $cm^{-1}$ in the spectrum of $C_{8b}$. In contrast, the lack of a peak for the carboxylic group at 1700 $cm^{-1}$ revealed that the purified cellulose is free of unreacted acetic acid.

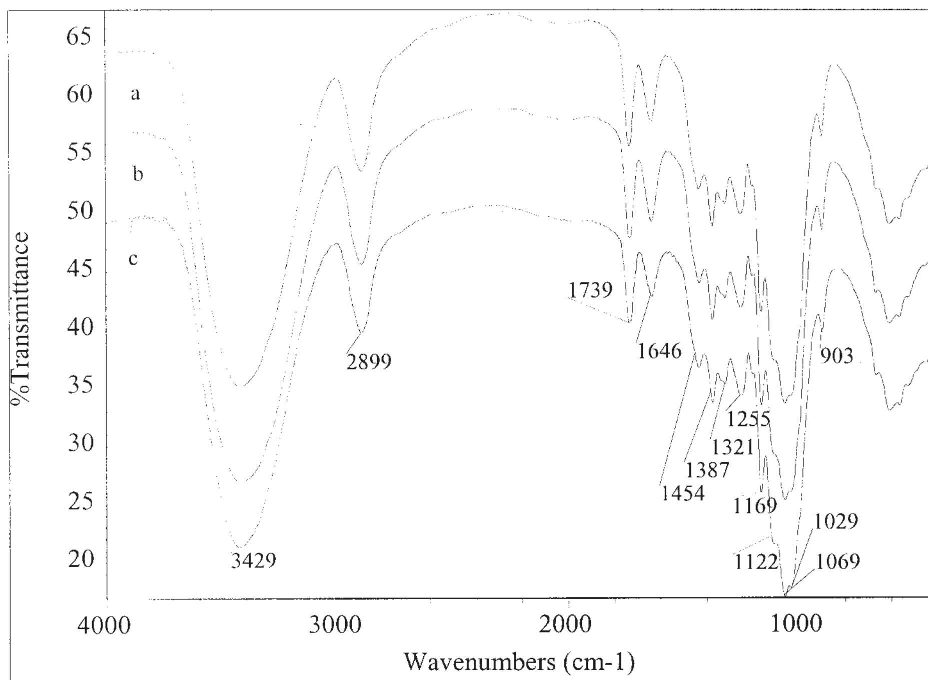


**Figure 3** FTIR spectra of the purified cellulosic preparations ($C_{2b}$, spectrum a), poplar wood ($C_{3b}$, spectrum b), and rye straw ($C_{7b}$, spectrum c) obtained by posttreatment with 80% acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min from the corresponding crude cellulosic samples $C_{2a}$,$C_{3a}$, and $C_{7a}$, respectively.

**CP/MAS $^{13}$C-NMR spectra**

CP/MAS $^{13}$C-NMR spectroscopy has proved particularly useful for examining changes in the nature of cellulose subjected to degradation processes. In contrast to wet chemical analyses, NMR spectroscopy provides information about molecular ordering in cel-

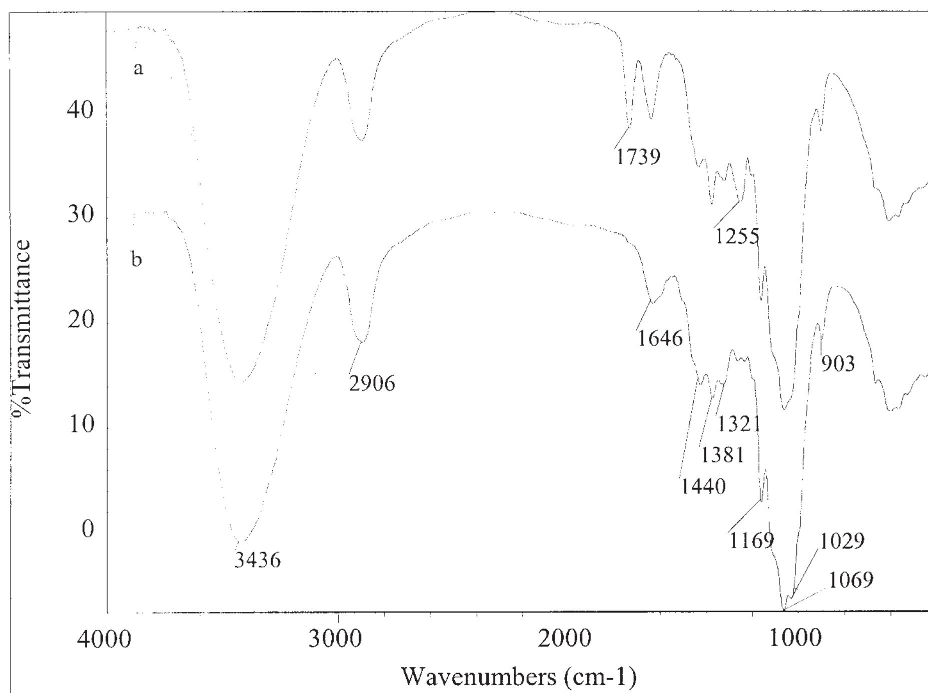


**Figure 4** FTIR spectra of the purified cellulosic preparations $C_{5b}$ (spectrum a) and $C_{8b}$ (spectrum b) obtained by posttreatment of crude cellulosic fraction $C_{5a}$ with 80% acetic acid–70% nitric acid (10/1, v/v) at 120°C for 15 min and 10% NaOH at 25°C for 6 h, respectively.

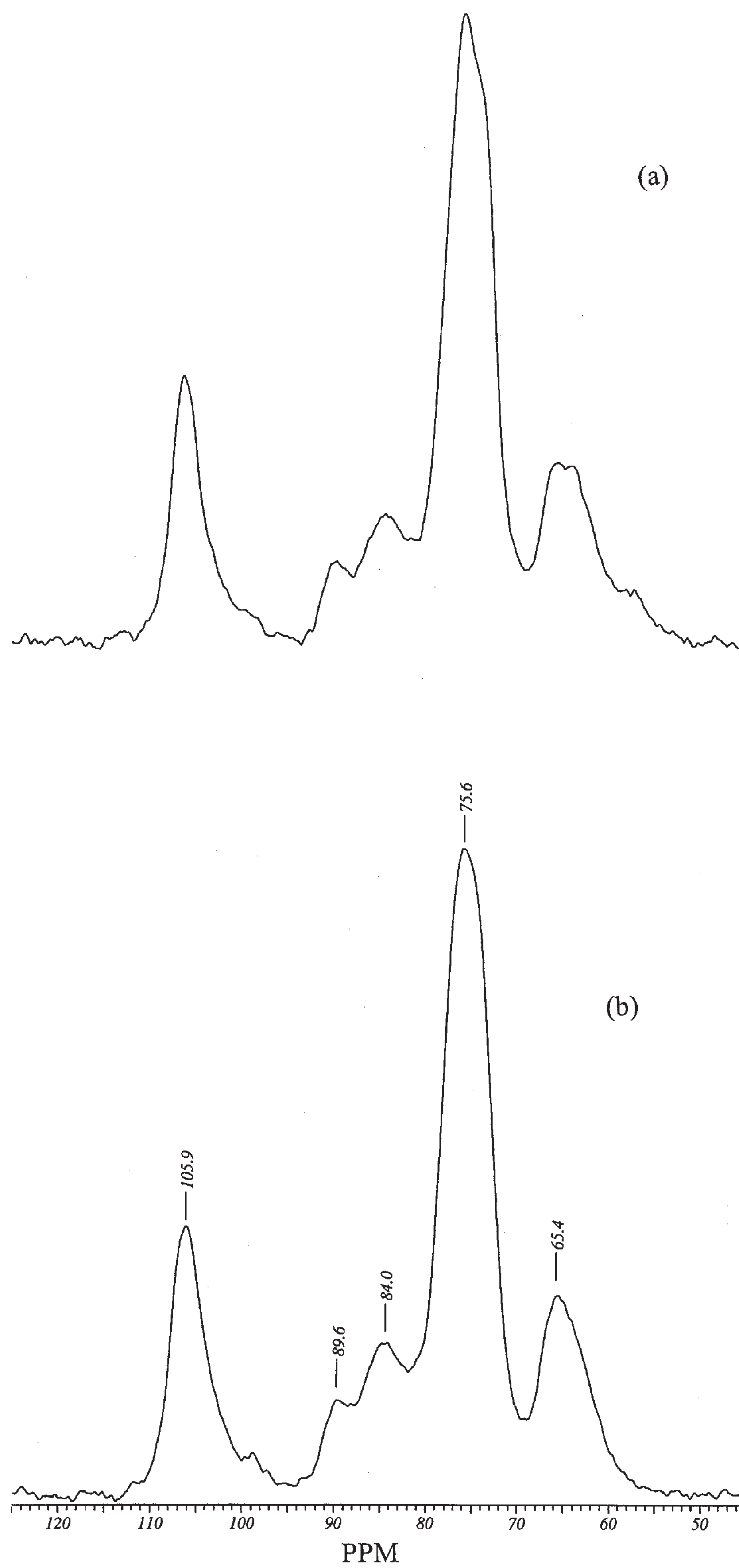


**Figure 5** CP/MAS $^{13}$C-NMR spectra of crude cellulose preparations (a) $C_{3a}$ and (b) $C_{4a}$.

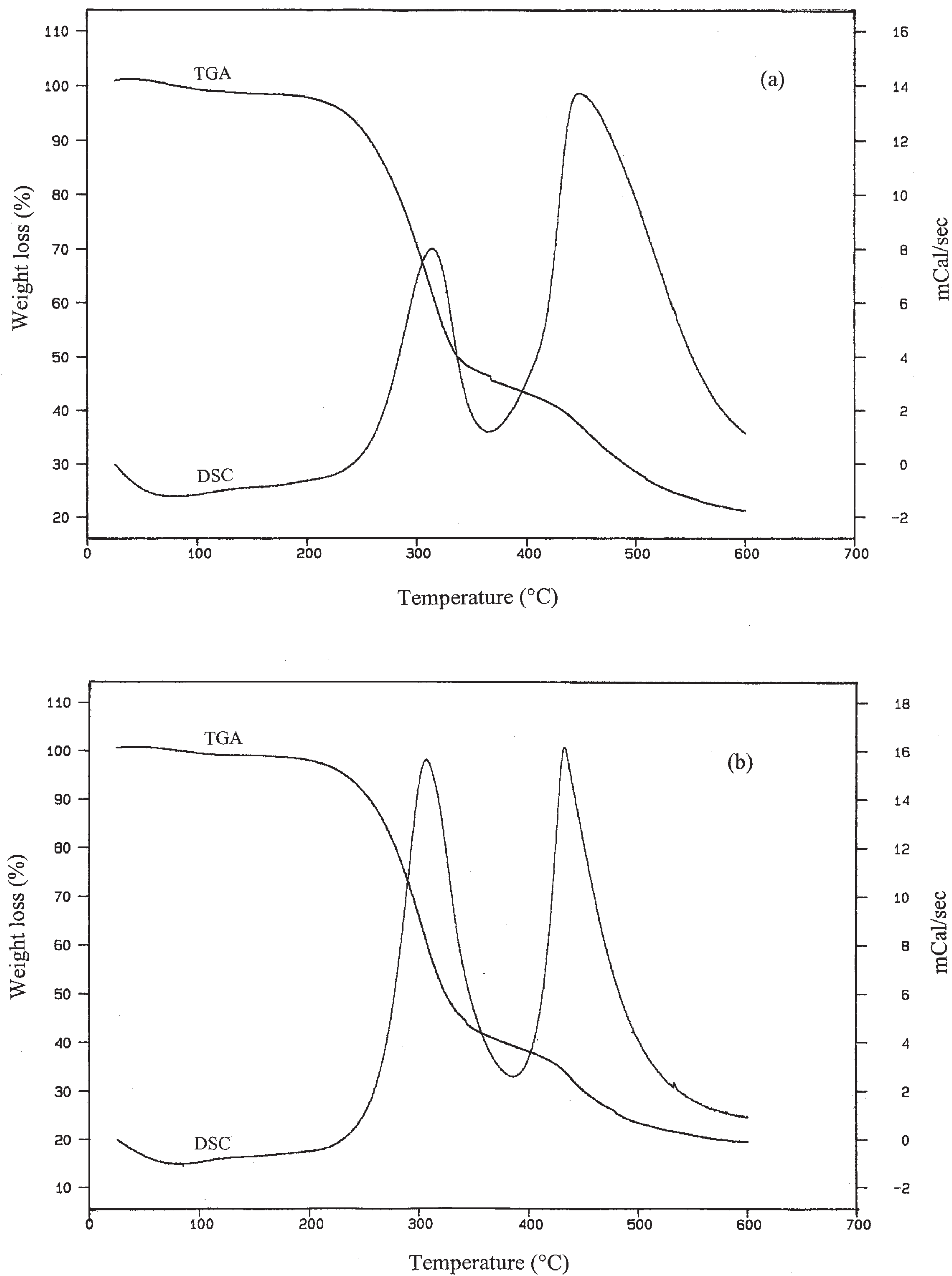


**Figure 6** TGA/DSC curves of crude cellulose preparations (a) $C_{4a}$ and (b) $C_{5a}$.

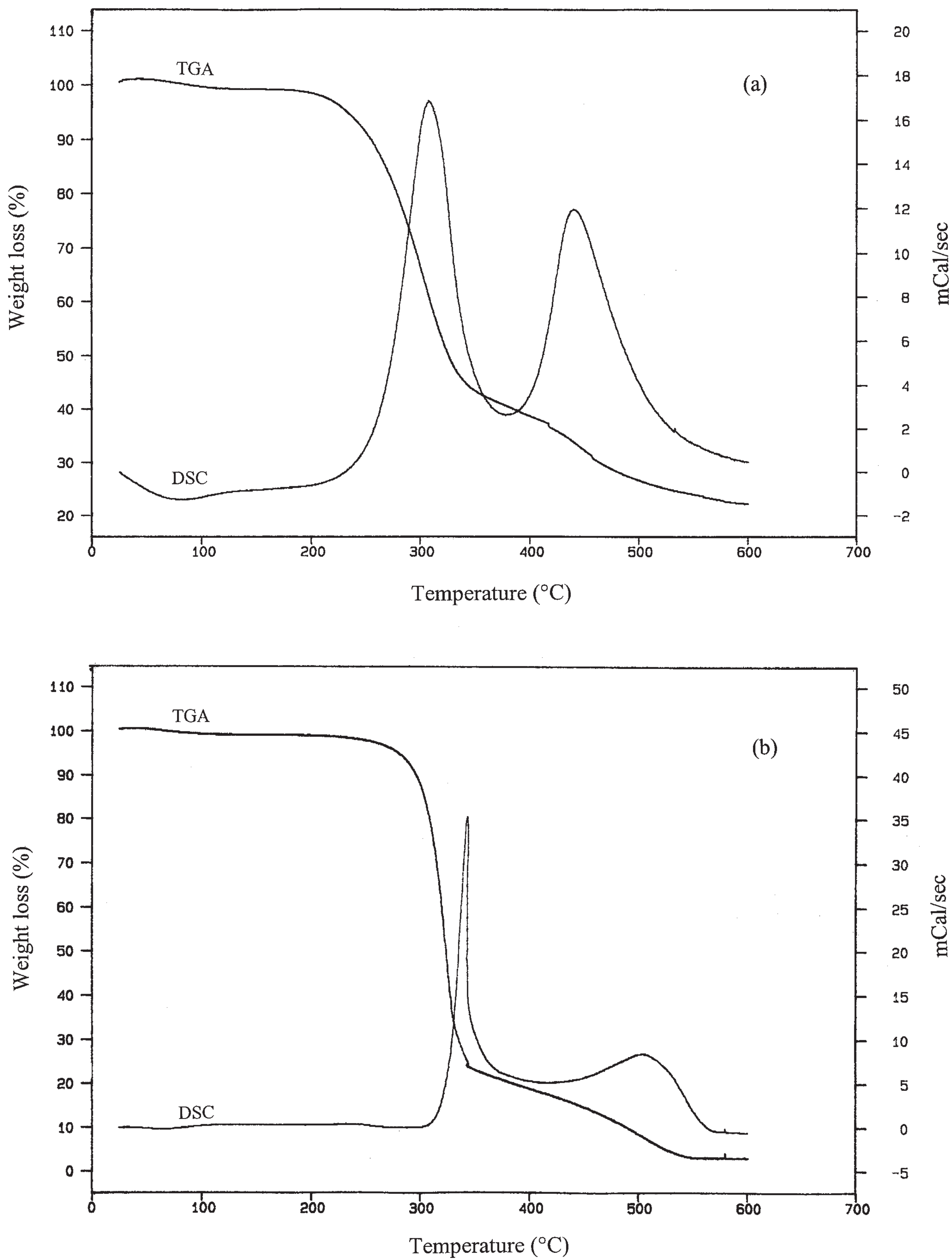


**Figure 7** TGA/DSC curves of crude cellulose preparations (a) $C_{3a}$ and (b) the corresponding purified cellulose fraction $C_{8b}$.

lulose as well as a description of any changes in the structure or content of hemicelluloses and lignin,[45] because the isolated cellulose fractions exhibit not only different lignin and hemicellulosic compositions but also different morphological features.[46] Leary et al.[47] found by CP/MAS $^{13}$C-NMR that fines isolated from spruce wood exhibited lower cellulose crystallinity than the whole wood. In contrast, Wistara and coworkers[48] found by FTIR that the crystallinity of cellulose in the fines was higher than that in the whole pulp.

To verify the structural changes during the pretreatment with 2.0% $H_2O_2$ at 45°C and pH 11.6 for 16 h and the differences between wood and straw celluloses, crude cellulosic samples $C_{3a}$ and $C_{4a}$ were analyzed by CP/MAS $^{13}$C-NMR spectroscopy and their spectra are illustrated in Figure 5. According to the literature,[49] the resonances of the carbon-13 of cellulose appear at 105.9 ppm for C-1, at 89.6 and 84.0 ppm for C-4, and at 65.4 ppm for C-6. The resonances of C-2, C-3, and C-5 overlap each other and occur in the 70–80 ppm region, which is centered on 75.6 ppm.[50] It should be noted that the peak at 89.6 ppm is attributed to crystalline cellulose and the peak at 84.0 ppm is assigned to the crystal surface or disordered cellulose. A small peak at 98.8 ppm corresponds to the residual hemicelluloses, which has been reported to arise from xylan deposited on cellulose crystallites.[51] Interestingly, a similar intensity of the signals between the spectra in Figure 5(a,b) indicated that the crude cellulose samples obtained from poplar wood and maize stem had a similar structure. Similarly, the occurrence of typical patterns of the cellulose signals verified that the pretreatment with alkaline peroxide under the conditions used did not cause any significant changes of the macromolecular structure of cellulose.

### Thermal analysis

It is well known that the thermolysis reaction of cellulose occurs by the cleavage of glycoside bonds (C—H, C—O, C—C bonds) and by dehydration, decarboxylation, and decarbonilation.[52] In this study the thermal stability of the crude and purified cellulose preparations were investigated by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). Figure 6 shows the TGA/DSC curves of crude cellulose preparations $C_{4a}$ [Fig. 6(a)] and $C_{5a}$ [Fig. 6(b)] obtained from maize stem and wheat straw, respectively. Note that the TGA curves of both crude cellulose samples started to decompose at 215°C. At 50% weight loss the decomposition temperature was observed at 329°C for both crude celluloses, indicating that crude cellulosic samples $C_{4a}$ and $C_{5a}$ had equal thermal stability. A similar phenomenon was observed in the two DSC curves of $C_{4a}$ and $C_{5a}$ as shown by two big exothermic peaks at 310 and 438°C.

Figure 7 illustrates the TGA/DSC curves of crude cellulose sample $C_{3a}$ [Fig. 7(a)], obtained by pretreatment of dewaxed poplar wood with 2.0% $H_2O_2$ (pH 11.6, 45°C, 16 h) at a liquid to solid ratio of 25 mL/g, and purified cellulose preparation $C_{8b}$ [Fig. 7(b)], obtained by posttreatment of crude cellulose sample $C_{3a}$ with 10% NaOH at 25°C for 6 h. As illustrated in the figure, the TGA curves of the crude cellulose and purified cellulose started to decompose at 214 [Fig. 7(a)] and 276°C [Fig. 7(b)]. Similarly, at 50% weight loss the decomposition temperature was observed at 319°C for the crude cellulose and at 324°C for the purified cellulose. This implied that the purified cellulose had higher thermal stability than the corresponding crude cellulose preparation, because the crude cellulose preparations contained some amounts of less thermally stable substances, such as hemicelluloses. Further, two exothermic peaks were observed at 300 and 438°C [Fig. 7(a)] for the crude cellulose. However, they appeared in much smaller peaks and were shifted to 343 and 510°C [Fig. 7(b)] for the purified cellulose.

## CONCLUSIONS

In conclusion, the technique described here for isolation of highly pure cellulose from barley straw, oil palm frond fiber, poplar wood, maize stems, wheat straw, rice straw, and rye straw by sequential treatments with alkaline peroxide and a mixture of acetic acid–nitric acid may be considered as an improvement on other published methods. The technique represents a TCF method and it may be used to isolate pure cellulose from any lignocellulosic materials for industries. The purified celluloses obtained were found to be relatively free of bound hemicelluloses and lignin and had molecular weights ranging from 39,030 to 48,380 g/mol. CP/MAS $^{13}$C-NMR spectroscopy observations confirmed that the pretreatment with alkaline peroxide under the conditions used did not cause any significant changes of the macromolecular structure of cellulose. Conversely, posttreatment of the corresponding crude cellulose with an acetic acid–nitric acid mixture under the conditions used resulted in somewhat of an acetylation of the cellulose, which may be hydrolyzed by alkali before use. The thermal stability of the purified cellulose was higher than that of the corresponding crude cellulose.

The authors are grateful for the financial support of this research from the National Natural Science Foundation of China (No. 30271061 and 30430550) Guangdong Natural Science Foundation (013034), and the Ministry of Education China for a major project.

### References

1. Lynd, L. R.; Wyman, C. E.; Gerngross, T. U. Biotechnol Prog 1999, 15, 777.

2. Lynd, L. R.; Weimer, P. J.; Van Zyl, W. H.; Pretorius, I. S. Microbiol Mol Biol R 2002, 66, 506.
3. Roncero, M. B.; Torres, A. L.; Colom, J. F.; Vidal, T. Bioresource Technol 2003, 87, 305.
4. Kirk, R. E.; Othmer, D. F. Encyclopaedia Chem Technol 1967, 4, 593.
5. Brown, Jr., R. M.; Saxena, I. M. Plant Physiol Biochem 2000, 38, 57.
6. VanderHart, D. L.; Atalla, R. H. Macromolecules 1984, 17, 1465.
7. Petitpas, T.; Oberlin, M.; Mering, J. J Polym Sci C 1963, 2, 423.
8. Sarko, A.; Mugli, J. Macromolecules 1974, 7, 486.
9. Marchessault, R. H.; Sundararajan, P. R. The Polysaccharides; Academic: New York, 1983; Vol. 2, p 11.
10. Isogai, A. Allomorphs of Cellulose and Other Polysaccharides. Cellulose Polymers, Blends and Composites; Hanser: Munich, 1994; p 1.
11. Sugiyama, J.; Persson, J.; Chanzy, H. Macromolecules 1991, 24, 2461.
12. Sugiyama, J.; Vuong, R.; Chanzy, H. Macromolecules 1991, 24, 4168.
13. Yamamoto, H.; Horii, F.; Odani, H. Macromolecules 1989, 22, 4130.
14. Kiessig, H.; Hess K.; Sobue, K. Z Phys Chem 1939, 43, 309.
15. Ago, M.; Endo, T.; Hirotsu, T. Cellulose 2004, 11, 163.
16. Okano, T.; Sarko, A. J Appl Polym Sci 1985, 30, 325.
17. Kai, A.; Xu, P. Polym J 1991, 23, 1.
18. Pappas, C.; Tarantilis, P. A.; Daliani, I.; Mavromustakos, T.; Polissiou, M. Ultrason Sonochem 2002, 9, 19.
19. Kadla, J. F.; Gilbert, R. D. Cellulose Chem Technol 2000, 34, 197.
20. Wilson, J. R.; Mertens, D. R. Crop Sci 1995, 35, 251.
21. Loader, N. J.; Robertson, I.; Barker, A. C.; Swistur, V. R.; Waterhouse, J. S. Chem Feol 1997, 136, 313.
22. Abad, S.; Santos, V.; Parajo, J. C. J Chem Technol Biotechnol 2001, 76, 1117.
23. Blakeney, A. B.; Harris, P. J.; Henry, R. J.; Stone, B. A. Carbohydr Res 1983, 113, 291.
24. Sun, X. F.; Sun, R. C.; Tomkinson, J.; Baird, M. S. Polym Degrad Stab 2004, 83, 47.
25. Evans, R.; Wallis, A. F. A. J Appl Polym Sci 1999, 37, 2331.
26. Guay, D. F.; Cole, B. J. W.; Fort, R. C.; Genco, J. M.; Hausman, M. C. J Wood Chem Technol 2000, 20, 375.
27. Pan, G. X.; Bolton, J. L.; Leary, G. J. J Agric Food Chem 1998, 46, 5283.
28. Taher, A. M. M.; Cates, D. M. Textile Chem Color 1975, 7, 220.
29. Brooks R. E.; Moore, S. B. Cellulose 2000, 7, 263.
30. Gould, J. M. U.S. Pat. 4,806,475, (1989).
31. Sun, R. C.; Tomkinson, J.; Ma, P. L. Carbohydr Polym 2000, 42, 111.
32. Dence, C. W. Pulp Bleaching—Principle and Practice; TAPPI Press: Atlanta, GA, 1996; p 349.
33. Scott, R. W. J Wood Chem Technol 1984, 4, 199.
34. Zawadzki, J.; Wisniewski, M. J Anal Appl Pyrol 2002, 62, 111.
35. Timell, T. E. Wood Sci Technol 1967, 1, 45.
36. Iwata, T.; Indrarti, L.; Azuma, J. I. Cellulose 1998, 5, 215.
37. Hansson, J. A.; Hartler, N. Svensk Papperstidning 1969, 72, 521.
38. Mora, F.; Ruel, K.; Comtat, J.; Joseleau, J. P. Holzforschung 1986, 40, 85.
39. Linder, A.; Roubroeks, J. P.; Gatenholm, P. Holzforschung 2003, 57, 496.
40. Saake, B.; Kruse, T.; Puls, J. Bioresource Technol 2003, 80, 195.
41. Tenkanen, M.; Tamminen, T.; Hortling, B. Appl Microbiol Biotechnol 1999, 51, 241.
42. Potthast, A.; Rosenau, T.; Buchner, R.; Roder, T.; Ebner, G.; Bruglachner, H.; Sixta, H.; Kosma, P. Cellulose 2002, 9, 41.
43. Michell, A. J. Carbohydr Res 1993, 241, 47.
44. Togrul, H.; Arslan, N. Carbohydr Polym 2003, 54, 63.
45. Kim, Y. S.; Newman, R. H. Holzforschung 1995, 49, 109.
46. Hardell, H. L.; Leary, G. J.; Stoll, M.; Westermark, U. Svensk Papperstidning 1980, 84, 44.
47. Leary, G. J.; Morgan, K. R.; Newman, R. H. Holzforschung 1986, 40, 221.
48. Wistara, N.; Zhang, X.; Young, R. A. Cellulose 1999, 6, 325.
49. Tang, H. R.; Wang, Y. L.; Belton, P. S. Solid State NMR 2000, 15, 239.
50. Pappas, C.; Tarantilis, P. A.; Daliani, I.; Mavromustakos, T.; Polissiou, M. Ultrason Sonochem 2002, 9, 19.
51. Liitia, T.; Maunu, S. L.; Hortling, B. Holzforschung 2000, 54, 618.
52. Schirs J.; Camino, G.; Tumiatti, W. Eur Polym J 2001, 37, 933.