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Estimation of Cellulose Crystallinity of Lignocelluloses Using Near-IR FT-Raman Spectroscopy and Comparison of the Raman and Segal-WAXS Methods

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Supporting Information

ABSTRACT: Of the recently developed univariate and multivariate near-IR FT-Raman methods for estimating cellulose crystallinity, the former method was applied to a variety of lignocelluloses: softwoods, hardwoods, wood pulps, and agricultural residues/fibers. The effect of autofluorescence on the crystallinity estimation was minimized by solvent extraction or chemical treatment or both. Additionally, when the roles of lignin and hemicellulose in the Raman crystallinity assessment were investigated, it was found that syringyl lignin containing lignocelluloses generated somewhat higher crystallinity, whereas the presence of hemicellulose reduced the crystallinity. Overall, when autofluorescence was minimized and corrections made for hemicellulose and syringyl lignin contributions, the univariate Raman method performed well and estimated cellulose crystallinity accurately. Moreover, when the Raman and Segal-WAXS methods were compared, we observed that in the absence of significant fluorescence, the Raman method was influenced mostly by hemicellulose and syringyl lignin, whereas the Segal-WAXS was affected by various types of lignin and hemicellulose. It was concluded that the near-IR FT-Raman method with corrections for influences of syringyl lignin and hemicellulose can be used to correctly estimate cellulose crystallinity.

KEYWORDS: *cellulose crystallinity, Raman, WAXS, wood, lignocellulose, lignin*

INTRODUCTION

Measurement of cellulose crystallinity is important for plant biomass materials because crystallinity has an effect on their physical, mechanical, and chemical properties. For example, with increasing crystallinity, tensile strength, dimensional stability, and density increase, whereas properties such as chemical reactivity and swelling decrease. Cellulose crystallinity is also believed to play a significant role when biomass is enzymatically hydrolyzed to bioethanol.[1] Therefore, the crystallinity needs to be measured accurately. Fiber crystallinity is an essential parameter for utilization of agricultural and wood products.

Some frequently used techniques for estimating cellulose crystallinity are wide-angle X-ray scattering (WAXS),[2−4] solid state ^{13}C cross-polarization/magic-angle spinning (CP/MAS), NMR spectroscopy,[5−7] and FT-IR spectroscopy.[8,9] Additionally, recently developed methods include near-IR FT-Raman[10,11] and sum frequency generation (SFG) vibration spectroscopy.[12] However, the presence of noncellulosic components in lignocellulosic biomass causes problems when most of the established methods are used to estimate crystallinity. For instance, in WAXS, scattering contributions of lignin, hemicellulose, and pectins cause extra X-ray scattering and lead to an inaccurate measurement of the cellulose crystallinity.[13] Such contributions affect both the width of the [002] peak of crystalline cellulose and the background under the cellulose diffraction region. The implication in the case of the Segal-WAXS method, for instance, would be that cellulose crystallinity of lignocelluloses will be underestimated compared to pure cellulose samples. There is no simple way to account for such interference/overlap or to remove the contributions of the noncellulosic components.[14] Similarly, in NMR analysis, where signals assigned to C-4 in the cellulose spectrum are used to calculate crystallinity, hemicellulose and lignin contributions in the spectra cause overlapping peaks and the calculation is based upon first separating the total NMR signal into two components, one belonging to cellulose and the other to the noncellulose contributors.[6,7] Moreover, the researchers found that the accuracy of the NMR method depends not only upon how well the spectra can be separated but also upon lateral dimensions of the crystallites.[7] An additional consideration in NMR crystallinity determination has to do with the use of curve-fitting methods that have shortcomings.[15,16] As far as the SFG is concerned, although the selective detection of crystalline cellulose in biomass was demonstrated,[17] the SFG signal intensity was found to vary nonlinearly with crystalline cellulose content in the samples. The latter aspect makes this technique more difficult to use. To deal with some of these problems, the authors developed the Raman methods to more accurately estimate cellulose crystallinity.[11]

Previous work[11] showed that both the univariate and multivariate Raman methods produced good correlations with crystallinities of the calibration-set cellulose samples. Raman crystallinities were correlated with modified Segal-WAXS crystallinities instead of the traditional Segal-WAXS data. The

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Table 1. Sample Identification, Raman Crystallinity, Fluorescence Level, and Change in Crystallinity[a]

1	2	3	4	5	6	7	8
sample no.	sample ID	av crystallinity extracted (X) ± SD (%); fluorescence[b]	crystallinity alkaline H_2O_2 treated (X_1) (%); 1096 − fluorescence	crystallinity acid chlorite treated (X_2) (%); 1096 − fluorescence	change 100 × ($X_1 - X$)/X (%)	change 100 × ($X_2 - X$)/X (%)	crystallinity after correction for hemicellulose contribution (%)
			Softwoods				
1	black spruce	**53.3** ± 0.8; low	56.6; higher	53.5; lower	6.3	0.4	58.5
2	Engelmann spruce	**52.6** ± 1.0; medium	58.3; higher	48.2 lower	10.8	−8.4	57.7
3	jack pine	**50.4** ± 1.9; low	52.6; similar	52.6; similar	4.2	4.3	55.3
4	loblolly pine	**46.6** ± 2.2; low	48.8; similar	48.0; similar	4.7	3.2	51.2
5	lodgepole pine	49.2 ± 3.2; high	54.7; much lower	**49.9**; much lower	11.2	1.5	55.9
6	red cedar	**52.4** ± 0.8; low	56.6; higher	51.7; lower	8.1	−1.3	57.5
7	red pine	**52.3** ± 1.1; low	59.0; higher	55.0; higher	12.9	5.2	57.4
8	Sitka spruce	**46.1** ± 0.9; low	52.6; lower	48.0; lower	14.2	4.3	50.6
9	western hemlock	**53.0** ± 0.8; low	59.4; lower	57.7; lower	12.1	5.2	58.2
			Hardwoods				
10	American elm	57.6 ± 0.5; low	65.9; higher	**50.9**; similar	14.4	−11.6	56.1
11	aspen	48.8 ± 0.7; low	53.1; lower	**45.3**; lower	8.9	−7.1	49.9
12	cottonwood	58.6 ± 0.6; low	64.6; lower	**51.4**; lower	10.3	−12.3	56.7
13	dogwood	51.7 ± 1.4; low	57.6; higher	**42.3**; lower	11.5	−18.1	46.6
14	hickory	55.7 ± 1.4; low	62.7; lower	**42.8**; lower	12.6	−23.1	47.2
15	madrone	62.5 ± 1.2 medium;	62.8; lower	**54.6;** lower	0.4	−12.7	60.2
16	red maple	61.5 ± 1.2; low	67.4; lower	**53.4**; lower	9.6	−13.2	58.9
17	sour orange	49.9 ± 1.7; low	61.1; similar	**50.6**; similar	22.6	1.6	55.8
18	sweetgum	63.0 ± 1.5; low	64.4; similar	**48.7**; similar	2.2	−22.7	53.7
19	white birch	55.2 ± 1.5; low	59.7; lower	**49.5**; lower	8.1	−10.4	54.6
20	willow	55.7 ± 1.3; low	60.7; higher	**51.8**; lower	9.1	−6.9	56.2
21	eucalyptus	67.3 ± 0.8;[c] medium	65.8; lower	**53.1**; lower	−2.3	−21.1	58.5
			Agricultural Residues/Fibers				
22	alfalfa	46.8 ± 1.1; medium	47.9; lower	**44.6**; lower	2.5	−4.6	49.6
23	corn stalk	57.3 ± 0.7; low	53.8; lower	**52.7**; lower	−6.1	−8.0	60.5
24	kenaf bast	56.7 ± 1.2; medium	58.7; lower	**49.7**; lower	3.6	−12.2	55.3
25	kenaf core	59.1 ± 2.5; medium	60.7; lower	**45.2**; lower	2.6	−23.6	49.6
26	oat straw	53.7 ± 1.6; high	51.7; lower	**50.0**; lower	−3.7	−6.8	55.6
27	sugar cane bagasse	51.4 ± 1.7; low	51.6; lower	**44.8**; lower	0.4	−13.0	50.4
28	flax	—;[d] very high	—[d]	**60.5**; lower	NA[e]	NA	63.9
29	ramie	**65.9** ± 1.1; low	69.7; lower	**66.1**; lower	5.7	0.3	66.1
30	banana stem	—;[d] very high	—[d]	**40.7**;[f] lower	NA	NA	45.1
			Wood Pulps				
31	unbleached softwood kraft pulp	83.9;[c] high	62.2; lower	**50.3**; lower	−25.8	−40.0	54.5
32	bleached softwood kraft pulp	**49.1** ± 0.4; low	56.9; similar[g]	55.4; similar[h]	15.9	12.9	53.2
33	bleached hardwood kraft pulp	**50.3** ± 0.4; low	54.0; similar	56.9; lower	7.4	13.2	54.5
34	spruce TMP[i]	54.8 ± 1.4; medium	62.9; higher	**54.2**; lower	14.7	−1.1	59.5

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Table 1. continued

1	2	3	4	5	6	7	8
sample no.	sample ID	av crystallinity extracted (X) ± SD (%); fluorescence[b]	crystallinity alkaline H_2O_2 treated (X_1) (%); 1096 − fluorescence	crystallinity acid chlorite treated (X_2) (%); 1096 − fluorescence	change 100 × $(X_1 - X)/X$ (%)	change 100 × $(X_2 - X)/X$ (%)	crystallinity after correction for hemicellulose contribution (%)
			Wood Pulps				
35	hemlock CTMP[j]	59.1 ± 0.6; low	58.1; lower	**48.5**;[f] lower	−1.6	−18.0	53.2
			Compression versus Opposite Woods				
36	red pine compression wood	**50.9** ± 2.2; low	53.8; higher	52.9; similar	5.7	3.9	55.9
37	red pine opposite wood	**50.1** ± 2.2; low	50.9; higher	53.7; lower	1.5	7.1	55.0
			Heartwood versus Sapwood				
38	white oak heartwood	63.2 ± 3.3; low	62.6; higher	**54.0**; lower	−0.9	−14.6	59.5
39	white oak sapwood	56.7 ± 1.5; low	59.8; similar	**53.3**; lower	5.4	−6.1	58.8
40	Pacific yew heartwood	47.8 ± 0.8; low	54.3; higher	**51.7**; lower	13.6	8.1	56.8
41	Pacific yew sapwood	51.7 ± 0.8; low	53.3; higher	**52.6**; lower	3.0	1.8	57.7

[a]The best crystallinity values are in bold. [b]Fluorescence level low, medium, high, and very high in the spectrum of the extracted sample. How the spectral fluorescence changed upon treatment is designated as lower, similar, or higher. [c]Crystallinity high due to significant level of fluorescence in the spectrum. [d]Raman spectrum of poor quality to calculate crystallinity. [e]Not applicable. [f]Treated with $NaBH_4$ after partial delignification to reduce fluorescence. [g]But has higher fluorescence at 380 cm^{-1}. [h]But has lower fluorescence at 380 cm^{-1}. [i]Thermomechanical pulp. [j]Chemithermomechanical pulp.

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two measurements differ because, in the former case, the contribution of amorphous cellulose was measured at $2\theta = 21°$ and subtracted from the [002] peak intensity.[11] In traditional Segal-WAXS, on the other hand, the amorphous contribution is measured at 2θ of 18°.[2] The reasoning behind choosing modified Segal-WAXS for the correlation was that the subtraction at 21° was more appropriate because that is where the peak position of amorphous cellulose scattering actually exists. As expected, this approach resulted in significantly lower values of cellulose crystallinity compared to Segal-WAXS. Nevertheless, Raman crystallinity values were reliable in the entire crystallinity range (0−90%), as was evident from estimated crystallinities of milled Whatman CC31 samples, Whatman CC41, Whatman 541, CF-11, and Avicel-PH-101.[11]

The objective of the present study was to use the univariate Raman method to determine cellulose crystallinity of lignin- or hemicellulose-containing materials, or both. Forty-one samples of various lignocelluloses that were fairly heterogeneous in composition were selected. The materials consisted of softwoods, hardwoods, wood pulps, and agricultural residue/fibers.

MATERIALS AND METHODS

Materials. Biomaterials investigated in the present project are listed in Table 1. The 41 samples are classified in groups of softwoods (9 samples), hardwoods (12 samples), agricultural residues/fibers (9 samples), wood pulps (5 samples), compression versus opposite woods (2 samples), and heartwood versus sapwoods (4 samples). All of these materials were either available in our laboratory or were obtained from other researchers at the Forest Products Laboratory. The dried materials were Wiley milled and sieved to pass a 1 mm (1000 μm) screen. The 1000 μm fraction was used in analyses. Crystallinities of Wiley-milled materials were measured in three states: after overnight extraction with acetone/H_2O (9:1), after alkaline peroxide bleaching (lignin retaining bleaching), and after significant delignification with acid chlorite. However, the acetone/water extracted sample of lodgepole pine (sample 5) was additionally extracted with toluene/ethanol (2:1) for 2 h to reduce the level of fluorescence further.

For the calibration set of samples, the set of eight samples used was as described in Agarwal et al.[11] These were produced using various mass ratios of control Whatman CC31 and 120 min ball-milled Whatman CC31 celluloses. Additionally, to study the effects of lignin and hemicellulose on the estimations of cellulose crystallinity by WAXS or Raman or both, samples with various mixture compositions of cellulose, lignin, and xylan were prepared. Cellulose was Whatman CC31 as used previously[11] and was purchased from Whatman International Ltd. (Maidstone, UK). Organosolv lignin (Sigma-Aldrich, Milwaukee, WI, USA) and xylan from birch wood (Sigma, St. Louis, MO, USA) were used to represent lignin and hemicellulose, respectively. Other chemicals used were from Sigma-Aldrich unless stated otherwise. Cellulose/xylan mixtures for Raman crystallinity measurements were made using $NaBH_4$ bleached xylan. This was done to reduce the fluorescence signal from the xylan.

Alkaline Peroxide Bleaching. Two grams of the extracted sample was placed in double ziplock bags and bleached as follows. First, 8.6 mL of 0.4% $MgSO_4$ solution was transferred to a test tube. Next, 2.5 mL of 4% H_2O_2 solution was added to the test tube, mixed well, and then added to the ziplock bag. This was followed by adding 8.6 mL of 8% Na_2SiO_3 solution to the bag. The bag was subsequently kneaded to disperse the solution in the sample. The bag was put into a 70 °C water bath for 90 min but was removed once at 45 min to mix the contents. The samples were then filtered (Buchner funnel) and washed first with water and then with dilute HCl until slightly acidic. Samples were dried overnight in a vacuum oven at 50 °C.

Acid Chlorite Delignification. Acid chlorite treatment of the lignin-containing samples was carried out by using the recommended procedure.[18] At about 70 °C, glacial acetic acid and sodium chlorite were added to the water-suspended samples, and chemicals was added three times over 7 h. The extent of delignification achieved was measured by the Klason method.[19] Data indicated that all samples were extensively delignified (Klason lignin 1−5%).

Sodium Borohydride Treatment. For some lignocelluloses for which fluorescence of the delignified samples was still high, samples were additionally treated for 2 h at room temperature, with 0.5 M $NaBH_4$. The treatment was found to be effective in reducing background in the spectra. Although exact natures of the fluorescing species are not known, carbonyl groups in lignocelluloses are likely to be reduced upon $NaBH_4$ treatment. Post $NaBH_4$ treatment, samples were thoroughly washed with water and then with dilute HCl until slightly acidic followed by additional water washes. Similarly, birch xylan was bleached with $NaBH_4$ prior to making mixture pellets for Raman analysis.

Chemical Composition Analysis. Selected lignocellulose samples were analyzed chemically to quantitate the amounts of Klason lignin[19] and carbohydrates.[20] Reproducibility for Klason lignin was 0.4%, and for the carbohydrate analysis, the standard deviation was <1%.[20]

Near-IR FT-Raman. All samples were analyzed with a MultiRam spectrometer (Bruker Instruments Inc., Billerica, MA, USA). This Raman system is equipped with a 1064 nm 1000 mW CW diode pumped Nd:YAG laser. Approximately 0.1−0.2 g of each sample was pressed into a pellet with a hydraulic press. The laser power used for sample excitation was between 300 and 600 mW, and 1024 scans were accumulated. Bruker's OPUS software program was used to find peak positions and process the data. Processing of the spectra included, among other things, selecting a specific region, baseline correction, and normalization.

X-ray. Wide-angle X-ray diffraction profiles were recorded on a Bruker X-ray diffractometer with a Hi-Star 2-D area detector at the Materials Research Science and Engineering Center, University of Wisconsin, Madison. For most samples, diffractograms were obtained on the same sample pellets that were analyzed in FT−Raman.

Crystallinity Calculation. *Raman.* Using the previously established correlation (eq 1), which was based on the Bruker RFS-100 instrument, the univariate Raman crystallinities of the samples (Table 1) were estimated. Using OPUS, the sample spectrum was cut in the 250−1850 cm^{-1} region, baseline corrected (rubber band correction, 64 points), and normalized (min−max) to the 897 cm^{-1} band intensity of the completely amorphous cellulose sample.[11] From the normalized sample spectrum, the 250−700 cm^{-1} amorphous cellulose spectrum was subtracted. This is illustrated in Figure 1 for black spruce wood. From the subtracted spectrum of the sample, peak intensities for the 380 and 1096 cm^{-1} bands were measured using the peak intensity relative to the baseline (between 358 and 396 cm^{-1}) and peak intensity relative to the horizontal baseline (from 944 cm^{-1}) methods,

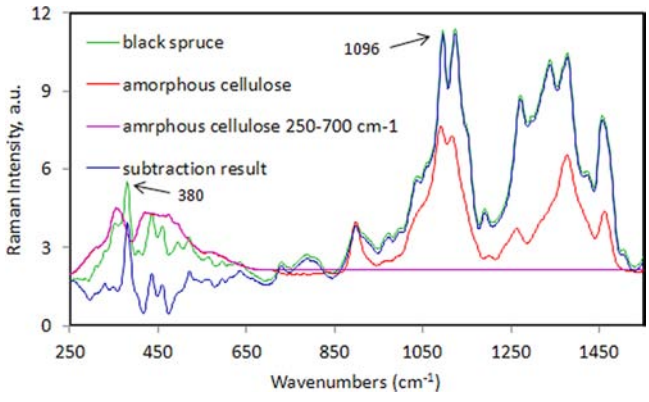

Figure 1. Normalized Raman spectra of black spruce and amorphous cellulose, region 250−1550 cm^{-1}; amorphous cellulose spectrum, region 250−700 cm^{-1} and subtraction spectrum (normalized black spruce minus "amorphous cellulose 250−700 cm^{-1}").

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Table 2. Chemical Composition Analysis of Selected Samples

1	2	3	4	5
sample no.	sample ID	Klason lignin (%)	glucan[a] (%)	hemicellulose[b] (%)
	Softwoods			
1	black spruce, extracted[c]	26.5	41.8	17.0
	black spruce, bleached[d]	24.3	43.3	17.3
	black spruce, delignified[e]	1.6	57.0	20.1
5	lodgepole pine	26.9	42.7	21.5
	lodgepole pine, bleached	26.5	44.0	19.0
	lodgepole pine, delignified	1.3	52.5	21.1
	Hardwoods			
11	aspen, extracted	18.7	46.9	18.0
	aspen, bleached	16.8	49.5	17.1
	aspen, delignified	3.1	52.3	18.4
17	sour orange, extracted	20.0	40.4	17.8
	sour orange, bleached	19.3	41.1	17.7
	sour orange, delignified	4.1	45.7	19.0
20	willow, extracted	24.4	40.6	14.2
	willow, bleached	23.4	42.2	15.0
	willow, delignified	4.3	40.4	16.6
	Agricultural Residues/Fibers			
25	kenaf core, extracted	19.9	39.0	17.3
	kenaf core, bleached	20.1	44.0	17.7
	kenaf core, delignified	3.3	44.6	19.5
26	oat straw	18.4	42.9	19.6
	oat straw, bleached	14.8	48.0	20.1
	oat straw, delignified	2.1	56.9	21.2
29	ramie, extracted	1.4	83.9	ND
	ramie, bleached	1.0	81.7	ND
	ramie, delignified	1.0	78.8	ND
30	banana stem, extracted	14.2	41.4	16.4
	banana stem, bleached	12.8	43.7	17.6
	banana stem, delignified	2.8	47.4	18.7
	Wood Pulps			
31	unbleached softwood kraft pulp, extracted	4.4	75.7	12.7
	unbleached softwood kraft pulp, bleached	4.3	78.6	13.3
	unbleached softwood kraft pulp, delignified	0.7	78.8	14.1
32	bleached softwood kraft pulp, extracted	0.4	77.2	14.1
	bleached softwood kraft pulp, bleached	0.5	81.3	13.0
	bleached softwood kraft pulp, delignified	0.6	78.1	11.2

[a]Can be taken as representing % cellulose. [b]Hemicellulose is the sum of xylan, mannan, arabinan, and galactanan. ND, not detected. [c]Extracted by acetone/water (9:1). [d]Bleached by alkaline H_2O_2. [e]Delignified by acid chlorite.

respectively. Intensity ratio I_{380}/I_{1096} was determined, and Raman crystallinity was calculated using eq 1.[11] However, because another spectrometer, MultiRam, was used in the present study, the issue of response variation between the two Raman instruments (RFS-100 and MultiRam) needed to be addressed. Difference in relative intensities I_{380}/I_{1096} were observed when the same set of calibration samples[11] were analyzed by the two instruments. The I_{380}/I_{1096} ratios for MultiRam were lower compared to those obtained by RFS-100.[11] Therefore, to adjust for this change in response, the RFS-100 equivalent crystallinity values ($X_{RFS\text{-}100}$) were obtained by using a correction factor (eq 2). The correction relationship was generated by correlating MultiRam and RFS-100 calibration set Raman crystallinities. The Raman crystallinity estimation equation (based on RFS-100) and the interinstrument (RFS-100 vs MultiRam) calibration correction were as follows.

$$X_{\text{MultiRam}} = ((I_{380}/I_{1096}) - 0.0286)/0.0065 \quad (1)$$

$$X_{\text{RFS}-100} = (X_{\text{MultiRam}} + 2.0212)/0.8222 \quad (2)$$

Additionally, sample spectra were corrected for change in response of the MultiRam optics over time. Using white light in the sample compartment, the "reference correction" was performed on each sample spectrum. Reference correction was needed because correlation between RFS-100 and MultiRam was developed at an earlier date (2010), and between 2010 and 2012 the response of the MultiRam optics changed slightly.

Standard deviation (SD) listed in Table 1 is based on six spectra from each sample that were obtained from three different pellets. Two spectra per pellet were acquired by changing the side that was exposed to the laser. However, for the cellulose/xylan mixtures, the reported SD is based on triplicate sampling from two sample pellets.

WAXS. For each of the samples, WAXS crystallinities were calculated by subtracting the amorphous contribution approximately at $2\theta = 18°$ [$(I_{22.5} - I_{18})/I_{22.5}$; called 18-Segal-WAXS] (Table 1). This was done after a horizontal line was drawn between the 2θ values of 10° and 40°. This method is also known as the [002] peak height ratio method. The 18-Segal-WAXS crystallinity is the same as Segal crystallinity in the literature.[2,14] However, in the interest of clarity, the former designation is favored.

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RESULTS AND DISCUSSION

Univariate Raman Crystallinities. For the extracted lignocelluloses, crystallinities obtained using the univariate-Raman method are reported in Table 1. Average values are listed along with the SD. The SD varied between 0.4 and 3.3% and seems to be material dependent. Previously, using calibration set cellulose samples, the Raman method has been correlated with the WAXS crystallinities that were obtained by subtracting out the amorphous contribution at $2\theta = 21°$.[11]

Extracted Samples. In Table 1, except for flax and banana stem (sample 28 and 30, respectively), Raman crystallinities are reported for all other extracted samples. Because of the overwhelming fluorescence signal, no Raman peaks could be detected in the spectra of extracted flax and banana stem. Reasons for poor quality of the spectra are not clear but are likely to be due to the presence of highly fluorescent extraneous constituents. Moreover, the crystallinities of a few lignocellulosic samples, for example, eucalyptus (sample 21) and unbleached kraft pulp (sample 31), were significantly higher compared to the rest of the lignocellulose samples in their class. This is likely to be due to the presence of significant medium to high fluorescence in the spectra of these samples (Table 1). For lignocelluloses, we have observed that spectral fluorescence reduces intensities of the bands at 1096 and 380 cm^{-1} and relative suppression depends upon the shape of the fluorescence curve. In such cases, based on eq 1, crystallinity estimation is likely to be inaccurate. The spectra of most other samples had a significantly lower level of fluorescence (Table 1). Generally speaking, spectra with the lowest level of fluorescence background should be used in estimating Raman crystallinity. This was the reasoning behind preparing samples with reduced concentrations of chromophores (alkaline H_2O_2 bleached) and significantly reduced lignin (acid chlorite delignified).

Role of Fluorescence and Lignin Removal. (a) Softwoods. To minimize the fluorescence background in the spectra, extracted samples were bleached with alkaline H_2O_2. It is well-known that such a treatment primarily removes chromophores and that the concentration of lignin or hemicellulose is not affected greatly.[21] Chemical composition data of selected samples further supported this information (Table 2). Nevertheless, post alkaline H_2O_2 treatment, fluorescence background was not necessarily lower for all bleached samples (Table 1, column 4). In Table 1, the terms higher, lower, or similar are used to qualitatively describe how the 2900 cm^{-1} band normalized spectral fluorescence changed (in the 1096 cm^{-1} region) compared to its level in the extracted sample. Therefore, for a biomaterial, these terms are the relative measure of fluorescence change in the spectra within the set of three samples (extracted, alkaline H_2O_2, and acid chlorite treated). For instance, in black spruce (sample 1) compared to extracted, alkaline hydrogen peroxide-treated sample produced higher background in the spectrum (Figure 2). This affected intensities of the 380 and 1096 cm^{-1} bands, and crystallinity for the bleached sample went up by 6.3% (Table 1, column 6). Changes in autofluorescence upon treatment with acid chlorite are similarly designated in Table 1 (column 5). The effect of peroxide and acid chlorite treatments on crystallinity estimation is listed Table 1 (percentage changes in columns 6 and 7, respectively). On the basis of the crystallinity data of peroxide bleached samples where most lignin was retained, changes in crystallinities are the net result of how changes in the

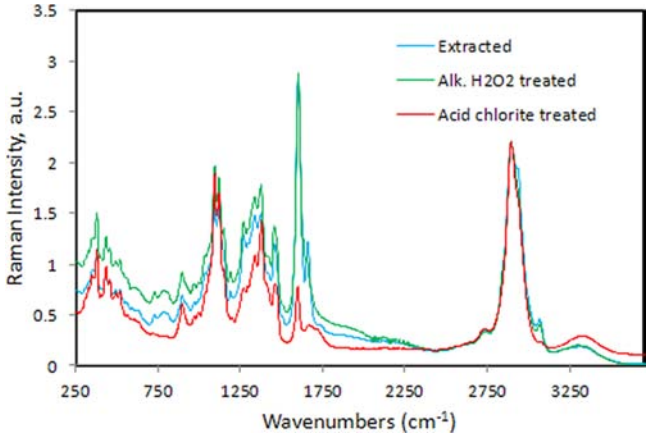

Figure 2. Near-IR FT-Raman spectra of extracted, alkaline H_2O_2 treated, and acid chlorite delignified black spruce.

fluorescence, in the 380 and 1096 cm^{-1} regions, affected Raman intensities. For softwoods (samples 1–9, Table 1), upon bleaching and delignification the average crystallinity increases were 9.4 and 1.6%, respectively. However, considering that acid chlorite treatment affected not only the fluorescence level but also lignin content (Table 2), its effect on crystallinity needed a more detailed explanation.

From earlier FT-Raman studies of softwood lignin[22,23] it is known that guaiacyl lignin does not have significant contribution at either of the band positions (380 and 1096 cm^{-1}) used in the crystallinity calculation (eq 1) and, therefore, lignin removal is not expected to change Raman crystallinity of softwoods. Near-IR FT-Raman spectra of milled-wood lignins are shown in Figure 3. This observation is further supported by the data of the softwood samples, which indicated that crystallinity did not change significantly upon lignin removal (Table 1, column 7). For example, for jack and loblolly pine (samples 3 and 4, respectively), where spectra of extracted, alkaline H_2O_2 bleached, and delignified samples contained similar levels of autofluorescence, the lignin removal has no effect on Raman crystallinities. Therefore, it can be concluded that as long as fluorescence levels are low, the lignin removal has no effect on Raman crystallinity of softwoods. In Table 1, the best crystallinity values are in bold, and most of them belonged to extracted samples that were not treated chemically. This was mostly because such softwood samples produced low levels of fluorescence.

Of the other softwoods listed in Table 1, Raman crystallinities were similar between compression wood and opposite wood (in conifers, wood formed opposite to compression wood) red pine (samples 36 and 37, respectively). Similarly, no significant crystallinity difference existed between Pacific yew heartwood and sapwood (samples 40 and 41, respectively). This suggested that in softwoods, cellulose crystallinity is similar between compression and opposite wood and between heartwood and sapwood. Red pine results are contrary to a published study on this topic[24] in which using WAXS, compared to the opposite and normal woods, compression wood was found to have lower crystallinity. Literature-reported crystallinities were on softwoods different from those we studied, and the presence of lignin lowers the crystallinity estimation by the X-ray technique. Furthermore, compression wood has higher lignin compared to normal wood; therefore, it is likely that the X-ray crystallinity of

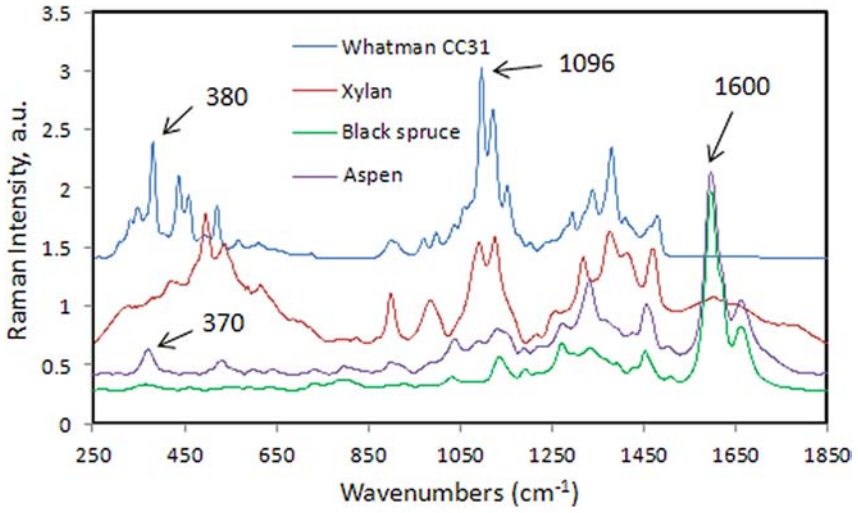

Figure 3. Near-IR FT-Raman spectra of Whatman CC31 cellulose, xylan, and aspen and black spruce milled-wood lignins.

compression wood would be lower. Nevertheless, our findings were supported by another investigation using ^{13}C NMR spectroscopy in which it was shown that for radiata pine crystallinity was homogeneous between normal and compression woods.[25] In any case, more research is needed to further investigate this topic.

(b) Hardwoods. Background fluorescence in the spectra of extracted samples varied depending upon the hardwood species and chemical treatment of a sample (Table 1). The most reliable values were obtained for delignified samples. This was due to both the reduced fluorescence background and the removal of a Raman contribution at 370 cm^{-1}. The band arises from syringyl lignin (Figure 3).[23] Its removal upon acid chlorite treatment is shown in Figure 4 for eucalyptus (sample 21)

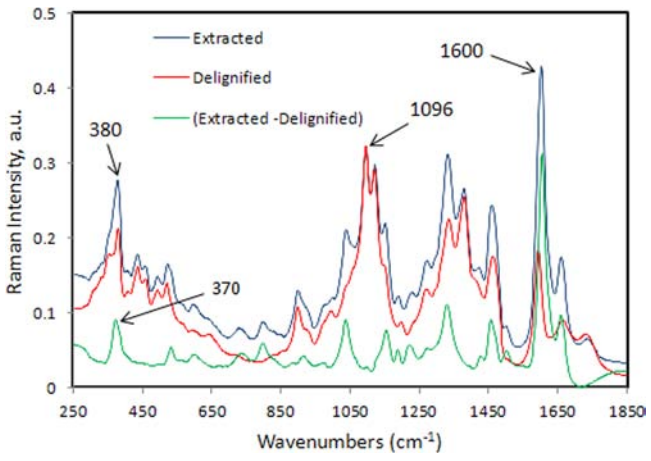

Figure 4. Near-IR FT-Raman spectra of extracted and delignified eucalyptus wood and the result of the spectral subtraction (extracted − delignified). Subtracted spectrum was shifted on intensity scale.

(Table 1). Whereas upon alkaline H_2O_2 bleaching the contribution at 370 cm^{-1} was retained (not shown in Figure 4), this contribution was absent in the spectrum of the delignified wood sample. When delignification-related percentage of change in crystallinity is compared between softwoods and hardwoods, we observed that the hardwoods showed a significantly higher decline (Table 1, column 7). This is because of the removal of the contribution at 370 cm^{-1} in the hardwoods (Figure 3). On the other hand, no such difference existed between the alkaline peroxide bleached softwoods and hardwoods (Table 1, column 6) due to the fact that in the spectra of the latter the 370 cm^{-1} band intensity was retained. As a group, on average, hardwood crystallinity increased by 10% upon bleaching and declined by 12.4% (Table 1) upon delignification.

Heartwood and sapwood of white oak (sample 38 and 39, respectively) are two additional hardwoods listed in Table 1. Samples were selected to determine if there was a difference between cellulose crystallinity of the heartwood and sapwood. As was the case with softwoods, crystallinity values were similar: 54.0 and 53.3% for heartwood and sapwood, respectively (Table 1, column 5).

(c) Agricultural Residues/Fibers. Crystallinities of most of the agricultural residues/fibers showed lower values when samples were treated with acid chlorite. This is attributed to removal of syringyl lignin. Therefore, analogous to the hardwoods, accepted values of crystallinities for most of the agricultural residues/fibers (in bold in Table 1, column 5) were obtained after contributions of both fluorescence and the 370 cm^{-1} band were minimized. In this class of samples, ramie fiber (sample 29) is an exception because it consisted of mostly cellulose (83.9% cellulose, 1.4% lignin, Table 2).

(d) Wood Pulps. Wood pulps are the remaining samples in Table 1. Upon bleaching, crystallinity of unbleached kraft pulp (sample 31) declined by 26% largely because of removal of chromophore-generated fluorescence. These chromophores are highly fluorescent even in the near-IR Raman spectroscopy (1064 nm excitation). An even more drastic reduction of 40% in Raman crystallinity is noted in the delignified sample (Table 1, column 7). This is explained by removal of choromophore-enriched residual lignin upon acid chlorite treatment (Table 2).

Crystallinities of bleached softwood and hardwood pulps (samples 32 and 33, respectively, in Table 1) went up significantly (7−16%) upon bleaching and acid chlorite treatments. Considering that concentration of hemicellulose was not altered significantly (Table 2), this is likely to be due to removal of fluorescence from the 380 cm^{-1} region. This leads to higher peak intensity at 380 cm^{-1} and slightly higher crystallinity. A similar outcome is expected for other samples provided that there is no corresponding increase in the peak height at 1096 cm^{-1}. Finally, when chemically treated, the two

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Table 3. Univariate Raman, Segal-WAXS, and Predicted Segal-WAXS Crystallinities

1	2	3	4	5	6	7	8
sample no.	corrected univariate Raman crystallinity, Y (%)	18-Segal-WAXS Y_1 (%)	predicted 18-Segal-WAXS Y_2 (%)	18-Segal-WAXS partially delignified Y_3 (%)	(predicted 18-Segal-WAXS − Raman) = 100 × $(Y_2 - Y)/Y$ (%)	(predicted 18-Segal-WAXS − 18-Segal-WAXS) = 100 × $(Y_2 - Y_1)/Y_1$ (%)	(delignified − control) = 100 × $(Y_3 - Y_1)/Y_1$ (%)
				Softwoods			
1	58.5	56.5	86.3	70.8	47.4	52.7	25.3
2	57.7	56.9	85.6	67.5	48.3	50.5	18.6
3	55.3	55.5	83.6	70.5	51.0	50.5	27.0
4	51.2	52.2	80.0	69.9	56.4	53.2	33.9
5	55.9	62.6	84.1	71.5	50.3	34.3	14.2
6	57.5	52.2	85.4	65.7	48.5	63.7	25.9
7	57.4	58.3	85.3	71.8	48.6	46.4	23.2
8	50.6	56.5	79.5	69.8	57.1	40.8	23.5
9	58.2	55.5	86.0	69.7	47.8	54.9	25.6
				Hardwoods			
10	56.1	58.6	84.2	66.2	50.1	43.7	13.0
11	49.9	60.8	79.0	67.4	58.1	29.9	10.9
12	56.7	61.4	84.7	68.6	49.5	37.9	11.7
13	46.6	59.9	76.1	66.0	63.2	27.1	10.2
14	47.2	60.6	76.6	65.8	62.3	26.4	8.6
15	60.2	62.2	87.7	64.1	45.7	41.0	3.1
16	58.9	58.1	86.6	68.0	47.0	49.0	17.0
17	55.8	65.4	83.9	62.2	50.5	53.5	9.7
18	53.7	58.8	82.2	64.0	53.0	39.7	8.8
19	54.6	55.1	82.9	65.4	51.9	50.5	18.7
20	56.2	61.7	84.3	68.5	50.0	36.6	11.0
21	58.5	56.6	86.3	65.5	47.4	52.5	15.7
				Agricultural Residues/Fibers			
22	49.6	56.7	78.6	59.7	58.6	38.7	5.3
23	60.5	59.3	88.0	65.8	45.4	48.4	11.0
24	55.3	65.7	83.5	70.2	51.1	27.1	6.8
25	49.6	56.3	78.7	60.9	58.6	39.8	8.2
26	55.6	66.1	83.8	69.3	50.7	26.7	4.8
27	50.4	51.1	79.4	58.4	57.4	55.3	14.3
28	63.9	78	90.9	83.4	42.2	16.5	6.9
29	66.1	86.6	78.6	88.6	40.3	7.1	2.3
30	45.1	44.5	88.0	60.1	66.0	68.1	35.1
				Wood Pulps			
31	54.5	76.5	92.7	77.9	52.0	8.3	1.8
32	53.2	70	74.8	79.6	53.6	16.8	13.7
33	54.5	70.4	82.9	79.5	52.0	17.7	12.9
34	59.5	64.2	81.8	67.5	46.4	35.7	5.1
35	53.2	62.6	82.9	63.5	53.6	30.6	7.2
				Compression versus Opposite Woods			
36	55.9	60.7	84.0	63.5	50.4	38.4	4.6
37	55.0	58.4	83.3	66.0	51.4	42.6	13.0
38	59.5	54.6	87.1	62.6	46.4	59.6	14.7
39	58.8	54	86.5	62.5	47.2	60.1	15.7
40	56.8	55.7	84.8	65.3	49.3	52.2	17.2
41	57.7	54.7	85.6	65.8	48.3	56.5	20.3

mechanical wood pulps, spruce TMP (sample 34) and hemlock CTMP (sample 35), behaved like softwoods; however, significant crystallinity decline was observed only for delignified hemlock CTMP (Table 1).

Because even in near-IR FT-Raman spectroscopy most isolated lignins are fluorescent, the role of lignin in Raman crystallinity was investigated only by removing it from the samples. Prepared cellulose/lignin composite samples were used to study the effect of lignin on Segal-WAXS crystallinity. On the basis of the discussion above, there are two ways in which the presence of lignin affects Raman crystallinity. First, for a large number of lignocelluloses, lignin-generated autofluorescence is detected in the spectrum, which alters Raman intensities at 1098 and 380 cm^{-1}, the two bands directly involved in crystallinity calculation (eq 1). Second, in the case of the syringyl lignin-containing materials, contribution at 380 cm^{-1} from the neighboring band at 370 cm^{-1} leads to somewhat higher estimation of the crystallinity. Both of these roles of lignin are important and need to be considered on the specific material basis.

Role of Hemicellulose. Hemicelluloses are plant cell wall polysaccharides and have a large number of Raman band

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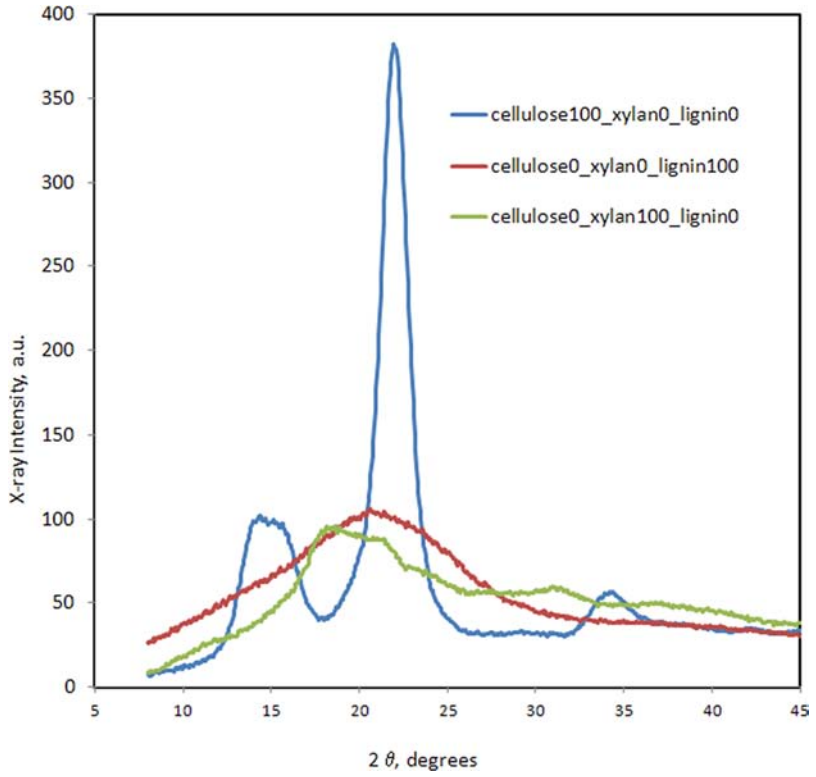

Figure 5. WAXS diffractograms of Whatman CC31 cellulose, organosolv lignin, and xylan.

positions that are similar to that of cellulose.[22] However, compared to cellulose, their Raman scattering is weak because they occur in the noncrystalline form. Some other aspects of the hemicelluloses are that they are difficult to completely remove from most lignocelluloses (alkali extraction yields only limited isolation) and, unlike lignin, are much less fluorescent in Raman spectroscopy. To study their role in Raman crystallinity estimation, xylan (a polysaccharide) was chosen to represent hemicelluloses, and several cellulose/xylan mixture powders were prepared. These mixtures had xylan content up to 50%. The reasoning behind choosing the upper xylan content limit of 50% was that in most lignocelluloses hemicellulose is present at significantly lower concentrations (for example, most woods are composed of <30% hemicelluloses (Table 2).[26] Results of the experiment on xylan mixture compositions showed that in the presence of xylan, Raman crystallinity of the mixture was reduced significantly and reduction was linearly related to concentration of xylan (R^2 = 0.97). The significant effect on crystallinity because of the presence of hemicellulose/xylan seems to arise from the scattering at 1096 cm^{-1} because xylan contribution at 380 cm^{-1} is virtually nonexistent (Figure 3).

On the basis of this finding, sample Raman crystallinities were corrected for hemicellulose contribution using the correlation developed. Hemicellulose content of the samples was taken from Table 2 and from the literature.[26−31] Corrected values are listed in Table 1 (column 8). These data are believed to be the most accurate measures of cellulose crystallinity using Raman spectroscopy because the originally estimated crystallinities are now corrected for both the syringyl lignin contribution, needed for hardwoods and most agricultural residues/fibers, and the effect of hemicellulose. A number of observations can be made from these data. In general, Raman crystallinities of softwoods and hardwoods are similar and were mostly in the range of 50−60%. This is also true for most materials classified in the categories of agricultural materials and wood pulps. The only exceptions are the samples of flax (sample 28), ramie (sample 29), and banana stem (sample 30). Crystallinities of flax (sample 28) and ramie (sample 29) were somewhat higher (63.9 and 66.1%, respectively) (Table 1, column 8), and that of banana stem (sample 30) was slightly lower (45.1%). Another observation for some specific syringyl lignin-containing materials is that the increment in crystallinity by the lignin unit can be fully offset by hemicellulose-related decline (Table 1; samples 10−12, 15, 16, 19, 20, 23, 24, 26, 27, 38, and 39). Although further investigation is required, it is possible that the behavior is related to the syringyl-to-guaiacyl ratio of the material.

Comparison to 18-Segal-WAXS. Before univariate Raman can be directly compared to 18-Segal-WAXS, Raman crystallinities need to be converted to the Segal-equivalent values. This is because Raman crystallinity is correlated with the 21-WAXS data (WAXS crystallinity calculated after subtracting amorphous contribution at 2θ = 21°) and not with 18-Segal-WAXS.[11] However, 18-Segal-WAXS equivalent (predicted 18-Segal-WAXS; Y_2 in Table 3) values can be easily estimated on the basis of the correlation between calibration set Raman and 18-Segal-WAXS crystallinities. Correlation was developed (R^2 = 0.925), and predicted 18-Segal-WAXS values for samples in Table 1 are reported in Table 3 (column 4). It is worth noting that the 18-Segal-WAXS crystallinity prediction accuracy of the correlation is 92.5% and, therefore, there is some error in the predicted data. Besides other data, Table 3 lists values of best Raman crystallinity (Y), 18-Segal-WAXS (Y_1), predicted 18-Segal-WAXS (Y_2), and differences between crystallinities [(predicted 18-Segal-WAXS − Raman) and (predicted 18-Segal-WAXS − 18-Segal-WAXS)].

For most samples, compared to Raman (Table 3, column 2) predicted 18-Segal-WAXS crystallinity data were 40−66%

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higher (Table 3, column 6). This is to be expected on the basis of the correlation. Moreover, when predicted 18-Segal-WAXS are compared with real 18-Segal-WAXS data, for most samples, the former was found to be significantly higher (Table 3, column 7). Only for five samples, flax (sample 28), ramie (sample 29), unbleached softwood kraft pulp (sample 31), bleached softwood kraft pulp (sample 32), and bleached hardwood kraft pulp (sample 33), was the difference between predicted-WAXS and Segal-WAXS <18%. These samples had either no or small amounts of lignin or hemicellulose, chemical composition of ramie and bleached and unbleached softwood kraft pulps (Table 2), and flax.[27] High values of predicted 18-Segal-WAXS suggested that for most lignocelluloses the actual 18-Segal-WAXS crystallinities are highly underestimated, and the latter seems to be related to the amounts of noncellulosic components in the samples. The latter proposition is supported by the WAXS diffractograms of Avicel-PH-101 and loblolly pine, where compared to Avicel, the [002] band shape for pine shows a slower decline as the 2θ decreases from the band maximum of 22.5°. This has primarily to do with broadness of the [002] peak in the diffractograms of pine (and of other lignin and hemicellulose rich materials in Table 1), due to which the contribution at 18° remains fairly high. Further evidence in support of low WAXS crystallinity in the presence of hemicellulose and lignin comes from the literature. For instance, in the case of aspen wood chips, Schwald et al.[32] reported that steam heating caused removal of lignin and hemicellulose and the 18-Segal-WAXS crystallinity increased from ∼58 to 80%, a large increase of 38%.

To explore this further, the WAXS diffractograms of cellulose (Whatman CC31), lignin, and xylan were obtained (Figure 5). Clearly, there is significant X-ray scattering in the 2θ region of 18−25° from noncellulosic components. Such contributions broaden the cellulose [002] peak and increase the underlying background in ways that are difficult to correct for while estimating cellulose crystallinity by 18-Segal-WAXS and other X-ray methods.[14]

Effect of Delignification on 18-Segal-WAXS. If indeed low Segal values are in part due to the contribution from lignin, delignified samples should give higher WAXS crystallinity. This was found to be true for most of the delignified samples (Table 3). All of the softwoods and most of the hardwoods showed increased crystallinity. The increase was anywhere from 3 to 34%. Among others, agricultural residues/fibers, wood pulps, and remaining samples had higher crystallinities as well. Because of the low lignin content of ramie (sample 29) and unbleached kraft pulp (sample 31; lignin contents of 1.4 and 4.4%, respectively, Table 2), crystallinity enhancement was quite small. However, for samples having similar lignin contents, significant variation in the crystallinity change was observed upon delignification, and this is likely to be because lignin was incompletely removed and hemicellulose was still present. Overall, though, the observation supported the proposition that lignin removal would lead to higher Segal-WAXS crystallinity.

Effect of Lignin and Hemicellulose on 18-Segal-WAXS. To further study how the presence of lignin and hemicellulose changes 18-Segal-WAXS measurements of Whatman CC31 cellulose, the 18 mixture samples were prepared using various mass ratios of cellulose, lignin, and xylan. The 18-Segal-WAXS crystallinity data indicated that in the presence of lignin or xylan or both, cellulose crystallinity declined in all cases. For cellulose/lignin mixtures, crystallinity declined with increasing lignin content. A similar observation was made for cellulose/xylan mixtures. The extent of crystallinity reduction depended upon the composition of the mixture but was as high as 15%. For the given cellulose content, the decline in crystallinity was similar when the mass ratio between lignin and xylan was varied. The latter indicated that both lignin and xylan were equally effective in reducing cellulose crystallinity.

When the mixture crystallinity data were plotted against the percentage of cellulose content in the mixtures, the two were found to be linearly correlated ($R^2 = 0.96$) and with the decline in cellulose concentration, crystallinity went down and vice versa. Moreover, the relationship clearly showed that as cellulose concentration is lowered, the error in the estimated 18-Segal-WAXS crystallinity increases. Using this correlation and considering that most woods in Table 1 have ∼40% cellulose, one expects 16% error in the 18-Segal-WAXS measurements with the assumption that the behaviors of the wood and Whatman CC31 celluloses are similar. However, it is possible that for wood cellulose, which has significantly lower crystallinity (compared to Whatman CC31), the error is even higher.

In this work, cellulose crystallinities of a large number of lignocelluloses were estimated using the univariate Raman method. The manner in which the presence of lignin and hemicellulose affects the Raman crystallinity estimation was investigated. Materials that contained syringyl lignin gave higher values of Raman crystallinity, whereas the presence of hemicellulose reduced the crystallinity values. For most samples, autofluorescence in spectra was not a concern, but accurate cellulose Raman crystallinity could be determined only after corrections were made for contributions from syringyl lignin (if present) and hemicellulose. Moreover, Raman and 18-Segal-WAXS crystallinity estimation methods were compared. We found that the Segal method underestimated true cellulose crystallinity significantly. This study indicated that with appropriate corrections, the univariate Raman method can be used to reliably measure the cellulose crystallinity of lignocellulose materials.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional results (in table and figures): composition and crystallinity data of prepared mixtures (Table S1); linear correlation plot between Raman crystallinity and percent xylan for cellulose:xylan mixtures (Figure S1); correlation plot between 18-Segal-WAXS and univariate Raman crystallinity for calibration set samples (Figure S2); WAXS diffractograms of loblolly pine wood and Avicel-PH-101 (Figure S3); 18-Segal-WAXS crystallinities of cellulose/lignin and cellulose/xylan mixtures as a function of cellulose/lignin and cellulose/xylan ratios, respectively (Figure S4); and 18-Segal-WAXS crystallinities of mixtures of cellulose, lignin, and xylan as a function of percent cellulose and lignin-to-xylan ratios (Figure S5). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

WAXS, wide-angle X-ray scattering; CP/MAS, cross-polarization magic angle spinning; SFG, sum frequency generation; TMP, thermomechanical pulp; CTMP, chemithermomechanical pulp

■ REFERENCES

(1) Fan, L. T.; Lee, Y.-H. Mechanism of the enzymatic hydrolysis of cellulose: effects of major structural features of cellulose on enzymatic hydrolysis. *Biotechnol. Bioeng.* **1980**, *22*, 177−199.

(2) Segal, L.; Creely, J. J.; Martin, A. E.; Conrad, C. M. An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. *Textile Res. J.* **1959**, *29*, 786−794.

(3) Jayme, G.; Knolle, H. The empirical X-ray determination of the degree of crystallinity of cellulosic material. *Papier* **1964**, *18*, 249−794.

(4) Leppanen, K.; Andersson, S.; Torkkeli, M.; Knaapila, M.; Kotelnikova, N.; Serimaa, R. Structure of cellulose and microcrystalline cellulose from various wood species, cotton and flax studied by X-ray scattering. *Cellulose* **2009**, *16*, 999−1015.

(5) Horii, F.; Hirai, A.; Kitamaru, R. CP/MAS ^{13}C NMR spectra of the crystalline components of native celluloses. *Macromolecules* **1987**, *20*, 2117−2120.

(6) Newman, R. H.; Hemmingson, J. A. Determination of the degree of cellulose crystallinity in wood by carbon-13 nuclear magnetic resonance spectroscopy. *Holzforschung* **1990**, *44*, 351−355.

(7) Newman, R. H. Estimation of the lateral dimensions of cellulose crystallites using ^{13}C NMR signal strengths. *Solid State NMR* **1999**, *15*, 21−29.

(8) Richter, U.; Krause, T.; Schempp, W. Untersuchungen zur Alkalibehandlung von Cellulosefasern. Teil 1. Infrarotspektroskopische und Rontgenographische Beurteilung der Anderung des Ordnungszustandes. *Angew. Makromol. Chem.* **1991**, *185/186*, 155−167.

(9) Hulleman, S. H. D.; Van Hazendonk, J. M.; Van Dam, J. E. G. Determination of crystallinity in native cellulose from higher plants with diffuse reflectance Fourier transform infrared spectroscopy. *Carbohydr. Res.* **1994**, *261*, 163−172.

(10) Schenzel, K.; Fischer, S.; Brendler, E. New method for determining the degree of cellulose I crystallinity by means of FT Raman spectroscopy. *Cellulose* **2005**, *12*, 223−231.

(11) Agarwal, U. P.; Reiner, R. S.; Ralph, S. A. Cellulose I crystallinity determination using FT-Raman spectroscopy: univariate and multivariate methods. *Cellulose* **2010**, *17*, 721−733.

(12) Barnette, A. L.; Bradley, L. C.; Veres, B. D.; Schreiner, E. P.; Park, Y. B.; Park, J.; Park, S.; Kim, S. H. Selective detection of crystalline cellulose in plant cell walls with sum-frequency-generation (SFG) vibration spectroscopy. *Biomacromolecules* **2011**, *12*, 2434−2439.

(13) Andersson, S.; Serimaa, R.; Paakkari, T.; Saranpaa, P.; Pesonen, E. Crystallinity of wood and the size of cellulose crystallites in Norway spruce (*Picea abies*). *J. Wood Sci.* **2003**, *49*, 531−537.

(14) Park, S.; Baker, J. O.; Himmel, M. E.; Parilla, P. A.; Johnson, D. K. Cellulose crystallinity index: measurement technique and their impact on interpreting cellulose data. *Biotechnol. Biofuels* **2010**, *3*, 10.

(15) Maddams, W. F. The scope and limitations of curve fitting. *Appl. Spectrosc.* **1980**, *34*, 245−267.

(16) Meier, R. J. On art and science in curve-fitting vibrational spectra. *Vib. Spectrosc.* **2005**, *39*, 266−269.

(17) Barnette, A. L.; Lee, C.; Bradley, L. C.; Schreiner, E. P.; Park, Y. B.; Shin, H.; Cosgrove, D. J.; Park, S.; Kim, S. H. Quantification of crystalline cellulose in lignocellulosic biomass using sum frequency generation (SFG) vibration spectroscopy and comparison with other analytical methods. *Carbohydr. Polym.* **2012**, *89*, 802−809.

(18) Browning, B. L. *Methods of Wood Chemistry*; Wiley-Interscience: New York, 1967; Vol. *II*.

(19) TAPPI. *Acid Insoluble Lignin in Wood and Pulp*; Official test method T-222 (Om); TAPPI: Atlanta, GA, 1983.

(20) Davis, M. W. A rapid method for compositional carbohydrate analysis of lignocellulosics by high pH anion exchange chromatography with pulsed amperometric detection (HPAEC/PAD). *J. Wood Chem. Technol.* **1998**, *18*, 235−252.

(21) Leary, G.; Schmidt, J. A. The chemistry of lignin-retaining bleaching: oxidative bleaching agents. In *Lignin and Lignans − Advances in Chemistry*; Heitner, C., Dimmel, D. R., Schmidt, J. A., Eds.; CRC Press: Boca Raton, FL, 2010; pp 439−469.

(22) Agarwal, U. P.; Ralph, S. A. FT-Raman spectroscopy of wood: identifying contributions of lignin and carbohydrate polymers in the spectrum of black spruce (*Picea mariana*). *Appl. Spectrosc.* **1997**, *51*, 1648−1655.

(23) Agarwal, U. P.; McSweeny, J. D.; Ralph, S. A. FT-Raman investigation of milled-wood lignins: softwood, hardwood, and chemically modified black spruce lignins. *J. Wood Chem. Technol.* **2011**, *31*, 324−344.

(24) Marton, R.; Rushton, P.; Sacco, J. S.; Sumiya, K. Dimensions and ultrastructure of growing fibers. *Tappi* **1972**, *55*, 1499−1504.

(25) Newman, R. H. Homogeneity in cellulose crystallinity between samples of *Pinus radiata* wood. *Holzforschung* **2004**, *58*, 91−96.

(26) Pettersen, R. G. The chemical composition of wood. In *The Chemistry of Solid Wood*; Roger, M. R., Ed.; Advances in Chemistry Series 207; American Chemical Society: Washington, DC, 1984; pp 57−126.

(27) Bochek, A. M.; Shevchuk, I. L.; Lavrentev, V. N. Fabrication of microcrystalline and powdered cellulose from short flax fiber and flax straw. *Russ. J. Appl. Chem.* **2003**, *76*, 1679−1682.

(28) Wedig, C. L.; Jaster, E. H.; Moore, K. J. Hemicellulose monosaccharide composition and in vitro disappearance of orchard grass and alfalfa hay. *J. Agric. Food Chem.* **1987**, *35*, 214−218.

(29) Lv, G.-J.; Wu, S.-B.; Lou, R. Kinetic study of the thermal decomposition of hemicellulose isolated from corn stalkaline. *Bioresources* **2010**, *5*, 1281−1291.

(30) Jonoobi, M.; Harun, J.; Shakeri, A.; Misra, M.; Oksman, K. Chemical composition, crystallinity, and thermal degradation of bleached and unbleached kenaf bast (*Hibiscus cannabinus*) pulp and nanofibers. *Bioresources* **2009**, *4*, 626−639.

(31) Rezende, C. A.; deLima, M. A.; Maziero, P.; deAzevedo, E. R.; Garcia, W.; Polikarpov, I. Chemical and morphological characterization of sugarcane bagasse submitted to a delignification process for enhanced enzymatic digestibility. *Biotechnol. Biofuels* **2011**, *4*, 54 DOI: 10.1186/1754-6834-4-54.

(32) Schwald, W.; Brownell, H. H.; Saddler, J. N. Enzymatic hydrolysis of steam treated aspen wood: influence of partial hemicellulose and lignin removal prior to pretreatment. *J. Wood Chem. Technol.* **1988**, *8*, 543−560.