Article

Determination of Cellulose Reactivity by Using Phosphitylation and Quantitative P NMR Spectroscopy

Ilari Filpponen, and Dimitris S. Argyropoulos

Ind. Eng. Chem. Res., **2008**, 47 (22), 8906-8910 • Publication Date (Web): 23 October 2008

Determination of Cellulose Reactivity by Using Phosphitylation and Quantitative ^{31}P NMR Spectroscopy

Ilari Filpponen and Dimitris S. Argyropoulos*

Organic Chemistry of Wood Components Laboratory, College of Natural Resources, North Carolina State University, Raleigh, North Carolina 27695-8005

The phosphitylation of cellulose with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane [P(II)], is proposed as a means to determine its reactivity via an evaluation of its accessible hydroxyl groups. A variety of cellulose samples were subjected to this phosphitylation reaction, and the consumption of phosphitylation reagent was followed by quantitative ^{31}P NMR spectroscopy. This consumption was found to be directly proportional to the amount of reactive hydroxyl groups on the cellulosic material. To further evaluate the quantitative reliability of this methodology, cellulose samples were subjected to a series of mechanical beating treatments, and the changes in the amount of accessible OH groups were evaluated. In addition, cellulose samples were equilibrated to various moisture contents, and their accessible OH groups were determined using the developed methodology. Both variables examined were found to affect the amount of reactive OH groups present on the samples with variations in the moisture content having a greater effect. For example, up to 6.5 mmol g^{-1}, of accessible OH groups were found to be created within the highly refined samples at the highest moisture content.

Introduction

Cellulose, the most abundant component of plant cell walls, exists as long fibers composed of aggregated cellulose chains called microfibrils.[1] These microfibrils consist of two distinctly different regions: a highly ordered crystalline region and less ordered amorphous regions. In nature, cellulose microfibrils are highly, albeit not completely, crystalline. As much as 70−80% of cellulose in cotton and about 60−70% of cellulose in wood is crystalline. The crystallinity of microfibrils is a combination of physicochemical factors such as linearity and structural uniformity of cellulose polymer that allows several individual cellulose chains to pack together and form a very ordered crystalline structure. Moreover, the cellulose polymer has a large number of hydroxyl groups capable of forming hydrogen bonds between (inter) and also within (intra) its polymeric chains. This extensive hydrogen bond network is known to greatly affect the accessibility of crystalline cellulose as it remains intact in the presence of most common solvents. Most aqueous reagents, however, can penetrate and swell the amorphous or noncrystalline regions of cellulose fibers. Thus, the concepts of crystallinity and accessibility of cellulose are closely related.

The accessibility of the hydroxyl groups within cellulose plays an important role in determining its reactivity toward the various chemical modifications of this material as far as its homogeneous chemical derivatization is concerned.[2] Furthermore, the accessibility of cellulose is one of the key parameters affecting the efficient production of bioethanol.[3,4]

Early attempts to quantitatively determine the accessible hydroxyl groups in cellulose were carried out in 1930s.[5,6] These early experiments were focused on the hydrogen−deuterium exchange reaction of D_2O with the OH groups of cellulose. The measurements were based on the density changes of D_2O caused by the simultaneous liberation of H_2O. Since then, deuterium exchange has prevailed as one of the most common approaches to determine the amount of accessible OH groups in cellulosic materials.

Additional methods for quantifying the accessible hydroxyls in cellulosic materials also include acetylation and differential scanning calorimetric-based methods (DSC).[7,8] Among these, deuterium exchange and acetylation are typically used in combination of IR and/or NMR spectroscopic techniques.[9,10] For example, Phuong et al. studied the accessibility of heat-treated wood by using hydrogen−deuterium exchange and ^{2}H NMR.[9] The amount of accessible OH groups was found to be significantly lower after the heat treatment, most likely, due to the decreased hygroscopicity of the heat-treated wood. Bertran and co-workers, on the other hand, were able to correlate the cellulose crystallinity and accessibility values by using a DSC technique for determining the amount of the absorbed water. In addition to above methodologies, both X-ray diffraction and iodine sorption measurements have been used for the determination of the crystallinity and accessibility of cellulose. X-ray diffraction data offers a measure of the crystalline component of cellulose while iodine sorption data provides information on the amorphous part or the accessible hydroxyl groups.[11,12] Yet, the correlation of these methods with the reactivity under conditions of industrial relevance and production has not been established.

Dyes have also been used for the determination of cellulose accessibility.[13,14] Although these measurements do not reveal the exact amount of OH groups, they can be used for resolving the specific surface area (SSA) of cellulose. The main advantage of using dyes derives from the possibility of determining the accessibility of wet swollen-state samples. As a matter of fact, the surface area determinations for a dry nonswollen sample and for a wet swollen cellulose sample have been reported to vary from 1.9 to 162 m^2 g^{-1}, respectively.[15,16]

One widely used method, especially for determining the reactivity of dissolving pulps, was described by Fock in the late 1950s.[17] This method is a microscale process similar to the viscose process, and it measures the amount of cellulose that is not soluble in sodium hydroxide when viscose is prepared. Recently, major efforts have been conducted with studies toward improving the reactivity of dissolved pulps by enzymatic treatment.[18−20] In these investigations, Fock's method has been

* To whom correspondence should be addressed. E-mail: dsargyro@ncsu.edu. Tel.: (919)515-7708. Fax: (919) 515-6302.

10.1021/ie800936x CCC: $40.75
Published on Web 10/23/2008

Table 1. Moisture Contents (%) of Cellulose Samples[a]

sample	oven dry	air dry	69% RH
control	n.d.	7.8	11.0
5000 rev	n.d.	7.9	10.9
30000 rev	n.d.	9.7	14.2

[a] n.d. = not determined. Oven dry samples were assumed to have near 0% moisture content; 69% RH samples were conditioned in a desiccator for 72 h at 23°C.

proven particularly useful for determining the reactivity of the various cellulosic materials.

Derivatization of surface hydroxyl groups followed by different analytical techniques has been the chosen approach in several investigations.[21−24] Chemicals such as trifluoroacetic anhydride or *N*,*N*-diethylaziridinium chloride can be effectively utilized for the derivatization of cellulose. Trifluoroacetic anhydride is used in a vapor phase reaction, and the derivatives are then characterized by various spectroscopic methods. More specifically, the analysis is carried out in the gaseous phase, thus offering some advantages over typical wet chemical methods. Another methodology, developed by Rowland, utilizes the mild reaction of *N*,*N*-diethylaziridinium chloride with the available hydroxyl groups to yield 2-diethylamino ethyl cellulose that can be further silylated and analyzed by gas liquid chromatography.

In this contribution, a new approach, based on phosphitylation followed by quantitative ^{31}P NMR measurements, is proposed for measuring the amount of accessible OH groups in cellulosic materials. This protocol is comprises phosphitylation of all OH groups present in cellulose using 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane [P(II)] followed by quantitative ^{31}P NMR spectroscopy using guaiacol as the internal standard under carefully selected NMR acquisition conditions.

Materials and Methods

Sample Preparation and Refining of Cellulose. Samples from cellulose filter paper (Whatman #1, cotton, 80% crystallinity) were refined for specific number of revolutions (5000 and 30000) according to TAPPI method T 248 cm-85. After the refining treatments the samples were first air-dried and then homogenized for 10 s using a blender. The homogenized samples were used as they were for all air-dry trials. Moreover, portions of samples were oven-dried or conditioned at 69% relative humidity to examine the effect of moisture content. Drying was carried out in a heating chamber under reduced pressure (15 mmHg) for 24 h. The 69% relative humidity was achieved by placing a saturated KI solution at the bottom of a desiccator in which the samples were conditioned over a period of three days prior to analyses. To better understand the effects of refining, control samples were always also examined. Control samples were prepared by first soaking the filter paper in water, then air-drying, and finally homogenizing in a Wearing blender.

Moisture Content Measurements. An electronic moisture analyzer (Sartorius MA 30) was used for the determination of the moisture contents of all the cellulose samples (Table 1). The moisture contents for the oven dry samples, however, were not measured as they were readily transferred to the reaction flasks to avoid the absorption of ambient moisture.

Phosphitylation of Cellulose. Reactions were carried out in 50 mL predried Schlenk flasks equipped with a magnetic stirrer, an argon inlet, and a septum for reagent addition via a stainless steel syringe. Cellulose sample (100 mg) was suspended in 15 mL of freshly distilled THF (distilled over the sodium lumps under argon atmosphere), 5 mL of dry pyridine containing 0.03 mmoles of 4-(dimethylamino) pyridine (DMAP), and 5 mL of dry chloroform containing 100 μL of guaiacol (0.9 mmol, internal standard). A 600 μL portion of 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane [P(II)] was then added slowly via the septum under slight agitation. The reaction kinetics was followed by taking aliquots (600 μL) from the reaction mixture and then determining the amount of remaining phosphitylation reagent using quantitative ^{31}P NMR spectroscopy. To ensure the sampling of clean and transparent samples for the NMR analysis, it is essential to let the cellulose settle on the bottom of the reaction flask prior to sampling. This was achieved by turning off the magnetic stirrer prior to withdrawing an aliquot. It is also worth mentioning that in all experiments one flask containing all the chemicals but no cellulose was kept on the side as a blank. The use of this reference sample allowed taking into account the slow decomposition kinetics of the phosphitylation reagent that was observed to occur.

Figure 1. Reaction between 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane [P(II)] and hydroxyl group of lignocellulosic material.

Quantitative ^{31}P NMR of remaining P(II). Aliquots of 600 μL of the reaction mixture, prepared as described above, were transferred into a NMR tube containing 0.57 mg of chromium(III) acetylacetonate dissolved in 50 μL of deuterated chloroform. This compound was used to reduce the relaxation time of the phosphorus nuclei, thus allowing for more signal acquisitions per unit time and spectra of greater signal-to-noise ratios. The quantitative ^{31}P NMR spectra were acquired immediately, using a Bruker 300 MHz spectrometer equipped with a Quad probe dedicated to ^{31}P, ^{13}C, ^{19}F, and ^{1}H acquisition. A total of 128 scans were acquired (via the use of a standards inverse gated decoupling pulse sequence) for each sample with the relaxation delay time (d1) of 5.0 s.[25,26]

Reactivity Calculations. The amount of reactive hydroxyls was calculated on the basis of the theoretical value of 18.5 mmol of hydroxyl groups present per gram of anhydroglucose unit (AGU). The hydroxyl groups in positions 2, 3, and 5 were considered to be reactive.

Results and Discussion

Phosphitylation of Cellulose. Phosphitylation followed by quantitative ^{31}P NMR analysis have been previously used for the determination of various chemical functionalities of lignocellulosic materials.[25,26] The methodology is based on the phosphitylation reaction of hydroxyl groups in lignocellulosics which in turn improves the solubility of the material and makes the various OH groups NMR detectable (Figure 1). The current approach, on the other hand, while based on the same reaction, does not focus on the analysis of the dissolved material but instead uses a reaction that is carried out in a heterogeneous nonswelling environment. The foundations of the methodology rest on the extreme reactivity of the cellulosic OH groups with the phosphitylating reagent, and the accurate detection of the unreacted (remaining) phosphitylating reagent. The latter value in turn provides information of the reactivity of the cellulosic material. Figure 2 shows a typical ^{31}P NMR spectrum of remaining phosphitylation reagent from a typical cellulosic reaction mixture. Three signals are apparent, one derived from the H_2O adduct of the phosphitylation reagent (132.2 ppm), one from phosphitylated guaiacol (139.9 ppm), and one from

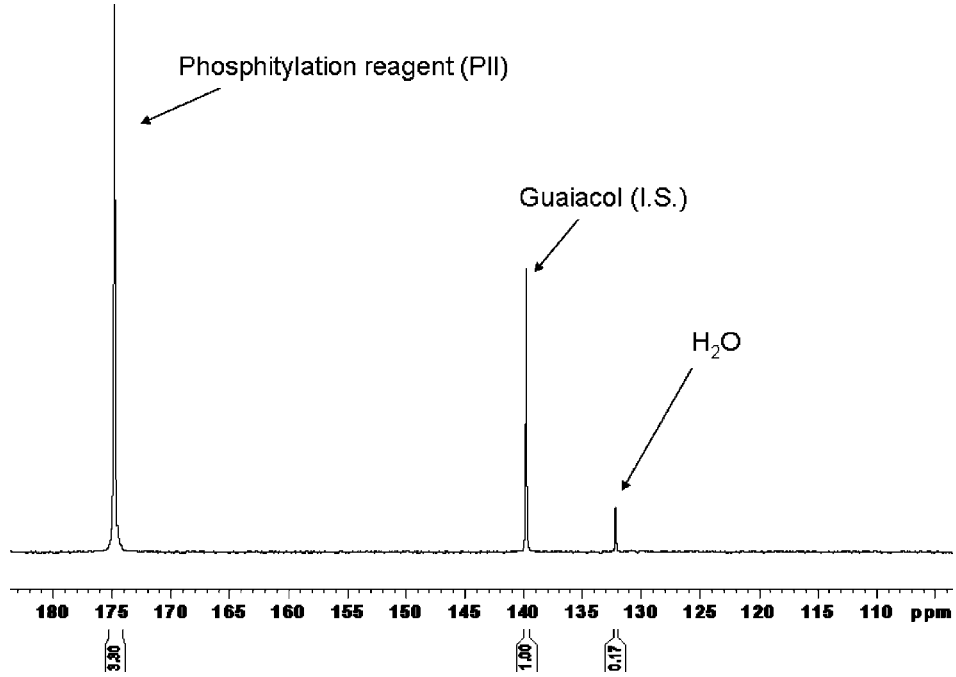

Figure 2. A typical quantitative ^{31}P NMR spectrum of remaining phosphitylation reagent. Signals: H_2O adduct of phosphitylation reagent (132.2 ppm), phosphitylated guaiacol (I.S., 139.9 ppm), and phosphitylation reagent (174.9 ppm).

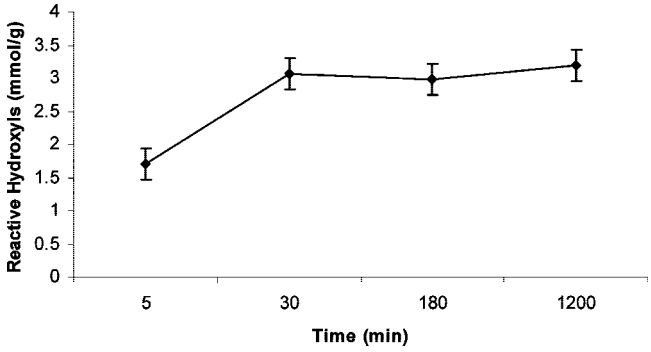

Figure 3. Preliminary phosphitylation experiments with an air-dried sample. Reaction was seen to level off after 30 min.

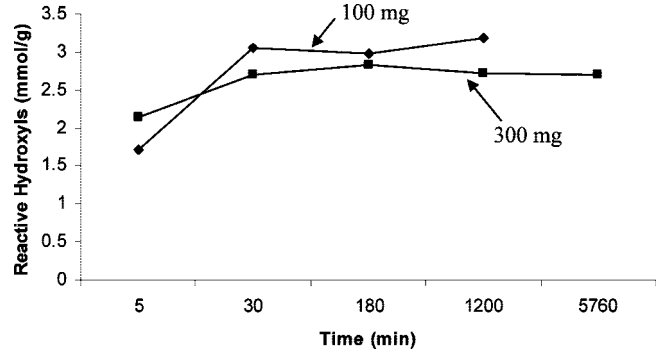

Figure 4. Scaling up experiments with air-dried cellulose samples with two different sample sizes.

remaining phosphitylation reagent (174.9 ppm), respectively. In fact, integration of the water signal at 132.2 ppm allows the accurate determination of water content in the system, and as such it becomes an additional way of evaluating the water content of such samples. Furthermore, its precise determination allows for accurate calculations to be made as they pertain to the various accessible OH groups in the various samples.

Preliminary Experiments with an Air-Dried Filter Paper. In an effort to determine the reaction time required for complete phosphitylation, an air-dried, Whatman #1 filter paper sample was subjected to the developed methodology as described in the experimental section. The consumption of phosphitylating reagent was seen to be complete after 30 min of reaction (Figure 3). The amount of phosphitylating reagent was found to remain at constant level for up to 20 h. Furthermore, in an effort to delineate any possible effects the sample size may have had on the determined reactivity, the amount of cellulose was increased to 300 mg. As shown in Figure 4, increased sample size did not affect the reactivity of cellulose. As a matter of fact the reaction profiles were found to be very similar with an error of approximately ± 0.25 mmol g^{-1}.

Effect of Laboratory Beating. To further investigate the validity of the proposed methodology, cellulose samples from two different beating treatments were subjected to these analyses. The mechanical treatment of fibrous material results in several actions on the fiber: it can fibrillate the surface of the fibers, it produces fines, and it also generates internal pores. All the aforementioned factors can be considered to increase the amount of available hydroxyl groups in cellulosic materials. This set of experiments clearly showed that intense (30000 rev) refining increased the reactivity of the cellulose exposing more hydroxyl groups for reaction with the phosphitylating reagent. Alternatively, the difference between a control and slightly refined sample (5000 rev) showed hardly any difference in the amount of available OH groups (Figures 5 and 6). However, at higher moisture contents, the slightly refined sample was seen to open up and the difference to the control became more distinguishable (Figure 7).

It is worth mentioning at this point that the starting filter paper sample had a crystallinity as high as 80%. Hence, in theory, 20% of its hydroxyl groups are to be located in the amorphous regions. This in turn implies that our method may determine up to 20% of the stoichiometrically present OH groups in the starting untreated control samples. By using the previously described theoretical maximum value of 18.54 mmol g^{-1} for all accessible hydroxyl groups, our experimental data shows a

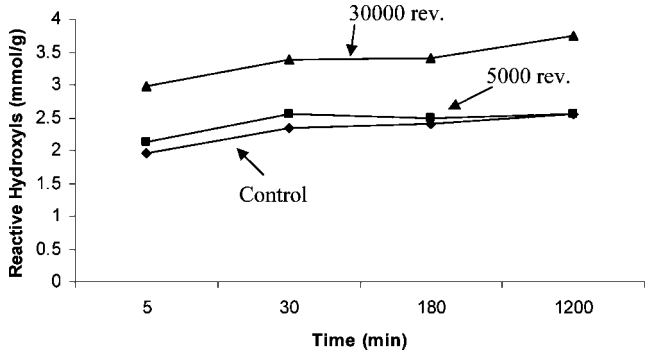

Figure 5. Reactivity of oven-dried cellulose samples. Control refers to untreated sample; 5000 rev refers to slightly refined sample; 30000 rev refers to the most refined sample.

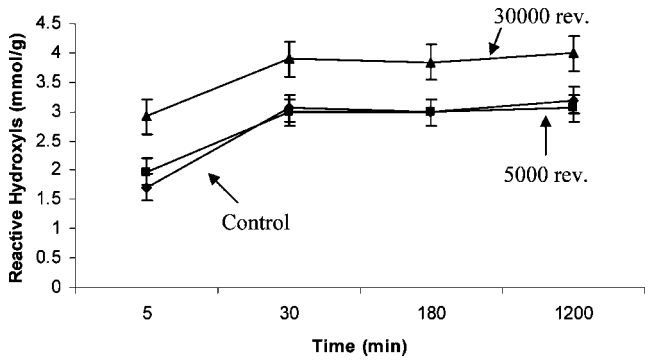

Figure 6. Reactivity of air-dried cellulose samples. Control refers to untreated sample; 5000 rev refers to slightly refined sample; 30000 rev refers to the most refined sample.

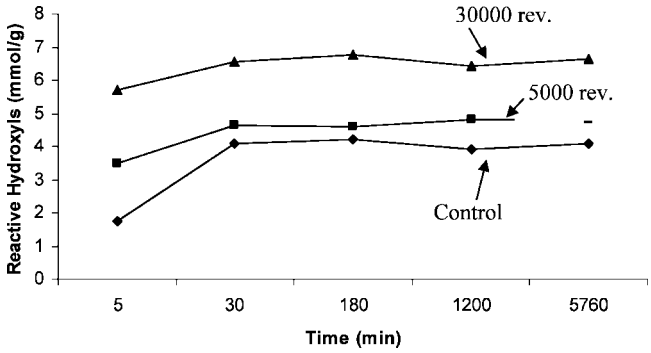

Figure 7. Reactivity of cellulose samples conditioned at 69% RH. Control refers to untreated sample; 5000 rev refers to slightly refined sample; 30000 rev refers to the most refined sample.

maximum reactivity of 2.5 mmol g^{-1} (13%) for the oven-dried sample, 3.2 mmol g^{-1} (17%) for the air-dried sample, and 4.1 mmol g^{-1} (22%) for the humidity-conditioned control sample. These values all vary consistently and within range of the calculated theoretical value of 20% reactivity. It is worth mentioning here that Phuong et al. has reported a reactivity as high as 6.8 mmol g^{-1} for the hydroxyl groups in nontreated wood samples.[9] Although the value is considerably higher than the reactivity observed for our cellulose samples it is not totally surprising when the lower crystallinity and other reactive hydroxyl groups present in wood are taken into consideration.

The highest reactivity, approximately 6.5 mmol g^{-1}, was observed for the most refined (30000 rev) sample with highest moisture content (Figure 7). Such reactivity is well above the amount of OH groups present within the amorphous region of the starting material and corresponds to 35% of the theoretically accessible maximum described above. One may assume that the mechanical treatment increased the reactivity of the sample by causing extensive fibrillation of the fibers and/or the production of fines, that is, more surface area/unit sample weight.

The reproducibility of the methodology was examined with the air-dry samples by conducting duplicate measurements for each type of starting cellulose samples (control, 5000 rev, and 30000 rev). As shown in Figure 6, the various measurements are very similar for all examined samples with the maximum experimental error of ± 0.3 mmol g^{-1}.

At this point, it can be stated that the developed protocol was shown to be highly sensitive for probing the morphological changes in cellulose as experienced by mechanical refining. Moreover, the methodology, despite the fact that it is carried out in a nonswollen environment, allows the monitoring of the effects of increased moisture content when in equilibrium with a cellulosic sample.

Effect of Moisture Content. Dry cellulose absorbs moisture from the air rather rapidly, to a given equilibrium moisture content. This absorption is particularly effective on the amorphous accessible regions of cellulose and it increases with the relative humidity (RH). Crystalline regions of cellulose, on the other hand, cannot be attacked by aqueous reagents, and therefore remain unchanged[1]. For the purposes of this work the data discussed is limited to a maximum moisture content of approximately 15% (Table 1). However, further investigations of extended moisture levels beyond the 15% level may also be examined either by increasing the amount of phosphitylation reagent or by decreasing the sample size.

The effect of increased moisture content can clearly be seen from the data of Figures 5, 6, and 7. Initially, the amount of accessible hydroxyl groups is seen to increase in tandem with increased moisture content. For example, the OH reactivities were found to be elevated by approximately 1.5−3.0 mmol g^{-1} when comparing the values of the oven-dried samples (Figure 5) to the ones that were conditioned at 69% RH (Figure 7). Second, the moisture content was found to have a greater effect to the accessibility of refined samples, that is, the difference between oven-dried and moist samples is seen to be larger for the refined samples than for the control samples. Furthermore, the difference between the control and the slightly refined (5000 rev) sample is more pronounced at the higher moisture content, most likely due to an increased swelling of fibers (Figure 7). The experimental data from the different moisture contents suggests that the structures subjected to the mechanical treatment are more susceptible to moisture changes because of the increased surface area produced by the mechanical treatment.

Conclusions

A newly developed sensitive methodology, based on a phosphitylation reaction of cellulose can be used for the determination of accessible hydroxyl groups in such materials. The sensitivity of the technique is such that it allows for changes to be observed as they are induced on cellulose via mechanical refining as well as variable equilibrium moisture contents.

Error analyses and the reproducible and sensitive nature of the developed technique will be of further use in a multitude of applications that require the absolute and accurate measurement of cellulosic surface development, that is, as a function of various industrially treatments (enzymatic, chemical, mechanical) as engaged in diverse applications of cellulose containing materials.

Acknowledgment

The authors would like to thank the College of Natural Resources at NCSU for the award of the Hofmann Fellowship (I.F.) that made graduate studies possible.

Literature Cited

(1) McGinnis, G. D.; Shafhadeh, F. *Pulp and Paper Chemistry and Chemical Technology*; Wiley-Interscience: New York, 1980.

(2) Gandini, A.; Belgacem, M. N. Modified cellulose fibers as reinforcing fillers for macromolecular matrices. *Macromol. Symp.* **2005**, *221*, 257.

(3) Frederick, W. J.; Lien, S. J.; Courchene, C. E.; DeMartini, N. A.; Ragauskas, A. J.; Iisa, K. Production of ethanol from carbohydrates from loblolly pine: A technical and economic assessment. *Bioresour. Technol.* **2008**, *99*, 5051.

(4) Galbe, M.; Zacchi, G. Pretreatment of lignocellulosic materials for efficient bioethanol production. *Adv. Biochem. Eng. Biotechnol.* **2007**, *108*, 41.

(5) Bonhoeffer, K. F. Reactions of heavy hydrogen. *Z. Elektrochem.* **1934**, *40*, 469.

(6) Champetier, G.; Viallard, R. Exchange reaction of cellulose and heavy water. Hydration of cellulose. *Bull. Soc. Chim.* **1938**, *5*, 1042.

(7) Mann, J.; Marrinan, H. J. The reaction between cellulose and heavy water. Parts 1, 2 and 3. *Trans. Faraday Soc.* **1956**, *52*, 481.

(8) Bertran, M. S.; Dale, B. E. Determination of cellulose accessibility by differential scanning Calorimetry. *J. Appl. Polym. Sci.* **1986**, *32*, 4241.

(9) Phuong, L. X.; Takayama, M.; Shida, S.; Matsumoto, Y.; Aoyagi, T. Determination of the accessible hydroxyl groups in heat-treated Styrax tonkinensis (Pierre) Craib ex Hartwich wood by hydrogen-deuterium exchange and ^{2}H NMR spectroscopy. *Holzforschung* **2007**, *61*, 488.

(10) Nishiyama, Y.; Isogai, A.; Okano, T.; Müller, M.; Chanzy, H. Intracrystalline Deuteration of Native Cellulose. *Macromolecules.* **1999**, *32*, 2078.

(11) Hessler, L. E.; Power, R. E. The use of iodine adsorption as a measure of cellulose fiber crystallinity. *Textile Res. J.* **1954**, *24*, 822.

(12) Racz, I.; Borsa, J.; Bodor, B. Crystallinity and accessibility of fibrous carboxymethyl cellulose by pad-roll technology. *J. Appl. Polym. Sci.* **1996**, *62*, 2015.

(13) Inglesby, M. K.; Zeronian, S. H. The accessibility of cellulose as determined by dye adsorption. *Cellulose.* **1996**, *3*, 165.

(14) Bairathi, A. Dyeing sorption isotherms of three direct dyes and their mixtures on purified cotton. *Text. Chem. Color.* **1993**, *25*, 41.

(15) Warwicker, J. O.; Jeffries, R.; Colbran, R. L.; Robinson, R. N. *A Review of the Literature on the Effect of Caustic Soda and Other Swelling Agents on the Fine Structure of Cotton;* Shirley Institute Pamphlet No. 93;The Cotton Silk and Man-Made Fibres Research Association: Manchester, U.K., 1966.

(16) Zeronian, S. H. (1985) *In Cellulose Chemistry and its Applications;* Nevell, T. P., Zeronian, S. H., Eds.; Ellis Horwood Ltd: Chichester, England, 1985; pp 138−158.

(17) Fock, W. A modified method for determining the reactivity of viscose-grade dissolving pulps. *Papier* **1959**, *13*, 92.

(18) Rahkamo, L.; Siika-aho, M.; Vehviläinen, M.; Dolk, M.; Viikari, L.; Nousiainen, P.; Buchert, J. Modification of hardwood dissolving pulp with purified Trichodserma reesei cellulases. *Cellulose* **1996**, *3*, 153.

(19) Henriksson, G.; Christiernin, M.; Agnemo, R. Monocomponent endoglucanase treatment increases the reactivity of softwood sulphite dissolving pulp. *J. Ind. Microbiol. Biotechnol.* **2005**, *32*, 211.

(20) Engström, A.-C.; Ek, M.; Henriksson, G. Improved accessibility and reactivity of dissolving pulp for the viscose process: pretreatment with monocomponent endoglucanase. *Biomacromolecules* **2006**, *7*, 2027.

(21) Östenson, M.; Järund, H.; Toriz, G.; Gatenholm, P. Determination of surface functional groups in lignocellulosic materials by chemical derivatization and ESCA analysis. *Cellulose* **2006**, *13*, 157.

(22) Tasker, S.; Badyal, J. P. S. Hydroxyl accessibility in celluloses. *Polymer* **1994**, *35*, 4717.

(23) Rowland, S. P.; Roberts, E. J.; Wade, C. P. Selective accessibilities of hydroxyl groups in the microstructure of cotton cellulose. *Text. Res. J.* **1969**, *39*, 530.

(24) Verlhac, C.; Dedier, J. Availability of surface hydroxyl groups in *Valonia* and bacterial cellulose. *J. Polym. Sci.: Part A* **1990**, *28*, 1171.

(25) Argyropoulos, D. S. Quantitative phosphorus-31 NMR analysis of lignins, a new tool for the lignin chemist. *J. Wood Chem. Technol.* **1994**, *14*, 45.

(26) Granata, A.; Argyropoulos, D. S. 2-Chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane, a reagent for the accurate determination of the uncondensed and condensed phenolic moieties in lignins. *J. Agric. Food Chem.* **1995**, *43*, 1538.

Received for review June 14, 2008
Revised manuscript received September 2, 2008
Accepted September 15, 2008

IE800936X