Cellulose Stability and Delignification after Alkaline Hydrogen Peroxide Treatment of Straw

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Synopsis

Neutron diffraction profiles for cellulose from different sources were compared before and after alkaline hydrogen peroxide (AHP) treatment. It was found that AHP treatment did not cause detectable changes in the structure of highly polymerized cellulose. In measurements on wheat straw, peaks were observed at the angles characteristic of pure cellulose. Changes, with AHP treatment, of the intensities, positions, and widths of these peaks were consistent with effects due to delignification, which frees the cellulose lattice from strains caused by the binding of lignin.

INTRODUCTION

The rate and extent of lignocellulose digestion by microorganisms present in the stomachs of ruminants are both greatly enhanced when the lignocellulose is first treated with an alkaline (pH 11.5) solution of hydrogen peroxide.[1] This treatment makes soluble a portion of the lignin that normally interferes with microbial degradation of structural carbohydrate present in plant cell walls.[2,3] Increasing the microbial digestibility of low value lignocellulosic materials such as straws could make available very large quantities of cellulosic biomass for food production from ruminants such as cattle and sheep.[4] The dramatic increase in the digestibility of lignocellulose following alkaline hydrogen peroxide (AHP) treatment has been attributed not only to delignification but also to a possible decrease in cellulose crystallinity.[1,3]

The precise structure of native cellulose is still a matter of controversy because of the difficulty of obtaining good fiber specimens of this polymer. In this paper we have chosen to index diffraction peaks using parameters from the monoclinic model structure proposed by Gardner and Blackwell.[5] It should be noted that the microfibril faces of cellulose correspond to the $(1\bar{1}0)$ and (110) planes of the Gardner and Blackwell unit cell. Cellulose microfibrils can be as thin as 35 Å in directions perpendicular to these planes.[6] Also, the

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glucose residues lie in (020) planes whose normals are perpendicular to the [001] fiber axis. It is therefore expected that, for intact cells, diffraction from these three planes will be sensitive to binding by lignin at the microfibril walls.

In the present experiment we have chosen neutron diffraction as our prime technique, although X-ray diffraction did indicate a similar but less consistent response. The advantage of neutron scattering in this instance was that it permitted measurements on significantly larger samples and thus provided better averaging in a system difficult to prepare in a perfectly homogeneous manner. With neutron diffraction the presence of significant incoherent scattering also permitted us to monitor removal of hemicellulose produced by different chemical procedures.

MATERIALS AND METHODS

In order to examine the molecular changes caused by AHP treatment, we have compared neutron diffraction measurements on various types of crystalline cellulose. These types include "Avicel" (a highly polymerized commercially available microcrystalline powder), ramie fibers, and native cellulose present *in situ* in kenaf and wheat straw. Avicel (average crystallite dimension = 20 μm) was a gift of the FMC Corporation of Montreal, Canada. The hairlike ramie fibers were kindly provided by Professor Iain Taylor of the Botany Department, University of British Columbia. Wheat straw and kenaf were obtained locally near the USDA Northern Regional Research Center, Peoria, IL, and were ground to pass a 2-mm screen prior to AHP treatment. Untreated kenaf and straw were ground to pass a 0.5-mm screen prior to neutron diffraction measurements.

For the AHP treatment, samples were suspended in distilled water containing 0.01 g/mL hydrogen peroxide. The suspension was initially adjusted to pH 11.5 with NaOH, and stirred gently for 16 h at room temperature. The concentration of solids in the reaction mixture was always less than 0.03 g/mL. By the end of 16 h, the pH was found to have risen above 12 in the wheat straw mixtures. This procedure yielded what we call type I specimens. A slight variation produced type II specimens of wheat straw. This variation involved maintaining the pH of the reaction mixture at 11.5 $\pm$ 0.1 by periodic addition of HCl. By this means hemicellulose was largely prevented from going into solution.[3] At the end of the reaction period, the insoluble residue was collected by filtration, rinsed with distilled water until the filtrate was neutral, and then oven-dried at 80°C. Dried treated wheat straw and kenaf were ground in a Wiley mill to pass a 0.5-mm screen.

Neutron diffraction measurements for each of the four types of cellulose consisted of dual diffraction runs, one on the untreated specimen and one after the AHP treatment. Samples weighing 2 g or more (dry weight) were placed in either quartz or vanadium containers for the neutron measurements. The samples were contained in the annular space between two concentric cylindrical shells. The diameter of the inner shell was 3.0 cm. Two thicknesses of annular space 3.0 and 1.5 mm were used. The measurements were carried out on a triple-axis spectrometer operated in a diffraction mode. The angular resolution was 0.50° full width at half maximum. The neutron wavelength was

1.54 Å. The transmission of most of the wheat straw samples was $\approx 75\%$. Measurements on samples with higher transmission ($\approx 88\%$) yielded the same results, but with poorer statistical accuracy. All measurements were corrected for fast neutron background. After subtraction of the scattering by the containers, the net scattering intensities were normalized to yield the same average value for both treated and untreated specimens in the range $8° \leq 2\theta \leq 10°$, where 2θ is the scattering angle

RESULTS

All types of cellulose yielded diffraction peaks that could be indexed in accordance with the known structure of native cellulose.[5] The Avicel specimens showed evidence of overlapping peaks [notably the $(1\bar{1}0)$ and (110) Bragg peaks], but no change in diffraction profile as a result of AHP treatment was observed. Typical results for Avicel are displayed in the upper panel of Figure 1. Measurements on ramie fibers confirmed the peak assignments for Avicel and also indicated no changes in peak widths, positions or intensities with AHP treatment. These data are consistent with earlier observations that AHP treatment has little effect on the digestibility of lignin-free celluloses such as Avicel and cotton.[7] The neutron diffraction results also cast doubt on the hypothesis[1] of a possible decrease in cellulose crystallinity with AHP treatment.

For the straw samples (lower panel, Fig. 1) several diffraction peaks which corresponded in angle to those for pure cellulose were found. This correspondence can readily be seen by comparing the upper and lower panels in Figure 1. In addition to an apparent increase in intensity in the region of the $(1\bar{1}0)$ and (110) peaks, there is a significant increase in the intensity of the (020) peak with AHP treatment. The AHP treatment employed here was type I (see above).

More detailed measurements of the low angle region for $9° < 2\theta < 29°$ are shown in Figure 2. Panels A, B, and C refer, respectively, to untreated, treated (type I) and treated (type II) samples of wheat straw. The full lines represent Gaussian fits to the data. Linear sloping backgrounds (dashed lines) were assumed and these were allowed to vary along with the six parameters describing the two Gaussians. The combined $(1\bar{1}0)$ and (110) peaks [referred to in the figure as (110)] were fitted with one Gaussian and the (020) peak with another. The results show several important features:

1. The integrated peak intensities in panel B are greater than in panel A. The fitting indicates increases in both peaks of $28 \pm 10\%$ as a result of type I treatment. The integrated intensities are the same (within experimental error) for the data in panels A and C.
2. The (110) peaks shift to lower values after either treatment. The arrows labelled a, b, and c indicate centroid positions obtained by the fitting procedure. Values for these centroid angles are listed as $2\theta m$ in Table I. Also listed there are the associated peak widths. As a result of the shifts and decreased peak widths, the (110) peaks appear more prominent after either AHP treatment.

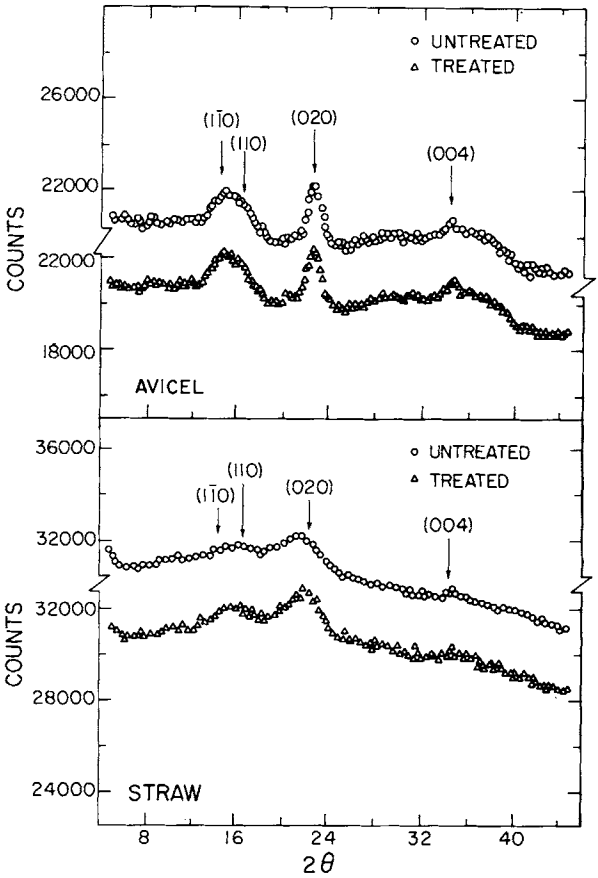

Fig. 1. Neutron diffraction patterns of pure microcrystalline cellulose (upper panel) and wheat straw (lower panel) before (○) and after (△) treatment with alkaline hydrogen peroxide. The indexing of the peaks is based on the structure suggested by Gardner and Blackwell.[5] The statistical errors are essentially the sizes of the data points. Spectra were normalized as described in the text. The scattering angle is denoted by 2θ.

3. The centroid (21.98 ± 0.05°) of the (020) peak did not shift significantly after either AHP treatment. The type I treatment led to a decrease in width from 3.70 ± 0.12 (untreated) to 3.34 ± 0.09°. After type II treatment the width was found to be 3.52 ± 0.11.

Another effect of AHP treatment is illustrated in Figure 3. This figure shows the results of detailed measurements of the (004) Bragg peak due to planes that are perpendicular to the fibril axis of cellulose.

The centroid of the (004) peak was found to increase slightly (from 34.74 ± 0.09° to 35.31 ± 0.11°) after type II treatment, but remained the same (within experimental error) after type I treatment. There were dramatic increases in integrated peak intensity after both types of treatment. Peak widths and intensities for the (004) peaks are listed in Table II.

Untreated and treated (type I) kenaf were also studied by neutron diffraction. The intensities of the (110), (1$\bar{1}$0), and (020) peaks were unchanged, but that of the (004) peak increased by a factor of ≈ 1.8 after type I treatment.

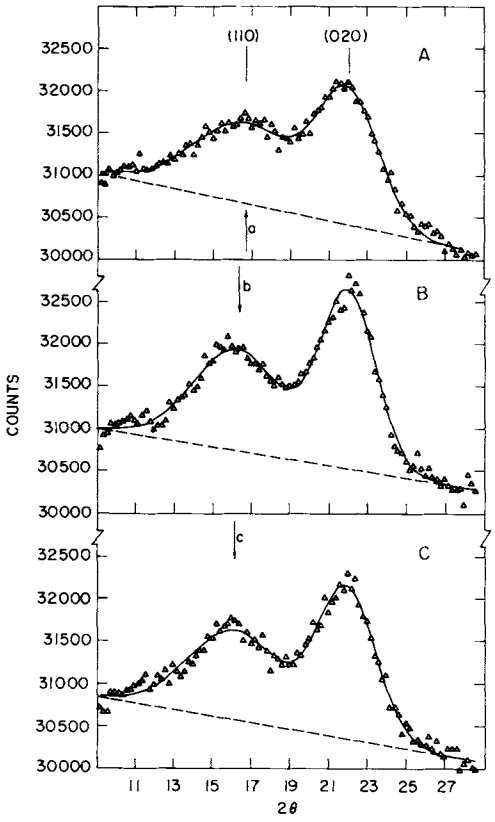

Fig. 2. Detailed measurements of diffraction profiles between 9° and 29°. The panel labels A, B, and C refer, respectively, to untreated wheat straw, treated (type I) and treated (type II) wheat straw as outlined in the text. The combined (110) and ($1\bar{1}0$) peaks are labeled simply as (110). The fitted values of the centroids obtained from Gaussian fits to the (110) peaks are designated by arrows labeled a, b, and c. Linear sloping backgrounds (obtained by the fitting procedure) are denoted by dashed lines. Note the increased prominence of the (110) peaks after treatment. Some parameters from the fitting procedure are listed in Table I.

The centroid of the combined (110) and ($1\bar{1}0$) peaks decreased from 15.90 ± 0.13° to 15.56 ± 0.14° after treatment, but the peak widths were the same within experimental error. As with wheat straw, the position of the (020) peak remained unchanged and its width (FWHM) decreased (from 3.92 ± 0.15 to 3.57 ± 0.15 degrees) after treatment.

TABLE I

Centroid Angles ($2\theta m$) and Full Widths at Half Maximum (FWHM) of the Peak Due to the Combined (110) and ($1\bar{1}0$) Reflections from Cellulose in Various Samples of Wheat Straw [a]

Sample	$2\theta m$	FWHM
Untreated	16.69 ± 0.14	6.37 ± 0.42
Treated, type I	16.33 ± 0.08	5.43 ± 0.27
Treated, type II	16.20 ± 0.10	5.61 ± 0.33

[a] Numerical values are in degrees.

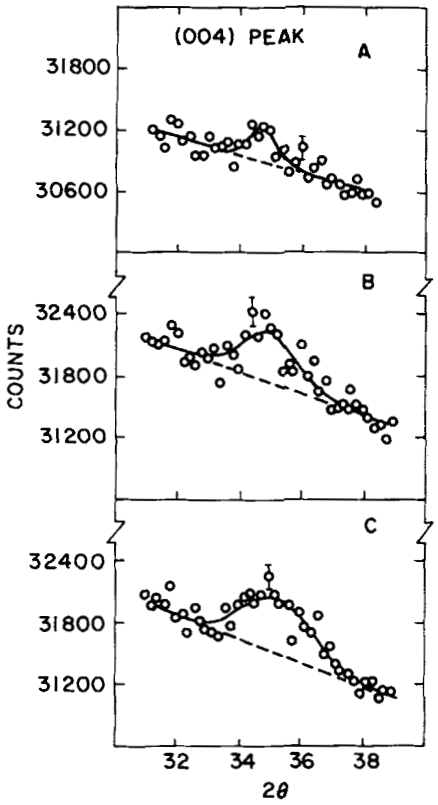

Fig. 3. The results of detailed measurements of the (004) diffraction peak obtained from cellulose in wheat straw: (A) untreated wheat straw; (B) treated wheat straw (type I); (C) treated wheat straw (type II). The lines have the same meanings as in Figure 2. Parameters from the fitting procedure are listed in Table II.

In order to assess the effects of AHP treatment on cellulose *in situ*, comparisons will be made in the next section with measurements on pure cellulose. For Avicel the $2\theta m$ and FWHM values for three peaks are listed in Table III. Except for the splitting of the $(1\bar{1}0)$ and (110) peaks and a much narrower (004) peak, the results for ramie fibers agree within experimental error with those listed for Avicel. Note that, even for ramie fibers, the $(1\bar{1}0)$

TABLE II
Full Widths at Half Maximum (FWHM) and Integrated Intensities (arbitrary units) of the (004) Peak from Cellulose in Various Samples of Wheat Straw [a]

Sample	FWHM	Intensity (arb. units)
Untreated	0.97 ± 0.22	345 ± 80
Treated, type I	2.04 ± 0.37	1030 ± 210
Treated, type II	2.52 ± 0.32	1445 ± 220

[a] Units of FWHM are degrees.

TABLE III
Centroids ($2\theta m$) and Full Widths at Half Maximum[a]

$2\theta m$ (110)	FWHM (110)	$2\theta m$ (020)
15.36 ± 0.05	3.52 ± 0.07	22.69 ± 0.02
FWHM (020)	$2\theta m$ (004)	FWHM (004)
1.46 ± 0.03	34.67 ± 0.04	1.33 ± 0.11

[a] FWHM of various peaks measured by neutron diffraction from Avicel treated or untreated. The combined (110) and ($1\bar{1}0$) peaks are listed together as (110). All values are in degrees.

and (110) peaks and the (020) peak are much broader than the experimental resolution because of finite fiber thickness.

DISCUSSION

In this study we have observed changes in the intensities, positions, and widths of different peaks. In this section we attempt to relate these to observed changes in molecular configuration inferred from previous biochemical experiments.[1-3]

The interpretation of intensity changes is complicated by the presence of short, tangled strands of the polysaccharide hemicellulose. These strands tend to hydrolyze with AHP treatment.[3] The removal of hemicellulose by type I treatment leads to a lower background causing the cellulose peaks to stand out more clearly in the treated samples. A 28% increase in peak intensity above background is consistent with the measured amount[8] of hemicellulose in wheat straw. With type II treatment the decrease in hydrogenous background scattering results from the removal of lignin only, a much smaller component[2] than hemicellulose. The lack of a measurable decrease in kenaf after type I treatment is consistent with a lower percentage of hemicellulose compared to wheat straw.[9]

The increase in intensity of the (004) peak is independent of the type of treatment and is therefore likely to be associated with the lignin rather than the hemicellulose becoming soluble. A similar increase in intensity of this peak was also observed after treatment of kenaf. A tentative interpretation is that only the central portion of the cellulose fiber contributes to the (004) intensity in untreated material. Individual cellulose polymer strands near the surface are so randomly bent by lignin that they do not contribute. After lignin is dissolved by the AHP treatment, however, polymer chains near fibril surfaces reorient and then contribute. The larger widths after AHP treatment are attributed to residual random lattice distortion along the [004] direction in the new regions which add to the diffraction after delignification.

It appears from Table I that decreases in centroid angle for the combined (110) and ($1\bar{1}0$) reflections are similar for the two treatment types. The same table also indicates a similar narrowing of this compound peak for the two treatments. An interpretation of these results is that again lignin is the important factor; our results suggest it is bound to the (110) and ($1\bar{1}0$) surfaces of the cellulose fibrils. The directions of the changes in $2\theta m$ and FWHM are such as to approach the pure cellulose values for Avicel listed in the first two columns of Table III.

The centroid of the combined (110) and ($1\bar{1}0$) peaks shifts with bonding to lignin but the (020) centroid does not. This is probably because the glucose residues form sheets in the (020) plane which can slide with respect to one another. Sliding is allowed by the weak interplanar hydrogen bonds. Thus the (020) plane spacing remains well defined.

As noted earlier, the (110) peak is composed of the ($1\bar{1}0$) and (110) reflections. Structure factor calculations were carried out using atomic coordinates taken from Table IV of the paper by Gardner and Blackwell.[5] In these calculations all the atoms in adjacent (020) planes of the unit cell were shifted by amounts of the order of 2–3% of the lattice parameter in opposite senses on going from one plane to another along the [100] direction. As a result of this procedure the (110) and ($1\bar{1}0$) peak positions remained constant but their intensities changed. Specifically it was found that when adjacent layers slide by each other in the [100] direction, the (110) peak at 16.68° decreases in intensity relative to the ($1\bar{1}0$) peak at 14.75°. This could account in part for the apparent decrease in angle observed for this compound peak. The decrease in the width of the (020) peak after type I treatment suggests that this peak is sensitive to the presence of hemicellulose (which may serve to bond adjacent fibrils to one another).

Similar trends in the diffraction results for kenaf serve to reinforce our conclusions about wheat straw. It may be noted that, for this fibrous plant, the centroid of the combined (110) and ($1\bar{1}0$) peaks is closer to that for Avicel than the centroid for straw is. Kenaf is somewhat more resistant than wheat straw to enzyme hydrolysis after AHP treatment, but the level of delignification was not found to be appreciably less.[2]

In conclusion, the AHP treatment of straw apparently "loosens" the lignocellulosic matrix, causing, at the molecular level, a more open three-dimensional relationship between the cellulose, lignin, and possibly hemicellulose polymers. As the results for Avicel show, highly crystalline cellulose is not affected by AHP treatment. Our diffraction measurements also indicate that, if anything, the crystallinity of cellulose within the cell wall of straw is increased by AHP treatment. The present results favor the contention that the principal result of AHP treatment is that it detaches and makes soluble the lignin,[3] thereby increasing the amount of cellulose available for hydrolysis by cellulolytic enzymes.

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