Changes in cellulose morphology of pretreated yellow poplar during enzymatic hydrolysis

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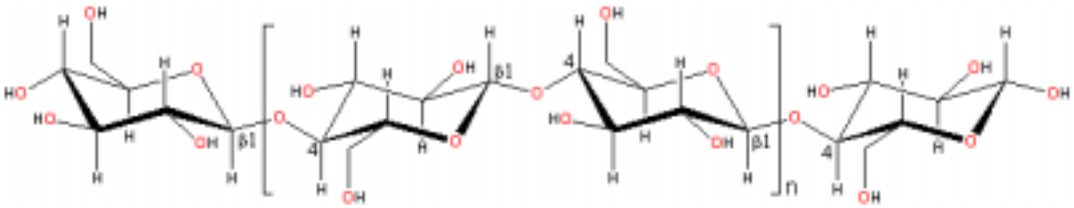
Abstract

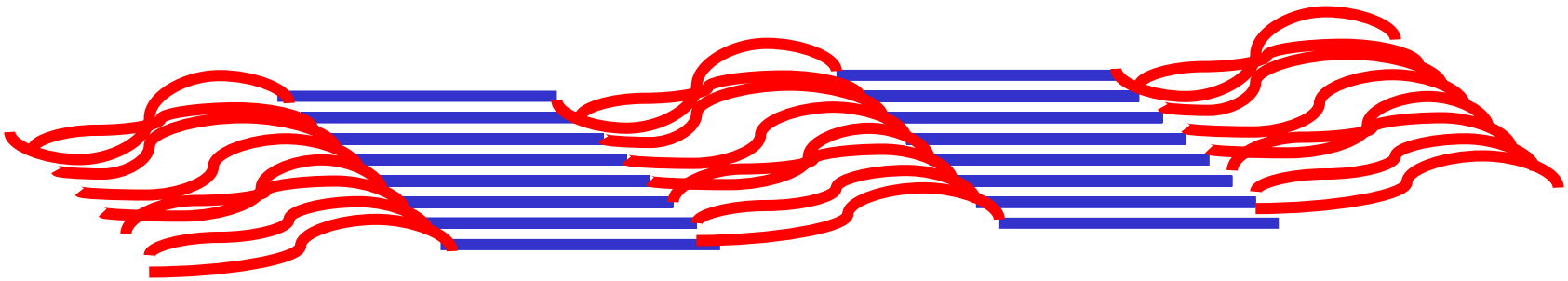
We have used solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy to study the changes in the cellulose morphology that occur during the enzymatic hydrolysis with an endoglucanase, E1, an exoglucanse, CBHI, and a binary mixture of E1 and CBHI (5/95 mixture). The NMR studies have observed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

Introduction



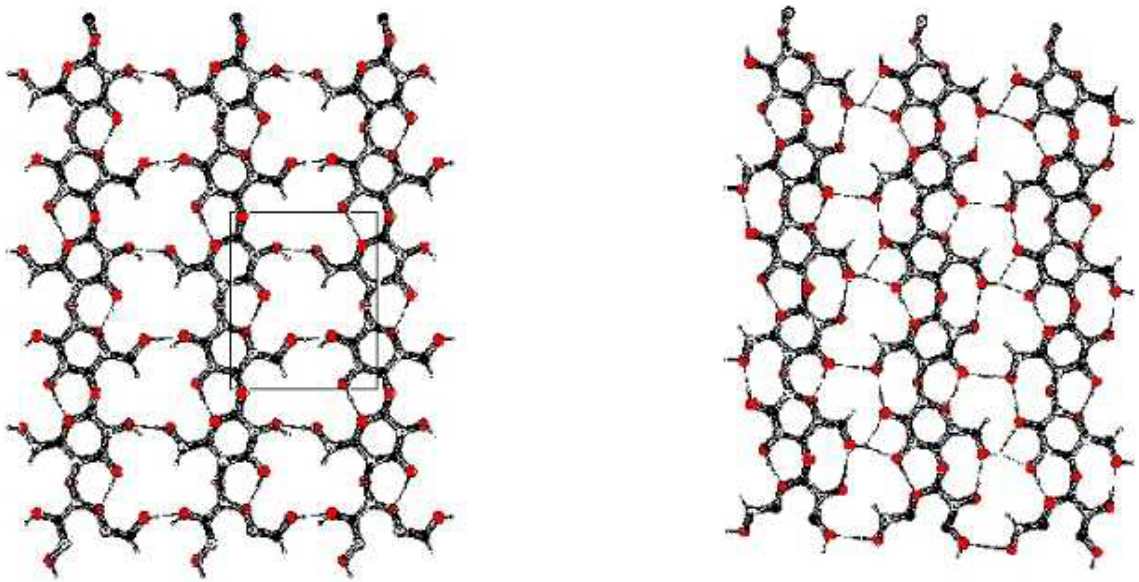
Cellulose

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).

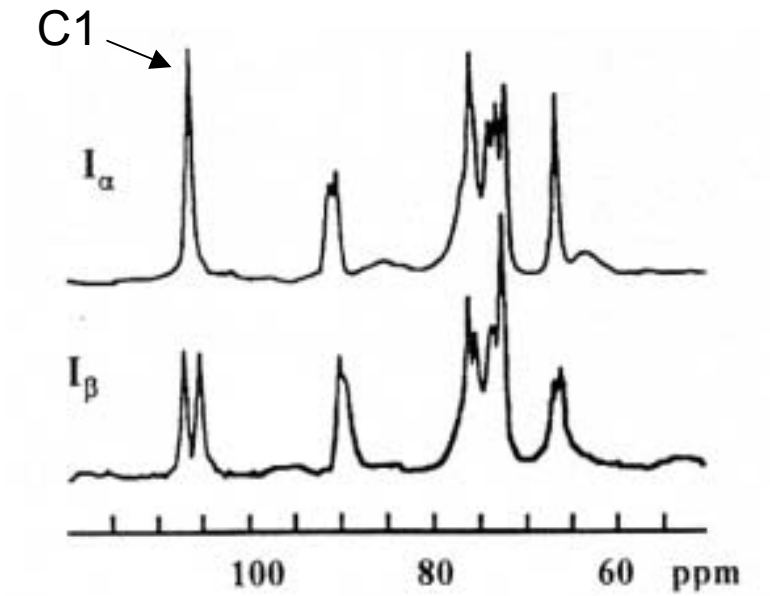
Native crystalline cellulose is generally found as a mixture of cellulose Iα and cellulose Iβ polymorphic forms. The proportions of the two allomorphs of crystalline cellulose vary depending on the source of the cellulose. Higher plants such as trees and corn believed to have a higher proportion of cellulose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



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

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

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

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

[3]J-F Sassi et al, (2000) , Cellulose, Relative susceptibility of the Iα and Iβ phases of cellulose towards acetylation, 7, 119-132.

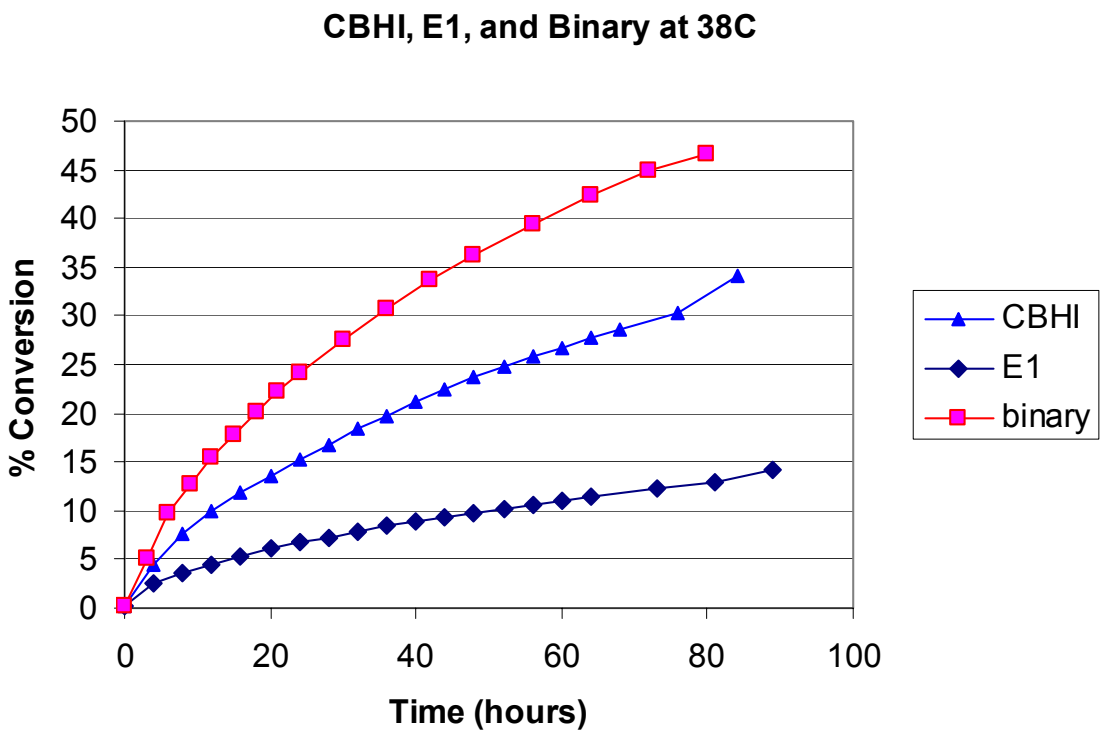
Sample Preparation

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.

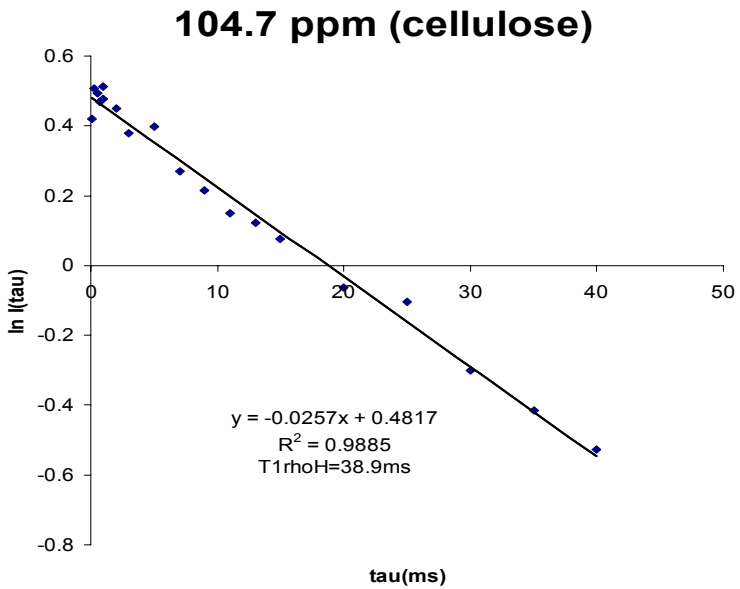
DSA curves

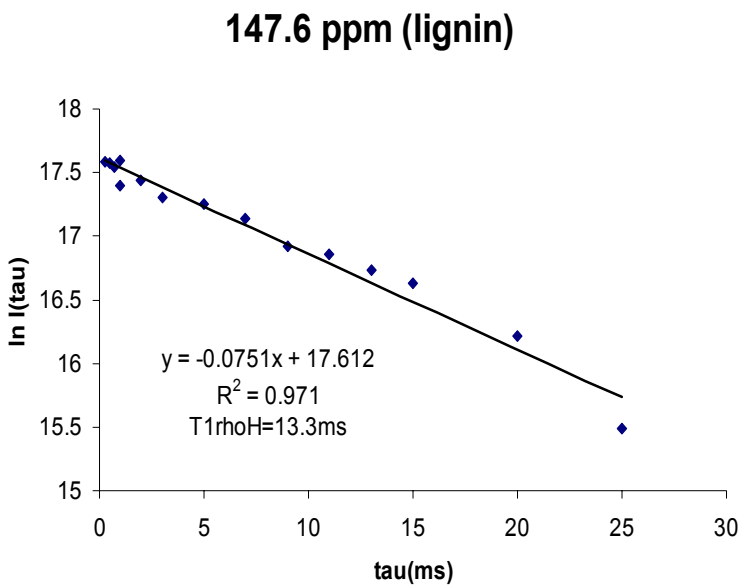


[3]J.O. Baker *et al*, (1997) , Applied Biochemistry and Biotechnology, Use of a new membrane-reactor saccharification assay to evaluate the performance of cellulases under simulated SSF conditions, 63-65, 565-595.

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 materials[2]. The different components can be distinguished because of differences in proton rotating-frame relaxation time constants ($T_{1\rho}H$).

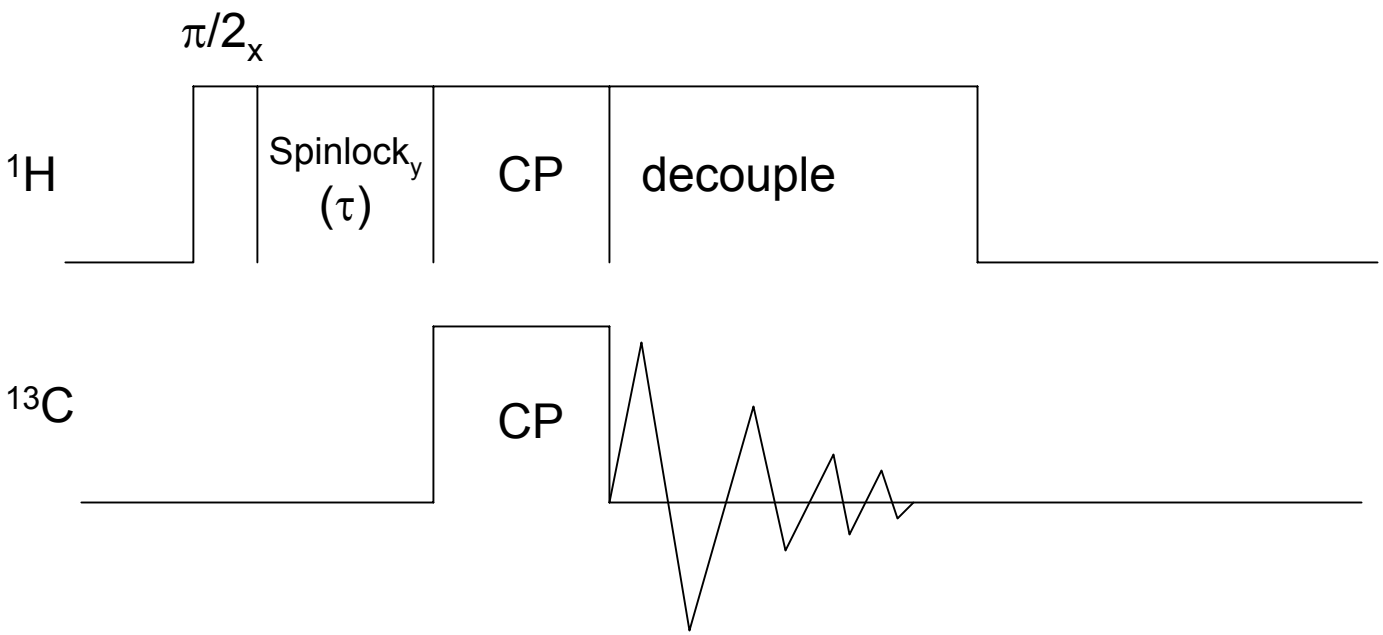




$T_{1\rho}H$ measurements of pretreated yellow poplar (PYP) indicated the presence of two distinct phases.

[2]R. 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

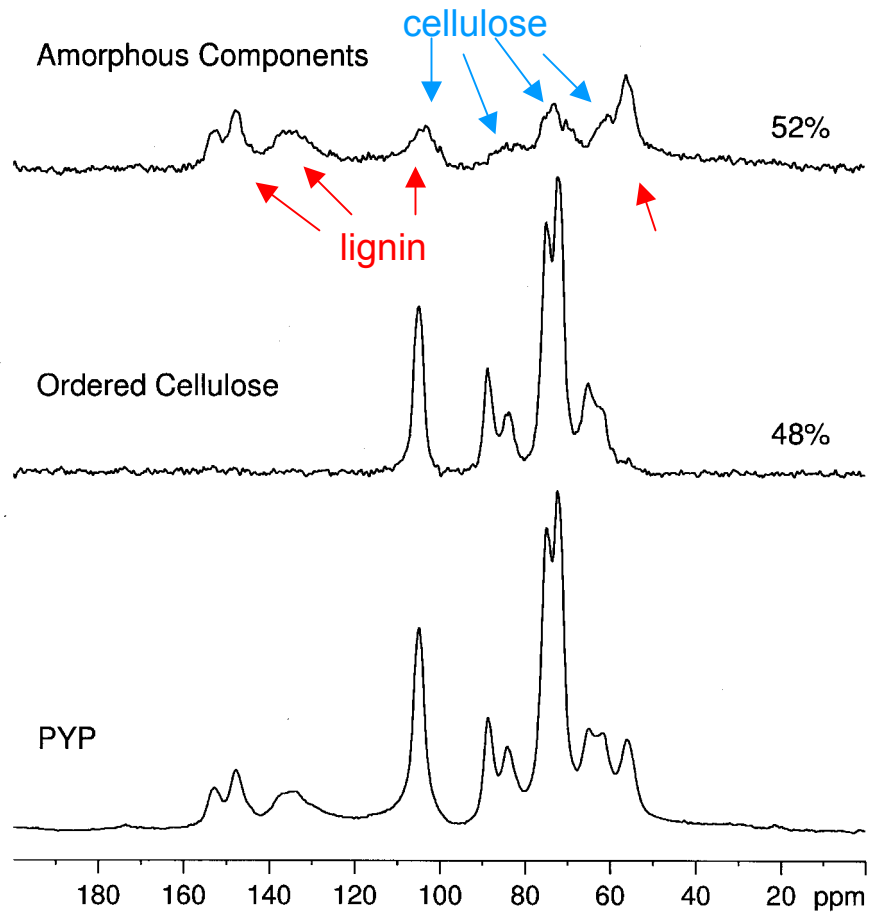
$I_{total} = I_{amorphous} + I_{crystalline}$

The intensity at $\tau\neq0$ depends on the $T_{1\rho}H$ values of each component

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

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.

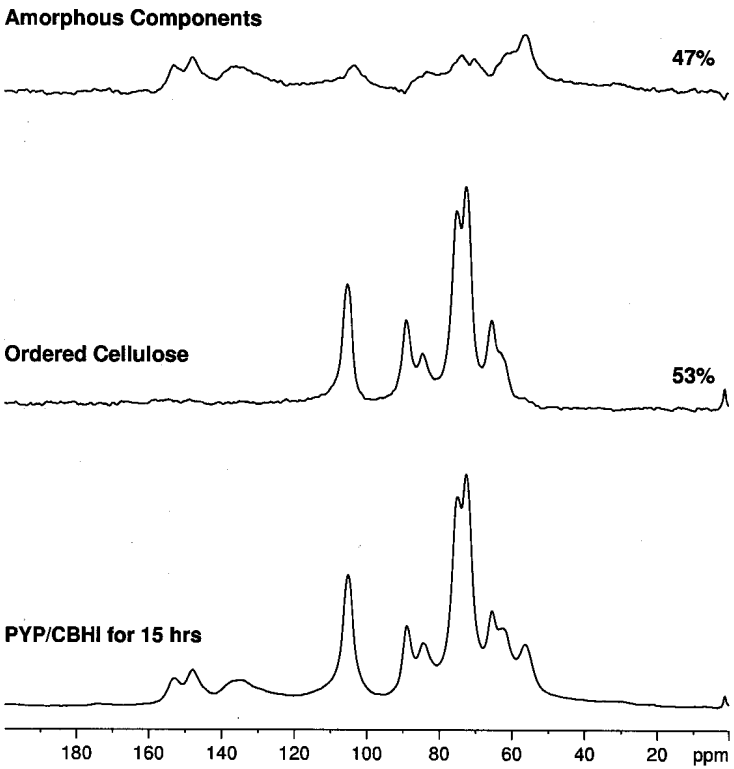
Component subspectra of PYP



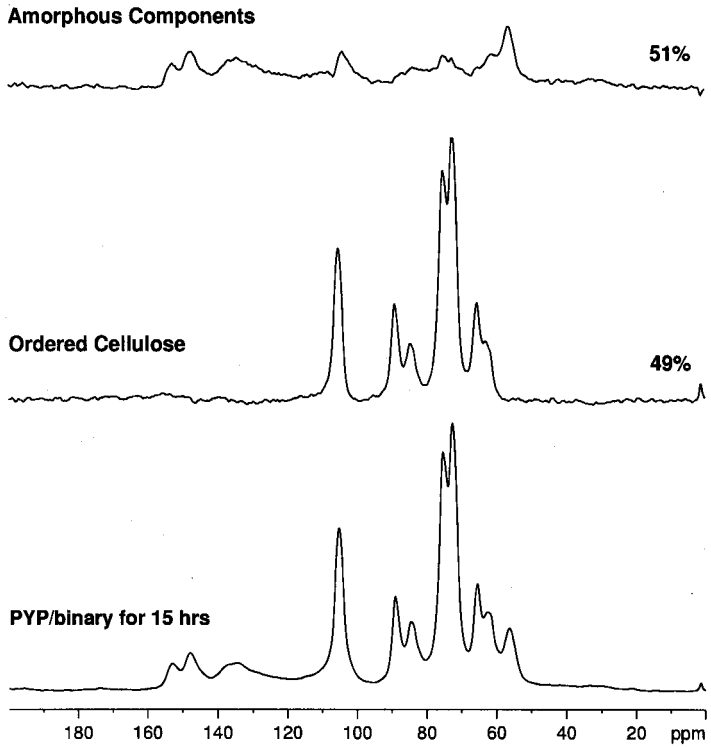
Calculations based on chemical composition data indicate there is ~10 to 15% amorphous cellulose

NMR determination of cellulose crystallinity (low conversion)

CBHI after 15hrs
(11% conversion)



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

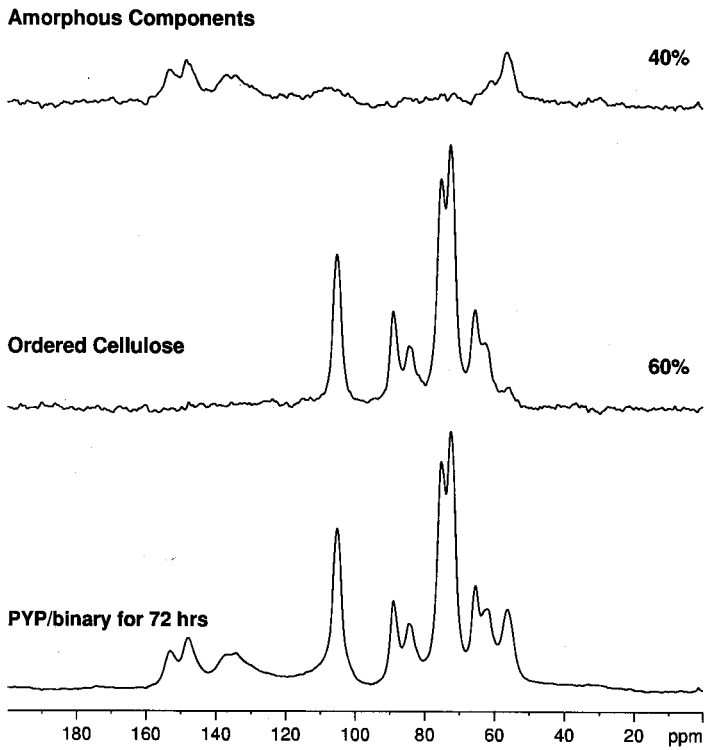


The proportions of crystalline and amorphous cellulose has not changed at lower conversion levels.

NMR determination of cellulose crystallinity (higher conversion)

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

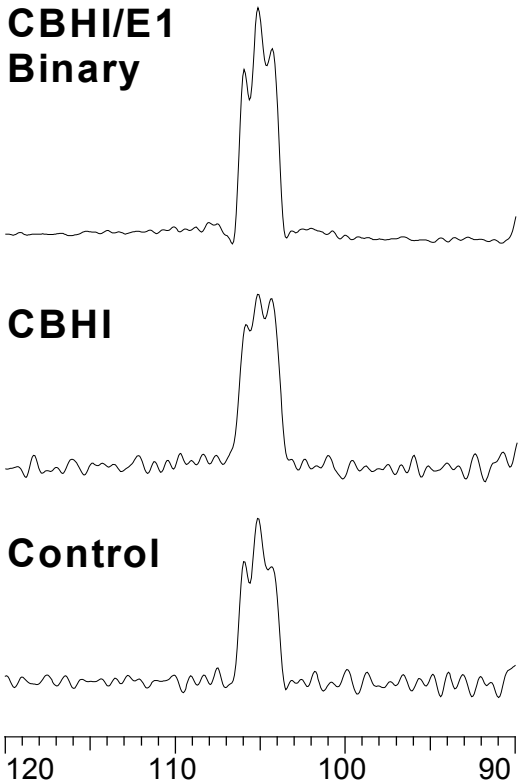
CBHI/E1 Binary mixture



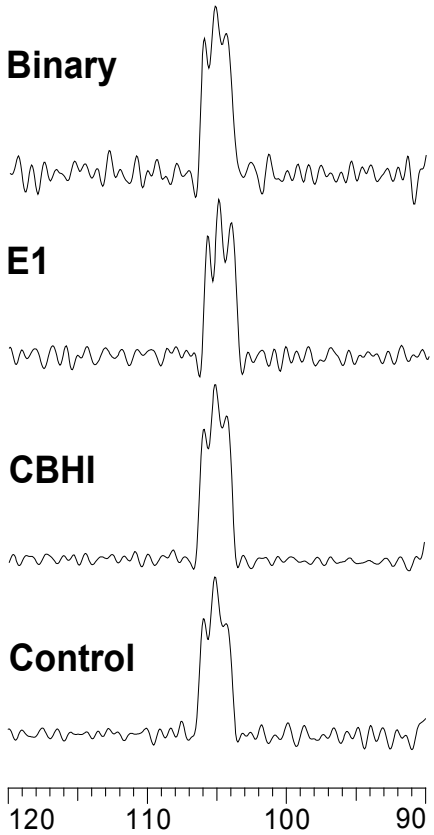
The proportion of crystalline cellulose has increased at higher conversion levels. Close examination of the amorphous components indicates almost complete removal of the amorphous cellulose.

Reactivity of cellulose polymorphic forms

Lower % conversion



Higher % conversion



Resolution enhancement of peaks at 105 ppm peak indicates cellulose Iβ increases with respect to cellulose Iα as the enzymatic digestion progresses. At lower conversions, the CBHI appears to be selectively digesting Iα. However, at the higher conversions, the change in Iα with respect to Iβ 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 and the remaining 85-95 % is highly ordered.

•The amorphous cellulose component are preferentially removed as the enzymatic digestion proceeds by both the endoglucanse, CBHI and exoglucanase, E1 .

•There is evidence for selective removal of cellulose Iα with respect to cellulose Iβ. 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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