An improved X-ray diffraction method for cellulose crystallinity measurement

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Abstract

We show in this work a modified X-ray diffraction method to determine cellulose crystallinity index (CrI). Nanocrystalline cellulose (NCC) derived from bleached wood pulp was used as a model substrate. Rietveld refinement was applied with consideration of March-Dollase preferred orientation at the (001) plane. In contrast to most previous methods, three distinct amorphous peaks identified from new model samples which are used to calculate CrI. A 2 theta range from 10° to 75° was found to be more suitable to determine CrI and crystallite structural parameters such as d-spacing and crystallite size. This method enables a more reliable measurement of CrI of cellulose and may be applicable to other types of cellulose polymorphs.

Keywords

Crystallinity index, nanocrystalline cellulose, X-ray diffraction, cellulose crystallite, cellulose polymorphs

1. Introduction

The presence of crystallinity in cellulose is one of the most important characteristics contributing to its physical, chemical and mechanical properties (Andersson, Serimaa, Paakkari, Saranpaa & Pesonen, 2003; Ryu, Lee & Tassinari, 1981; Soltes, 1983; Tanahashi, Goto, Horii, Hirai & Higuchi, 1989; Weimer, Hackney & French, 1995). Crystallinity index (CrI) is a parameter commonly used to quantify the amount of crystalline cellulose present in cellulosic materials and has also been applied to interpret changes in cellulose structures after physicochemical and biological treatments (Ai-Zuhair, 2008; Andersson et al., 2003; Cao & Tan, 2005; Chanzy, Marechal & Nishiyama, 1999; Hall, Bansal, Lee, Realff & Bommarius, 2010). Analytical methods to determine CrI include ^{13}C nuclear magnetic resonance (NMR) spectroscopy, Fourier transform (FT)-IR spectroscopy, and X-ray diffraction (XRD). Among these, XRD is the most prevailing method currently employed (Agarwal, Reiner & Ralph, 2010; Bansal, Hall, Realff, Lee & Bommarius, 2010; Deraman, Zakaria & Murshidi, 2001; Evans, Newman, Roick, Suckling & Wallis, 1995; Kljun, Benians, Goubet, Meulewaeter, Knox et al. , 2011; Liitia, Maunu & Hortling, 2000; Liitia, Maunu, Hortling, Tamminen, Pekkala et al. , 2003; Park, Baker, Himmel, Parilla & Johnson, 2010; Teeaar, Serimaa & Paakkari, 1987).

There are three methods commonly applied to calculate the CrI of cellulose based on the XRD data (Bansal et al., 2010; Cao & Tan, 2005; Driemeier & Calligaris, 2011; French & Santiago Cintron, 2013; Park et al., 2010; Rietveld, 1969; Rowe, Mckillop & Bray, 1994; Ruland, 1961; Segal, Creely, Jr & Conrad, 1962; Tanahashi et al., 1989; Thygesen, Oddershede, Lilholt, Thomsen & Stahl, 2005). The first was developed by (Segal et al., 1959), based on the ratio of the height of the (200) peak (I_{200}) and the height of the minimum (I_{AM}) between the (200) and (110) peaks. This is the simplest and most frequently used technique. However, as the exact

amount of crystalline fraction is proportional to the peak area rather than its height, the peak height calculation presents an obvious flaw to accurate measurement of CrI. In addition, a recent study (French & Santiago Cintron, 2013) has shown that the CrI obtained using this method is dependent on crystallite size and cellulose polymorph, for materials with the same fraction of crystalline cellulose. The second method is based on peak deconvolution of crystalline and amorphous peaks. The crystalline cellulose is represented by several intense peaks at (1 $\bar{1}$ 0), (110), (102), (200), and (004) for cellulose I $_{\beta}$. The positions and areas of these peaks may be arbitrarily chosen (Garvey, Parker & Simon, 2005) or calculated from a known crystal structure. The amorphous fraction is typically modeled by a single broad peak and CrI is calculated from the ratio of the area of the crystalline peaks to the total area. The accuracy of this method is challenged by the difficulty in selecting peaks that correctly correspond to the actual diffraction contributed by each fraction. The third common technique is the amorphous subtraction method first described by Ruland (Ruland, 1961) and later modified by Vonk (Vonk, 1973). The method fits an intensity profile of an amorphous component which is scaled so that it remains just below all the observed intensity from the experimental sample pattern. The CrI is determined from the ratio of the area above the amorphous profile to the total area. The amorphous profile is obtained either from a polynomial function or a pattern measured from experimentally prepared material believed to be entirely amorphous (e.g., ball-milled cellulose, regenerated cellulose, xylan or lignin powder) (Bansal et al., 2010; Thygesen et al., 2005).

Detailed review of these methods is given by (Park et al., 2010). These wide angle X-ray scattering (WAXS) based methods are reliable and commonly used for comparative determination of CrI between the cellulosic samples from same sources. Yet there is still a challenge in quantification and comparative analysis of CrI among cellulose samples obtained

from different origins and process methods. The crystallites of natural celluloses are small in size (< 10 nm) compared to typical crystalline solids like metals or ceramics. Because of the small crystallites and the variations in crystalline structures within cellulose chains, XRD analyses of cellulose always result in broad diffraction peaks which are difficult to distinguish from the diffractions of amorphous cellulose and background scattering. This is the main reason behind the long-term-challenge to apply XRD to obtain an exact quantification of cellulose CrI, especially when comparing samples from different origins or sources.

Recognizing the lack of accurate crystallinity measurement method, Driemeier and Calligaris (Driemeier & Calligaris, 2011) have recently developed new protocol to improve CrI measurement by systematically considering preferred fiber orientation, incoherent scattering, moisture, and other compositional deviations. However, this method is based on transmission data from capillary samples which is a relatively uncommon instrument set up. It requires a separate measurement of an empty capillary and uses a complex background which could substitute for amorphous cellulose. The objective of this study is to modify existing XRD procedures to improve the reliability of WAXS for CrI determination using general XRD instrumentation. The Rietveld method was chosen due to its broad applicability to all cellulose polymorphs with an available crystal structure and its consistency in treating all the crystalline peaks. Although we used commercial Rietveld software in this study, the same functionality is widely available in a range of other programs, some of which are available free of charge. The following approaches were taken in this study: 1) select the model substrates which closely represent the nanolevel size cellulose crystallites and amorphous cellulose; 2) determine the diffraction background in the absence of amorphous material; 3) determine a suitable 2θ range for better coverage of amorphous and crystalline diffraction.

2. Material and methods

2.1 Substrate preparation

The brown stock kraft pulp was first prepared from poplar wood chips by cooking with a mixture of NaOH and Na₂S solution at 170°C for 160 minutes. Bleached kraft pulp (BKP) was prepared by subsequent treatment of brown stock kraft pulp using acid sodium chlorite solution at room temperature for 24 h following a procedure previously described (Ju, Engelhard & Zhang, 2013). Nanocrystalline cellulose was prepared by acid extraction of BKP (Hamad & Hu, 2010). In brief, 64.5% sulfuric acid was used to extract nanocrystalline cellulose from BKP, followed by dilution to stop reaction. The mixture was filtered through 3K Dalton RC membrane using AMICON ultrafiltration 8400 system. Nanocrystalline cellulose collected above the membrane after repeated washing and filtering was freeze-dried for further analysis. Microcrystalline cellulose (Avicel PH101) was purchased from Sigma-Aldrich (St Louis, USA). Phosphoric acid swollen amorphous cellulose was prepared using phosphoric acid treating NCC as well as Avicel following procedure as previously described (Zhang, Cui, Lynd & Kuang, 2006).

2.2 XRD

XRD samples were front-loaded into shallow (ca. 0.5 mm) wells machined into zero background holders manufactured from off-axis single crystal quartz. XRD patterns were collected using a Panalytical Bragg-Brentano goniometer with Cu K α radiation, variable divergence and anti-scatter slits (illuminated length = 10 mm), and a post-diffraction monochromator. Data were collected from 10 to either 75 or 140 ° 2 θ (see text), counting for 3 s at 0.05 ° intervals.

Crystallinity index (CrI) based on the “Segal method” was calculated from the height ratio between the intensity of the crystalline peak ($I_{200}-I_{AM}$) and total intensity (I_{200}) after subtraction of the background signal. Nonlinear least squares fitting of the data was carried out using TOPAS (v4.2, Bruker AXS) with the crystalline component calculated from the cellulose I β structure published by Nishiyama et al. (2002). Pseudo-Voigt line shapes were used for the peaks of both crystalline and amorphous (see later) cellulose. A three-parameter 2nd order polynomial function was used for the background and the degree of crystallinity calculated from the area of crystalline cellulose divided by the total area of crystalline + amorphous cellulose. The measured patterns were used without any corrections or other processing; Lorentz-polarization, absorption, and sample displacement corrections were applied to the calculated patterns. The *d-spacing* were calculated using the Bragg’s equation (1) and the crystallite sizes were calculated from the widths of diffraction peaks using the Scherrer equation (2):

$$n\lambda = 2d \sin \theta \quad (1)$$

$$L = 0.9\lambda / (H \cos \theta) \quad (2)$$

where n is an integer; λ is the wavelength of incident wave length; d is the spacing between the planes in the atomic lattice, θ is the angle between the incident ray and the scattering planes; L is the crystallite size perpendicular to the plane and H is the full width at half-maximum (FWHM) in radians.

3. Results and discussion

To attain suitable representative peaks for the crystalline component of cellulose, nanocrystalline cellulose (NCC) prepared from bleached wood pulp (Dong, Revol & Gray, 1998) was used as a model compound in this study. In nature, cellulose does not occur as an individual molecule, it is composed of crystalline and amorphous components. During NCC preparation,

the amorphous regions of cellulose are removed inducing a transverse cleavage of cellulose fibers into rodlike nanoparticles (Habibi, 2014). This transformation consists of disruption of the amorphous regions surrounding the cellulose microfibrils, as well as those embedded between them, while leaving the crystalline segments intact. The NCC fibers are 5-20 nm in width, with length up to 200 nm based on the measurement by both dynamic light scattering particle size analyzer and atomic force microscope (Ju et al., 2014). This dimension closely represents the unit size of 1-4 cellulose crystallites in a cross section (Ju et al., 2013). These NCC particles closely represent highly pure individual cellulose crystallite.

A lack of an appropriate "amorphous" calibration standard is another reason hindering precise measurement of CrI (Fernandes et al., 2011) To generate an amorphous substrate that directly correlates to crystallite counterpart found in a microfibril, phosphoric acid treatment of NCC was applied to produce regenerated amorphous cellulose (RAC). Concentrated phosphoric acid was used in the treatment to dissolve and swell NCC.

Clearly distinguishing between the intensity from the sample and background scattering is critical to obtain an accurate measurement of CrI. Typical X-ray background intensity patterns arise from a variety of coherent and incoherent scattering, including Compton scattering, thermal vibrations of atoms in the sample, and interaction of the X-ray beam with air. Inelastic scattering was not significant in this study, since a monochromator was used to filter out radiation scattered at energies different from the incident radiation.

Figure 1 illustrates the need to separate the background scattering from other contributions to the XRD data of NCC. In this refinement we followed typical procedures reported in the literature (Thygesen et al., 2005) and used a 10-parameter Chebychev polynomial background. This background has a complex shape which could be attributed to amorphous

cellulose, for example the relatively rapid rise near 30-40° 2θ. To see if this was a realistic background, the diffraction pattern of glucose monohydrate was measured under identical conditions (Figure 2). This material has a similar chemical composition to cellulose but is entirely crystalline and thus should reveal the background without any contribution from amorphous material. The result was a smooth background rising from ca. 10 counts/s at 10° 2θ to 150 counts/s at 100° 2θ, which could be modelled satisfactorily with a 2-parameter Chebychev polynomial.

Figure 1. Calculated XRD pattern compared to raw data. Rietveld fit of data collected from NCC cellulose. Contributions from crystalline (green) and amorphous (black) cellulose are added to the background (grey) to give the total calculated pattern (red). Nishiyama cellulose is shown to confirm the cellulose peaks.

The obtained background was then applied to the pattern measured from RAC, with good results (Figure 3). In this figure, it is clear that amorphous cellulose contributes three broad signals to the diffraction pattern, at approximately 20.5, 38.9, and 80.9° 2θ. This is in contrast to previous research which has predominantly used a single amorphous peak centered at 20°

(Bansal et al., 2010; Driemeier & Calligaris, 2011; Park et al., 2010). The other two peaks were not considered in the majority of published studies, presumably because the peaks at higher diffraction angle are broader and have lower maxima than the peak near 20° 2θ . However since the CrI calculation for peak deconvolution methods relies on areas, ignoring these additional peaks from amorphous cellulose would introduce substantial errors. In our work we used all three peaks to model the amorphous fraction, fixing their positions and widths and constraining their intensities to the same ratio as found in the RAC sample.

Figure 2. Diffraction pattern of crystalline glucose with expanded vertical scale to show the shape of the background. The fitted background is shown in blue and matches the shape of the pattern recorded from an empty sample holder (gray, offset for clarity). These patterns are recorded with a variable divergence slit which increases the x-ray flux with in increasing diffraction angle, which will contribute to the background shape.

Figure 3. Experimentally generated amorphous cellulose material shows broad peaks centered at 20.53°, 38.87° and 80.90°.

Diffraction from the crystalline cellulose was calculated from published cellulose I β crystal structures published by Nishiyama et al, 2002 (Nishiyama, Langan & Chanzy, 2002). Since cellulose frequently forms in long fibrils, it is important to account for the preferred orientation that is almost invariably present in diffraction samples. The Rietveld program employed contains two computational options which are also widely available in other Rietveld software: 1) a spherical harmonics approach and 2) the March-Dollase approach (Dollase, 1986). After numerous experimentation, it was found that more reliable results were obtained using a single (001) March-Dollase correction. This approach had the advantage of a single refinable parameter and avoided the negative peak intensities which were occasionally observed from

applying spherical harmonic corrections. A pseudo-Voigt line shape was used to calculate the diffraction peaks.

Having established suitable methods for calculating the background intensity and the crystalline and amorphous peaks, we sought a suitable diffraction range to calculate cellulose CrI. This range should cover the significant areas where amorphous and crystalline components diffract, but be sufficiently small to allow reasonable data collection times. Figure 4 shows the XRD data from the crystalline model substrate, NCC, over the range of 10° to 140° 2θ . This wide range should capture all of the intensity from both the amorphous and crystalline fractions. The CrI was determined from the area of the crystalline portion divided by the total of crystalline and amorphous peak areas.

Figure 4. Rietveld fit of NCC up to 140° with three experimentally defined amorphous peaks (Inset: variation of determined CrI vs. the maximum 2θ angle used for calculation).

In order to see whether a smaller 2θ range gives comparable results, the diffraction profile fit and CrI calculation were repeated as the data were truncated at various diffraction angles. The resulting CrI values are plotted in Figure 4 inset. The estimated fraction of crystalline cellulose remained consistent at approximately 77% when the diffraction range was from 10 to 70-90 $^{\circ}2\theta$. When the diffraction range was restricted at less than 70 $^{\circ}2\theta$, the CrI rose, reaching 84% when the range ended at 40 $^{\circ}2\theta$. This is because crystalline cellulose has its most intense peaks below 30 $^{\circ}2\theta$, whereas amorphous cellulose shows broad features with substantial intensity extending beyond 70 $^{\circ}2\theta$ (see Figures 1 and 2). Unexpectedly, CrI dropped when the diffraction range extended past 100 $^{\circ}2\theta$. There was a broad region of fairly weak intensity near 100 $^{\circ}2\theta$ in the NCC sample which was not observed in the amorphous sample and did not appear in the pattern calculated from the crystal structure (Figure 3). This unknown intensity affected the Rietveld fit for the larger diffraction ranges, and produced unreliable results.

Most of the studies of cellulose CrI examined XRD pattern only to a maximum 2θ of 50 $^{\circ}$, partially because the last major crystalline peaks are centered at 35 $^{\circ}$ (Bansal et al., 2010; Driemeier & Calligaris, 2011; French & Cintron, 2013; Hall et al., 2010). However, our findings illustrated that considerable intensity from amorphous cellulose can extend beyond even 70 $^{\circ}$, making a significant contribution when calculating CrI. Therefore we recommend using a range of 2θ from 10 $^{\circ}$ to 75 $^{\circ}$. This is a practical range that likely covers significant intensities that are

representative of both crystalline and amorphous components of cellulose.

Figure 5. Measured XRD patterns of the cellulose samples, scaled to the same maximum intensity and offset for clarity.

To examine the applicability of this method on different cellulose samples, three cellulose model compounds, bleached kraft pulp (BKP) (Ju et al., 2013) prepared from poplar, microcrystalline cellulose Avicel and nanocrystalline cellulose were analyzed. Figure 5 shows the measured XRD patterns of the model compounds, using the improved refinements. Table 1 summarizes the CrI values for these samples obtained from this method and common Segal peak-height method. It is well recognized that peak height based CrI overestimates the true crystallinity of cellulose I β (Park et al., 2010). As shown in Table 1, lower CrI values were observed for all three tested materials calculated by our peak-fitting method compared to peak height method. . It is noteworthy that the Segal CrI difference between NCC and Avicel is very

small (90% vs 88%), while our method also showed a significant difference in CrI between NCC and Avicel, by 17%. This difference is partly due to the sharp (200) peak of NCC which resulted from the highly ordered crystallite structure of NCC. In Avicel, the peak width was decreased without significantly increasing the I_{200} intensity. The higher peak intensity near 35° in Avicel also contributed to the lower CrI compared to NCC.

Crystallinity index (CrI, %)	NCC	Avicel	BKP
Segal method	90 $\pm$ 1	88 $\pm$ 1	71 $\pm$ 2
This method	77 $\pm$ 3	60 $\pm$ 4	50 $\pm$ 3

Table 1. Crystallinity index (CrI) calculated for model compounds NCC, Avicel and BKP.

	Planes	NCC	Avicel
<i>d</i> -spacing	($\bar{1}10$)	0.593	0.607
	(110)	0.531	0.544
	(200)	0.389	0.399
Average crystallite size (nm)		4.5 $\pm$ 0.1	5.6 $\pm$ 0.1

Table 2. Crystallite structures of model cellulose compounds calculated from our proposed improved method.

Besides CrI, several key parameters associated with crystallite structure such as crystallite size and *d*-spacing between glucan chains in the cellulose crystallite were also determined from this method (Table 2). Avicel has been previously used as a model substrate to estimate cellulose crystallite structural parameters. There is little experimental data on crystallite structure of nanocrystallite cellulose. It is apparent that the *d*-spacings along $\bar{1}10$, 110, 200 planes

are all smaller in NCC than Avicel (Table 2). The average particle size of Avicel is in micrometer scale. It is conceivable that cellulose crystallite cores are entwined with amorphous and paracrystalline cellulose layers in Avicel. d -spacing value from XRD is the average measurement of entire crystalline structure of the bulk material, and it is difficult to differentiate between paracrystalline layers and crystalline cores on XRD. The presence of paracrystalline cellulose, a transition phase between crystalline and amorphous region, is a recognized factor interfering the accurate measurement of CrI and crystallite structure parameters (Park et al., 2010; Nishiyama et al., 2012). A relationship between d -spacing values in (200) planes (d_{200} -spacing) in cellulose I_β and the amount paracrystalline cellulose was investigated. An increased amount paracrystalline cellulose layers will yield larger d_{200} -spacing while a perfect cellulose crystallite has a minimal value perhaps between 0.384 nm - 0.3866 nm (Ioelovich et al., 2010; Nishiyama et al. 2002). The d_{200} -spacing of NCC is clearly smaller than that of Avicel confirming NCC is a substrate more closely representing compact cellulose crystallite. Table 2 also showed the crystallite size obtained from XRD analysis. NCC has a higher CrI with a smaller crystallite size compared to Avicel. This is consistent with the closer d -spacing values and a more compact crystallite structure of NCC. This result also suggests that amorphous and crystalline cellulose regions are not starkly divided, further supporting the presence of paracrystalline cellulose in cellulose macromolecules. A model of cellulose crystallite structure from I_α with 36 glucan chains has been previously illustrated (Lehtio et al., 2010). Based on the data obtained from this study, a similar 36 glucan chains arrangement for cellulose I_β is proposed as shown in Figure 6. Each block presents one glucan chain in the crystallite with c pointing to the axis of glucan chains (French 2014).

Figure 6. Proposed crystallite structure of nanocrystalline cellulose

4. Conclusions

In conclusion, we have shown an improved XRD method for better quantification of cellulose CrI. The method utilizes a simple 3-parameter background model obtained from a representative crystalline sample and extension of the 2θ angle to 75° . This new method uses three distinct amorphous peaks identified from representative amorphous samples to calculate CrI instead of using only one peak representative amorphous peak. This method enables a more reliable measurement of CrI of cellulose I_β as well as d-spacing and crystallite size of cellulose crystallites, offering a useful tool to investigate plant cellulose crystallite structural changes during various biological and chemical treatments. The new background and amorphous peaks treatment procedures developed in this study may also be applied to different cellulose polymorphs to improve the quantification of CrI.

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References

- Agarwal, U.P., Reiner, R.S. & Ralph, S.A. (2010). Cellulose I crystallinity determination using FT-Raman spectroscopy: univariate and multivariate methods. *Cellulose*, 17, 721-733.
- Ai-Zuhair, S. (2008). The effect of crystallinity of cellulose on the rate of reducing sugars production by heterogeneous enzymatic hydrolysis. *Bioresource Technology*, 99, 4078-4085.
- Andersson, S., Serimaa, R., Paakkari, T., Saranpää, P. & Pesonen, E. (2003). Crystallinity of wood and the size of cellulose crystallites in Norway spruce (*Picea abies*). *Journal of Wood Science*, 49, 531-537.
- Bansal, P., Hall, M., Realff, M.J., Lee, J.H. & Bommarius, A.S. (2010). Multivariate statistical analysis of X-ray data from cellulose: A new method to determine degree of crystallinity and predict hydrolysis rates. *Bioresource Technology*, 101, 4461-4471.
- Cao, Y. & Tan, H.M. (2005). Study on crystal structures of enzyme-hydrolyzed cellulosic materials by X-ray diffraction. *Enzyme and Microbial Technology*, 36, 314-317.
- Chanzy, H., Marechal, Y. & Nishiyama, Y. (1999). Toward a better knowledge of the crystalline structure of cellulose. *Abstracts of Papers of the American Chemical Society*, 217, U264-U264.
- Deraman, M., Zakaria, S. & Murshidi, J.A. (2001). Estimation of crystallinity and crystallite size of cellulose in benzylated fibres of oil palm empty fruit bunches by X-ray diffraction. *Japanese Journal of Applied Physics Part 1-Regular Papers Short Notes & Review Papers*, 40, 3311-3314.
- Dollase, W. (1986). Correction of intensities for preferred orientation in powder diffractometry: application of the March model. *Journal of Applied Crystallography*, 19, 267-272.
- Dong, X.M., Revol, J.F. & Gray, D.G. (1998). Effect of microcrystallite preparation conditions on the formation of colloid crystals of cellulose. *Cellulose*, 5, 19-32.
- Driemeier, C. & Calligaris, G.A. (2011). Theoretical and experimental developments for accurate determination of crystallinity of cellulose I materials. *Journal of Applied Crystallography*, 44, 184-192.
- Evans, R., Newman, R.H., Roick, U.C., Suckling, I.D. & Wallis, A.F.A. (1995). Changes in Cellulose Crystallinity during Kraft Pulping - Comparison of Infrared, X-Ray-Diffraction and Solid-State Nmr Results. *Holzforschung*, 49, 498-504.
- French, A.D. & Santiago Cintrón, M. (2013). Cellulose polymorphy, crystallite size, and the Segal Crystallinity Index. *Cellulose*, 20, 583-588.
- French AD (2013) Idealized powder diffraction patterns for cellulose polymorphs. *Cellulose*, 21:885-896.

375 Fernandes A. N., Thomas L. H., Altaner C. M., Callow P., Forsyth V. T., Apperley D. C.,
 376 Kennedy C. J. & Jarvis M. C. (2011) Nanostructure of cellulose microfibrils in spruce wood.
 377 *Proc. Natl Acad. Sci. USA* 108, E1195–E1203.

378 Garvey, C.J., Parker, I.H. & Simon, G.P. (2005). On the interpretation of X-ray diffraction
 379 powder patterns in terms of the nanostructure of cellulose I fibres. *Macromolecular Chemistry*
 380 *and Physics*, 206, 1568-1575.

381 Habibi, Y. (2014). Key advances in the chemical modification of nanocelluloses. *Chemical*
 382 *Society Reviews*, 43, 1519-1542.

383 Hall, M., Bansal, P., Lee, J.H., Realff, M.J. & Bommarius, A.S. (2010). Cellulose crystallinity -
 384 a key predictor of the enzymatic hydrolysis rate. *Febs Journal*, 277, 1571-1582.

385 Hamad, W.Y. & Hu, T.Q. (2010). Structure-Process-Yield Interrelations in Nanocrystalline
 386 Cellulose Extraction. *Canadian Journal of Chemical Engineering*, 88, 392-402.

387 Ioelovich, M., Leykin, A. & Figovsky, O. (2010) Study of cellulose paracrystallinity.
 388 *Bioresources* 5, 1393-1407.

389 Ju, X., Engelhard, M. & Zhang, X. (2013). An advanced understanding of the specific effects of
 390 xylan and surface lignin contents on enzymatic hydrolysis of lignocellulosic biomass.
 391 *Bioresource Technology*, 132, 137-145.

392 Kim, U.J., Eom, S.H. & Wada, M. (2010). Thermal decomposition of native cellulose: Influence
 393 on crystallite size. *Polymer Degradation and Stability*, 95, 778-781.

394 Kljun, A., Benians, T.A.S., Goubet, F., Meulewaeter, F., Knox, J.P. & Blackburn, R.S. (2011).
 395 Comparative Analysis of Crystallinity Changes in Cellulose I Polymers Using ATR-FTIR, X-ray
 396 Diffraction, and Carbohydrate-Binding Module Probes. *Biomacromolecules*, 12, 4121-4126.

397 Liitia, T., Maunu, S.L. & Hortling, B. (2000). Solid state NMR studies on cellulose crystallinity
 398 in fines and bulk fibres separated from refined kraft pulp. *Holzforschung*, 54, 618-624.

399 Liitia, T., Maunu, S.L., Hortling, B., Tamminen, T., Pekkala, O. & Varhimo, A. (2003). Cellulose
 400 crystallinity and ordering of hemicelluloses in pine and birch pulps as revealed by solid-state
 401 NMR spectroscopic methods. *Cellulose*, 10, 307-316.

402 Nishiyama, Y., Langan, P. & Chanzy, H. (2002). Crystal structure and hydrogen-bonding system
 403 in cellulose I beta from synchrotron X-ray and neutron fiber diffraction. *Journal of the American*
 404 *Chemical Society*, 124, 9074-9082.

405 Nishiyama Y, Johnson GP, French A (2012) Diffraction from nonperiodic models of cellulose
 406 crystals. *Cellulose* 19(2):319–336.

407
 408 Park, S., Baker, J.O., Himmel, M.E., Parilla, P.A. & Johnson, D.K. (2010). Cellulose
 409 crystallinity index: measurement techniques and their impact on interpreting cellulase
 410 performance. *Biotechnology for Biofuels*, 3,

- 411 Rietveld, H. (1969). A profile refinement method for nuclear and magnetic structures. *Journal of*
412 *Applied Crystallography*, 2, 65-71.
- 413 Rowe, R.C., Mckillop, A.G. & Bray, D. (1994). The Effect of Batch and Source Variation on the
414 Crystallinity of Microcrystalline Cellulose. *International Journal of Pharmaceutics*, 101, 169-
415 172.
- 416 Ruland, W. (1961). X-ray determination of crystallinity and diffuse disorder scattering. *Acta*
417 *Crystallographica*, 14, 1180-1185.
- 418 Ryu, D., Lee, S.B. & Tassinari, T. (1981). Effect of Crystallinity of Cellulose on Enzymatic-
419 Hydrolysis Kinetics. *Abstracts of Papers of the American Chemical Society*, 182, 58-Micr.
- 420 Segal, L., Creely, J.J., Jr, A.E.M. & Conrad, C.M. (1959). An Empirical Method for Estimating
421 the Degree of Crystallinity of Native Cellulose Using the X-Ray Diffractometer. *Tex Res J*, 29,
422 786-794.
- 423 Soltes, E.J. (1983). Cellulose in the Plant-Cell Wall - Crystallinity and Accessibility Issues.
424 *Abstracts of Papers of the American Chemical Society*, 186, 9-Cell.
- 425 Tanahashi, M., Goto, T., Horii, F., Hirai, A. & Higuchi, T. (1989). Characterization of Steam-
426 Exploded Wood .3. Transformation of Cellulose Crystals and Changes of Crystallinity. *Mokuzai*
427 *Gakkaishi*, 35, 654-662.
- 428 Teeaar, R., Serimaa, R. & Paakkari, T. (1987). Crystallinity of Cellulose, as Determined by
429 Cp/Mas Nmr and Xrd Methods. *Polymer Bulletin*, 17, 231-237.
- 430 Thygesen, A., Oddershede, J., Lilholt, H., Thomsen, A.B. & Stahl, K. (2005). On the
431 determination of crystallinity and cellulose content in plant fibres. *Cellulose*, 12, 563-576.
- 432 Vonk, G.G. (1973). Computerization of Ruland's X-ray Method for Determination of the
433 Crystallinity in Polymers. *Journal of Applied Crystallography*, 6, 148-153.
- 434 Weimer, P.J., Hackney, J.M. & French, A.D. (1995). Effects of Chemical Treatments and
435 Heating on the Crystallinity of Celluloses and Their Implications for Evaluating the Effect of
436 Crystallinity on Cellulose Biodegradation. *Biotechnology and Bioengineering*, 48, 169-178.
- 437 Zhang, Y.H.P., Cui, J.B., Lynd, L.R. & Kuang, L.R. (2006). A transition from cellulose swelling
438 to cellulose dissolution by o-phosphoric acid: Evidence from enzymatic hydrolysis and
439 supramolecular structure. *Biomacromolecules*, 7, 644-648.