

Changes in cellulose morphology of pretreated yellow poplar during enzymatic hydrolysis

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Abstract

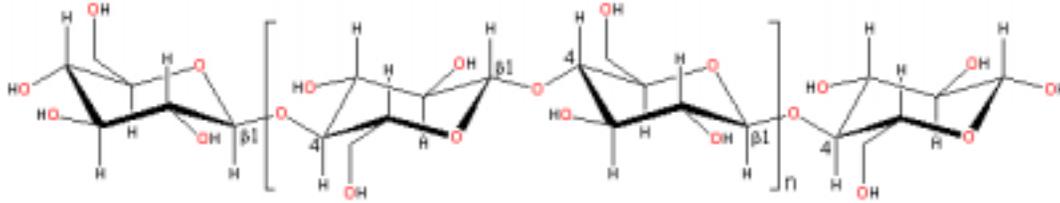
We have used solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy to study the changes in the cellulose morphology that occur during the enzymatic hydrolysis with an endoglucanase, E1, an exoglucanase, CBHI, and a binary mixture of E1 and CBHI (5/95 mixture). The NMR studies have observed increases in the crystallinity of the cellulose and an increase in cellulose I β with respect to cellulose I α as the enzymatic digestion progresses.



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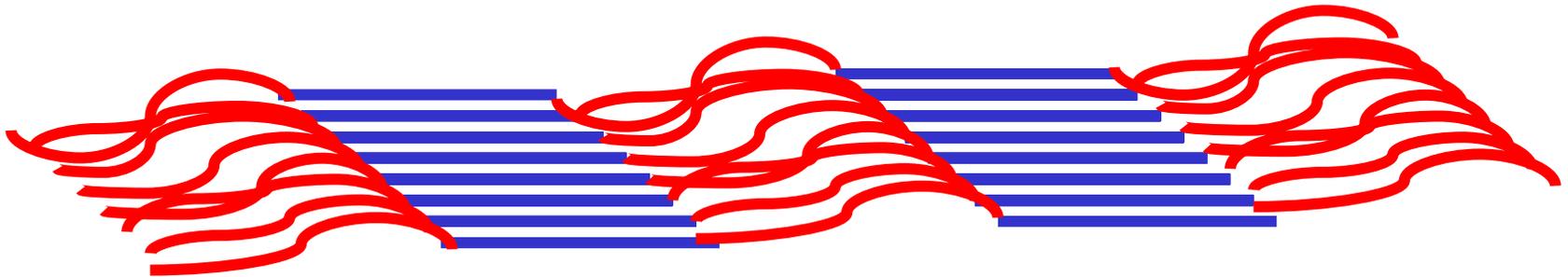


Introduction



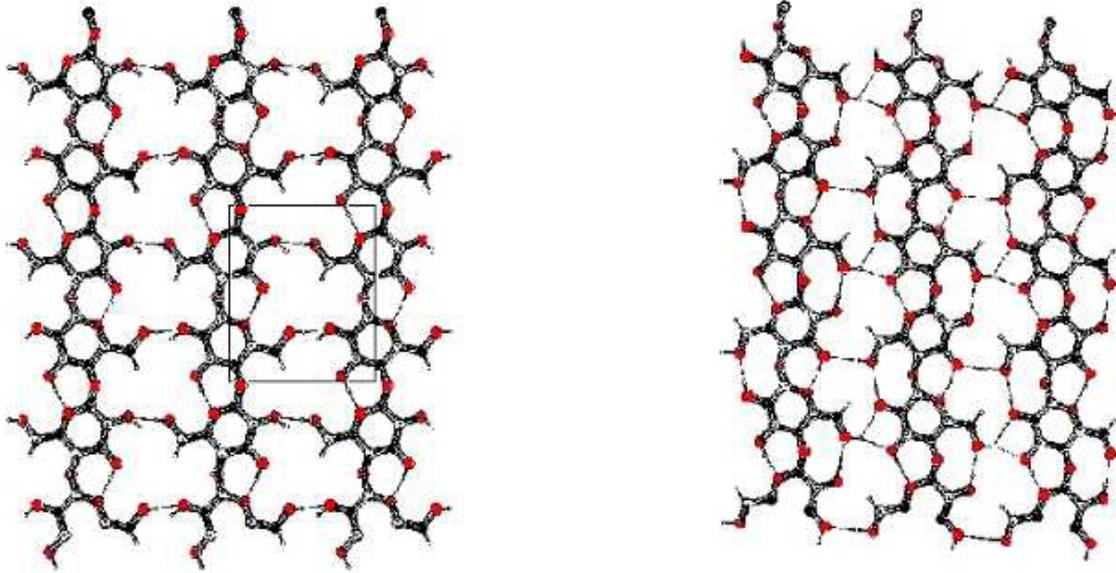
Cellulose

Cellulose is a polymer of β-D-glucose units linked together by (1→4) glycosidic bonds to form cellobiose residues that are the repeating units in the cellulose chain. The cellulose structure favors organization into bundles with the crystalline order held together by hydrogen bonds.



Cellulose consists of crystalline regions (blue) and disordered or amorphous regions (red).

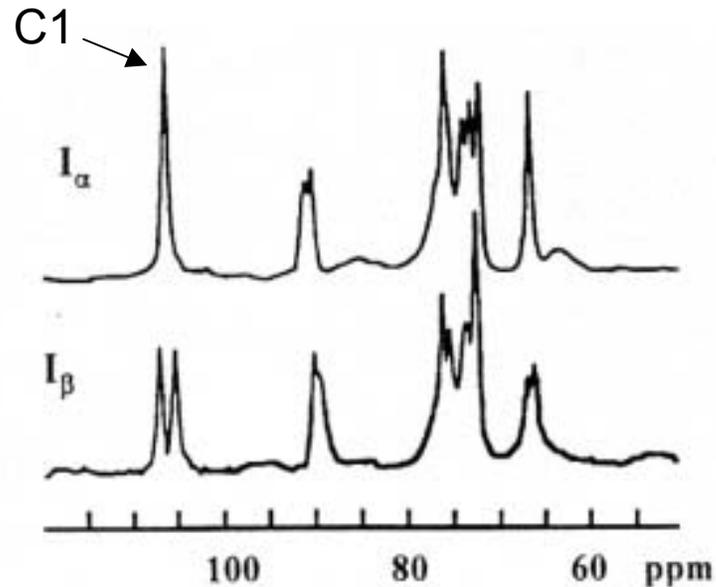
Native crystalline cellulose is generally found as a mixture of cellulose I α and cellulose I β polymorphic forms. The proportions of the two allomorphs of crystalline cellulose vary depending on the source of the cellulose. Higher plants such as trees and corn believed to have a higher proportion of cellulose I β . Studies have determined cellulose I β is more stable than cellulose I α and the conversion from type I α to I β is irreversible.



Theoretical models of cellulose I α (left) and cellulose I β (right) indicate different hydrogen-bonding patterns. Electron diffraction patterns show type I α to have a triclinic unit cell and type I β to have a monoclinic unit cell.

Studies of the enzymatic digestion of algal-bacterial cellulose (*Cladophora*) have indicated that the I α component to be selectively hydrolyzed with respect to the I β component.² Other research has demonstrated that the I α phase is more reactive towards acetylation than the I β phase.³

^{13}C cross polarization with magic angle spinning (^{13}C CP/MAS) spectroscopy confirmed the existence of cellulose I_α and cellulose I_β .¹ The ^{13}C spectra of the two allomorphs are unique, especially the peak assigned to the C1 carbon at ~ 106 ppm.



^{13}C CP/MAS spectra of cellulose I_α and cellulose I_β

¹D. L. VanderHart, R. H. Atalla, (1984), *Macromolecules*, Studies of microstructure in native celluloses using solid state ^{13}C NMR, 17, 1465-1472.

²N. Hayashi *et al.*, (1998), *Carbohydrate Research*, Selective degradation of cellulose I_α component in *Cladophora* cellulose with *Trichoderma viride* cellulase, 305, 109-116.

³J-F Sassi *et al.*, (2000), *Cellulose*, Relative susceptibility of the I_α and I_β phases of cellulose towards acetylation, 7, 119-132.

Sample Preparation

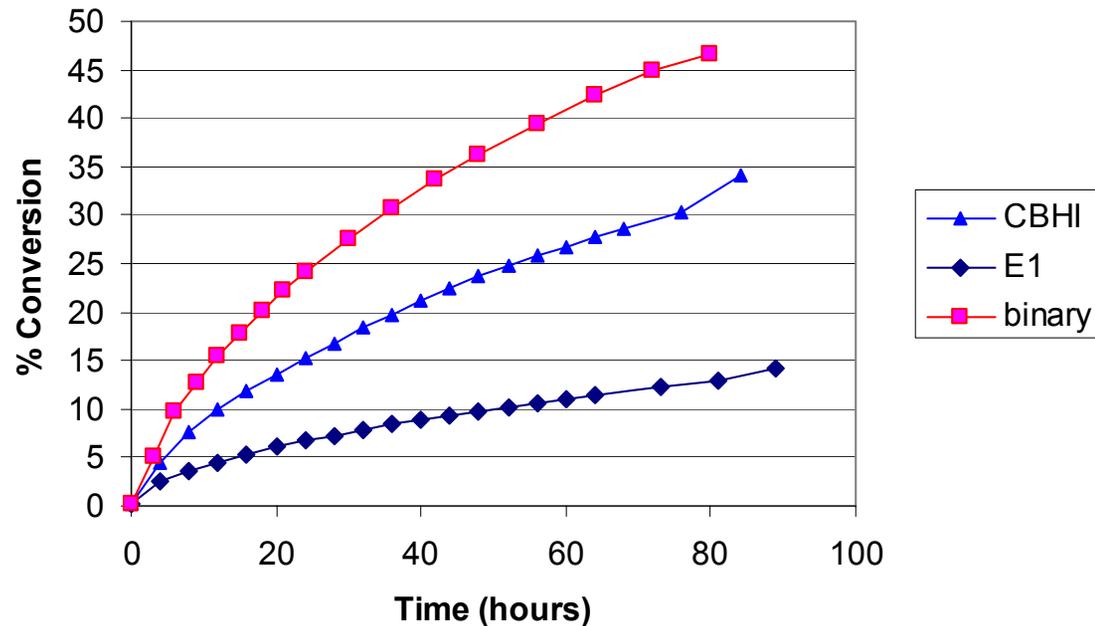
Residues were collected from diafiltration saccharification assays.³ (DSA) using endoglucanase, E1, an exoglucanase, CBHI, and a binary mixture of E1 and CBHI (5/95 mixture). Residual enzyme was removed before NMR analysis.

Residues were collected after 15hrs for CBHI and the binary mixture.

Residues were collected after 72 hrs for the binary mixture and 84 hrs for E1 and CBHI.

DSA curves

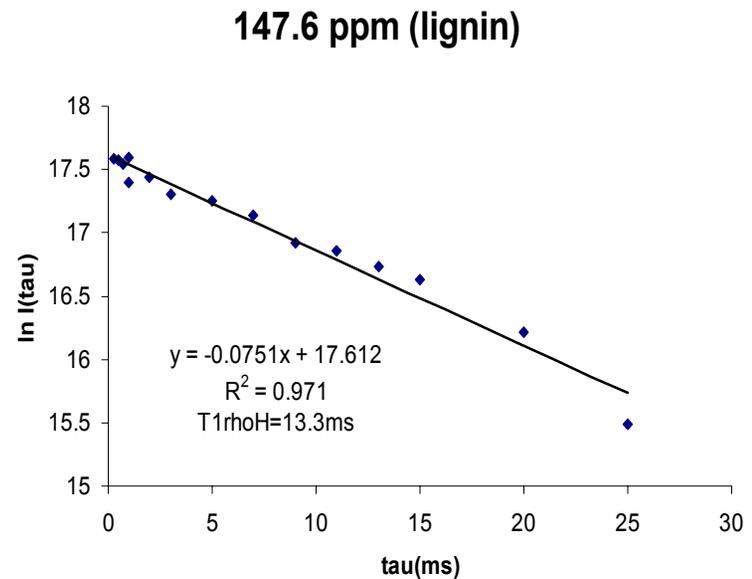
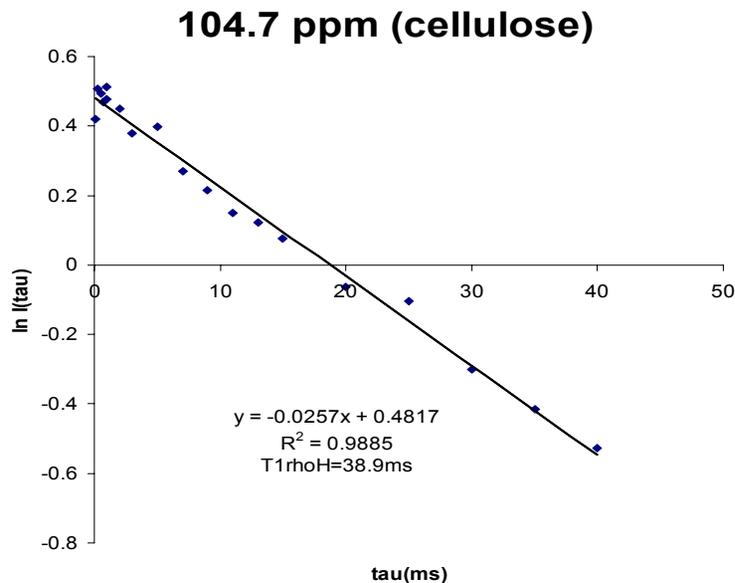
CBHI, E1, and Binary at 38C



³J.O. Baker *et al*, (1997) , Applied Biochemistry and Biotechnology, Use of a new membrane-reactor saccharification assay to evaluate the performance of cellulases under simulated SSF conditions, 63-65, 565-595.

Determination of Crystallinity

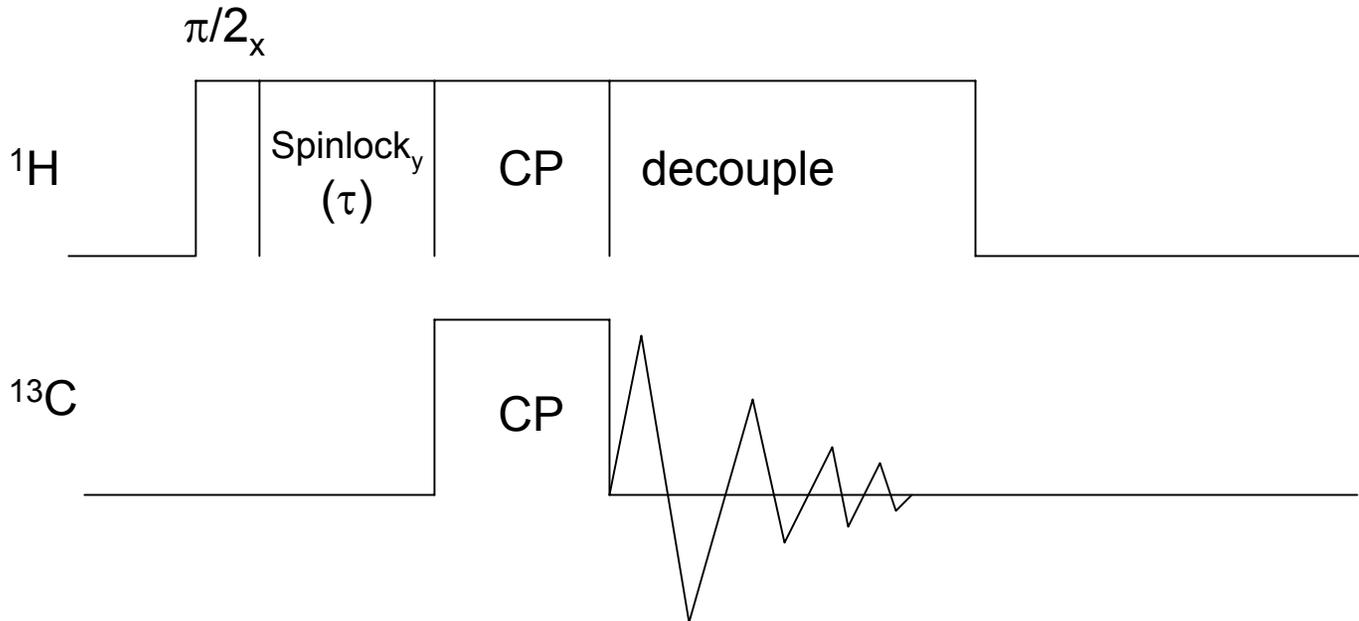
^{13}C CP/MAS spectroscopy has been used to determine the proportions of crystalline (highly ordered) and disordered components (amorphous cellulose, hemicellulose and lignin) present in biomass materials². The different components can be distinguished because of differences in proton rotating-frame relaxation time constants ($T_{1\rho}\text{H}$).



$T_{1\rho}\text{H}$ measurements of pretreated yellow poplar (PYP) indicated the presence of two distinct phases.

²R. H. Newman and J. A. Hemmingson, (1990), *Holzforschung*, Determination of the degree of cellulose crystallinity in wood by carbon-13 nuclear magnetic resonance spectroscopy, 44, 351-355.

NMR experiment



The total intensity of the NMR signal at $\tau=0$ is equal to the sum of the crystalline and amorphous components

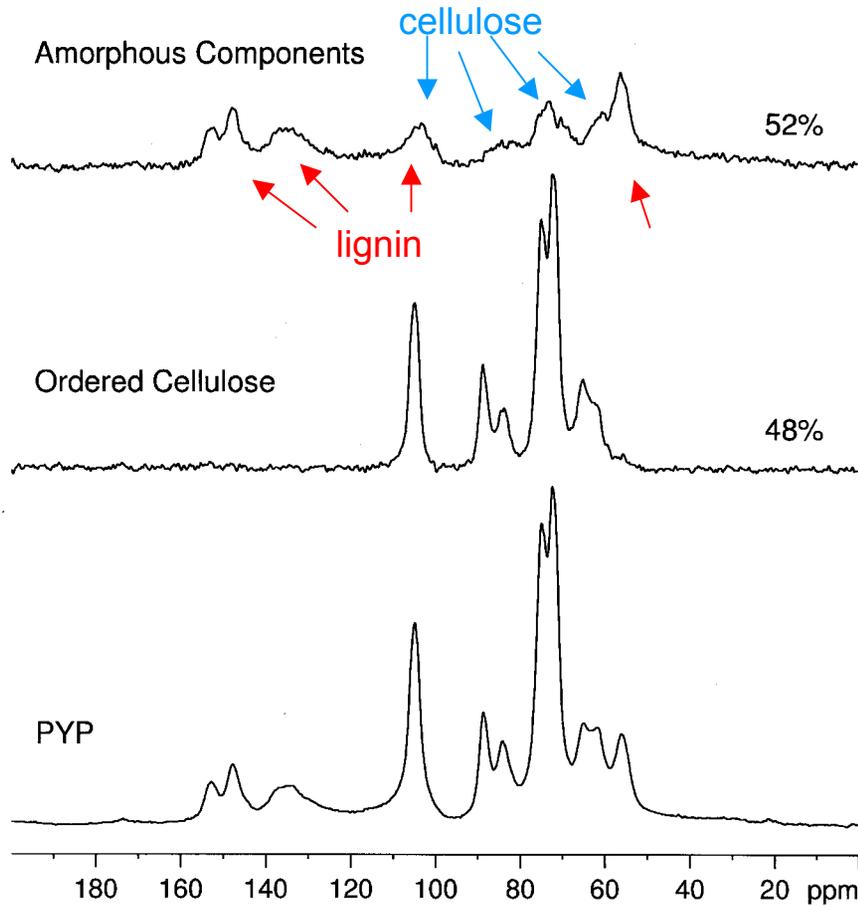
$$I_{\text{total}} = I_{\text{amorphous}} + I_{\text{crystalline}}$$

The intensity at $\tau \neq 0$ depends on the $T_{1\rho}\text{H}$ values of each component

$$I(\tau) = \exp(-\tau / T_{1\rho}\text{H}_{\text{amorphous}}) I_{\text{amorphous}} + \exp(-\tau / T_{1\rho}\text{H}_{\text{crystalline}}) I_{\text{crystalline}}$$

A component “subspectrum” can be found by solving the two equations and using the resulting constants in a linear combination of spectra generated with 2 τ values.

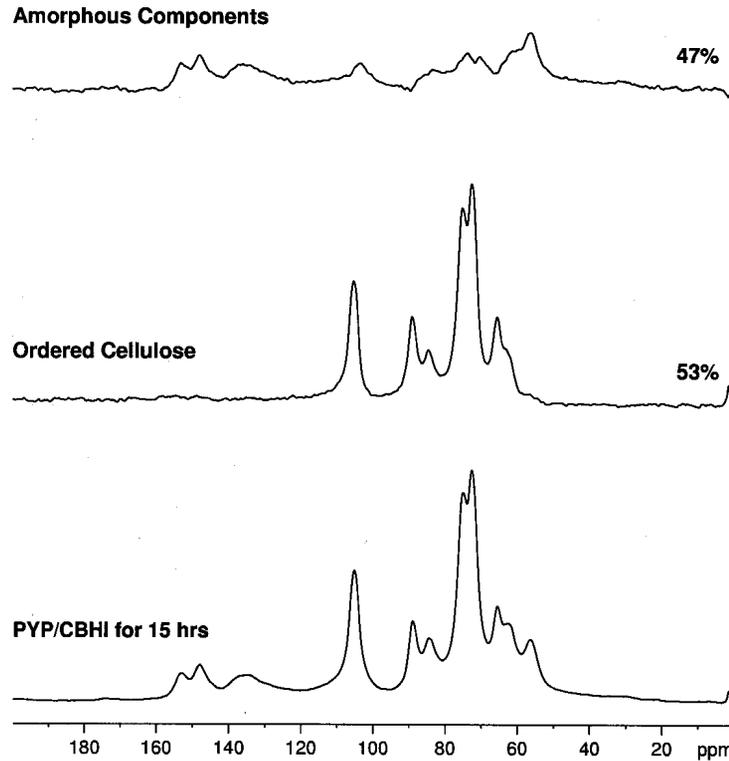
Component subspectra of PYP



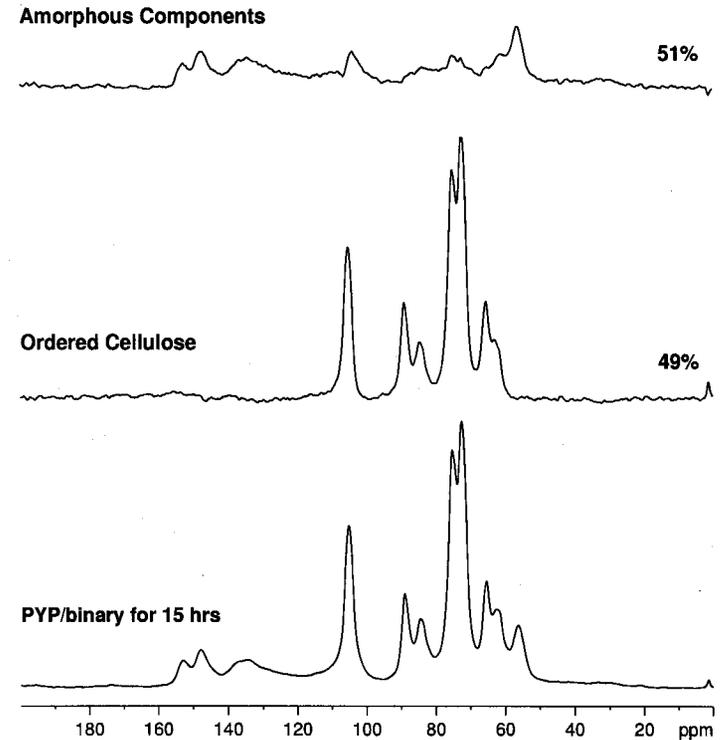
Calculations based on chemical composition data indicate there is ~10 to 15% amorphous cellulose

NMR determination of cellulose crystallinity (low conversion)

CBHI after 15hrs
(11% conversion)



CBHI/E1 Binary after 15hrs
(16% conversion)

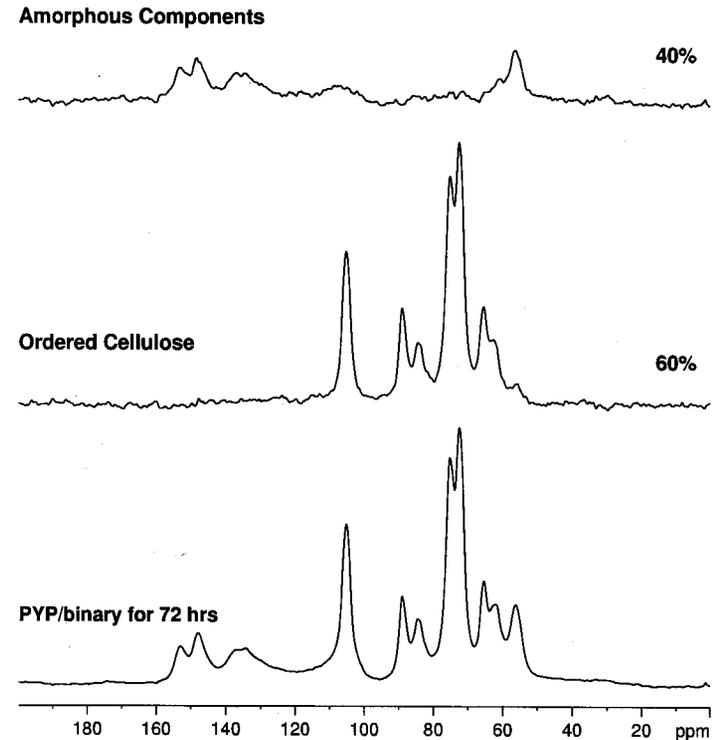


The proportions of crystalline and amorphous cellulose has not changed at lower conversion levels.

NMR determination of cellulose crystallinity (higher conversion)

CBHI/E1 Binary mixture

sample	% conversion	% crystallinity
CBHI	34	66
E1	13	60
Binary	45	60

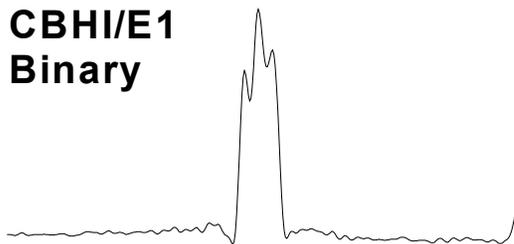


The proportion of crystalline cellulose has increased at higher conversion levels. Close examination of the amorphous components indicates almost complete removal of the amorphous cellulose.

Reactivity of cellulose polymorphic forms

Lower % conversion

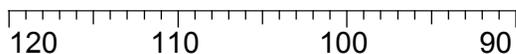
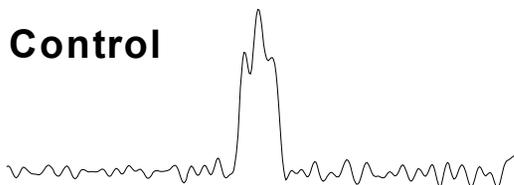
**CBHI/E1
Binary**



CBHI



Control

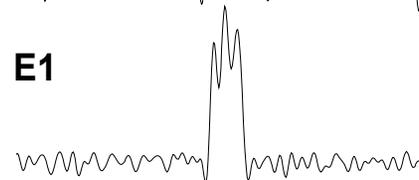


Higher % conversion

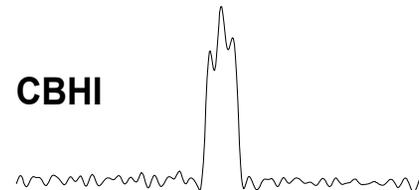
Binary



E1



CBHI



Control



Resolution enhancement of peaks at 105 ppm peak indicates cellulose I β increases with respect to cellulose I α as the enzymatic digestion progresses. At lower conversions, the CBHI appears to be selectively digesting I α . However, at the higher conversions, the change in I α with respect to I β does not seem to be as large.

Summary

Solid state ^{13}C NMR studies on the enzymatic digestion of pretreated yellow poplar have demonstrated:

- Cellulose in PYP is ~ 10 to 15% amorphous and the remaining 85-95 % is highly ordered.
- The amorphous cellulose component are preferentially removed as the enzymatic digestion proceeds by both the endoglucanase, CBHI and exoglucanase, E1 .
- There is evidence for selective removal of cellulose $\text{I}\alpha$ with respect to cellulose $\text{I}\beta$. This is most evident at lower conversion (10-15%) for both E1 and CBHI. At the higher conversions, the selective removal was less evident for CBHI and the E1/CBHI binary mixture.

Acknowledgments

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