

Mechanism of Nanocrystalline Cellulose Decrystallization during Enzymatic Hydrolysis

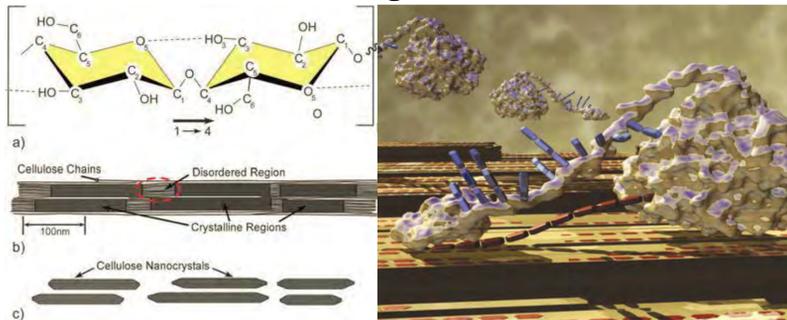


Project Overview

This project involves using Nanocrystalline cellulose (NCC) to probe the decrystallization mechanism of cellulose during enzymatic hydrolysis, providing experimental evidence in the hope of contributing to the fundamental understanding of the native cellulose deconstruction at molecular level during biofuel production process.

- ❖ Characterization of NCC— dimensions and crystallinity
- ❖ Improved X-ray diffraction analysis—Crystallinity Index (CrI)
- ❖ Enzymatic decrystallization of NCC—Enzyme-cellulose interaction
- ❖ Discussion on mechanism of NCC decrystallization at molecular level during enzymatic hydrolysis

Background



Moon, R. J. et al. *J. Chem Soc Rev* **2011**, 40, 3941-3994

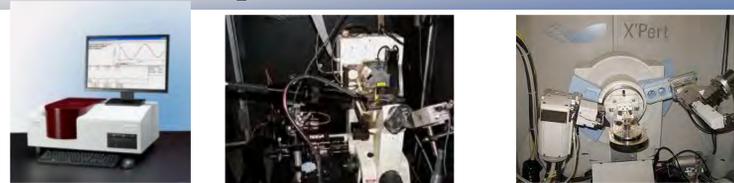
Himmel, M. E et al. *Science* **2007**, 315,804-7

Dimensions of cellulose nanocrystals from various sources

Cellulose type	length	Cross section
Tunicate	100nm –several μ m	10-20 nm
Bacterial	100nm –several μ m	5-10 nm by 30-50 nm
Algal (Valonia)	> 1000 nm	10 to 20 nm
Cotton	200-350 nm	5 nm
Wood	100-300 nm	3-5 nm diameter

Beck-Candanedo, S. et al. *Biomacromolecules* **2005**, 6, 1048-1054

Experimental Procedure



Particle size analyzer

AFM

X'pert XRD



¹³C NMR



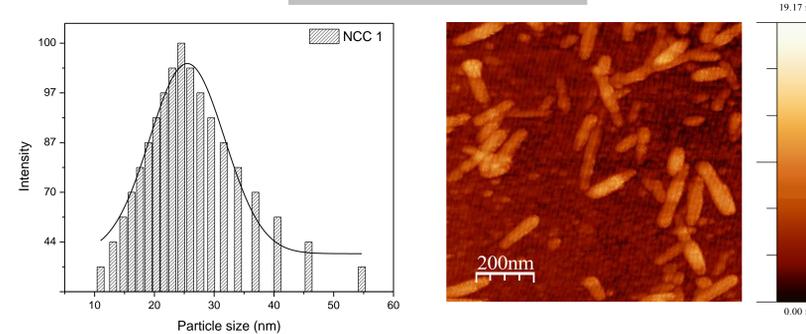
Enzymatic hydrolysis incubator



96-well microplate sugar measurement

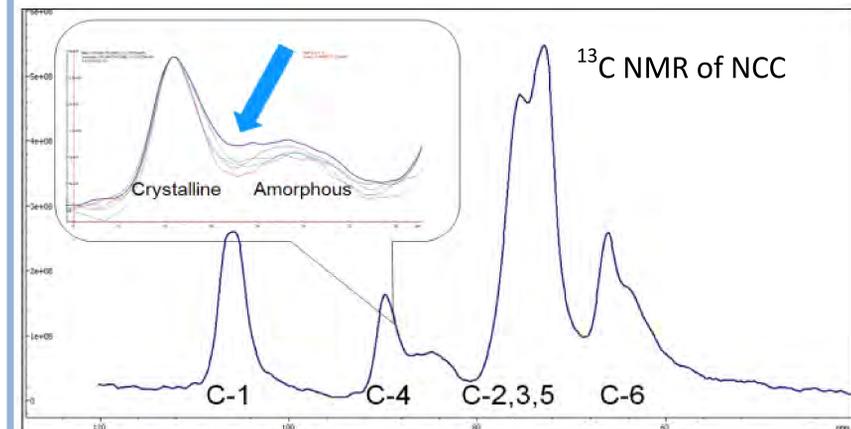
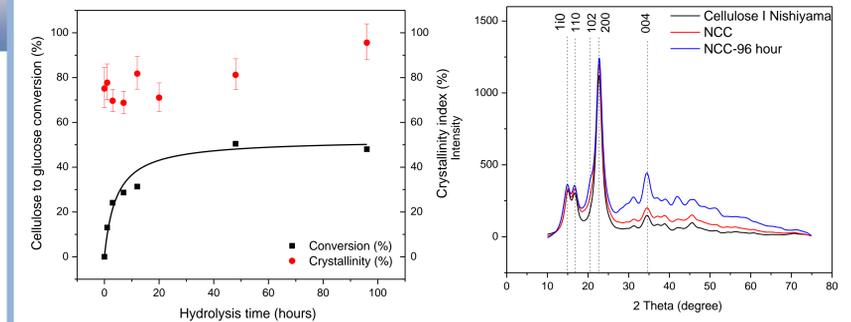
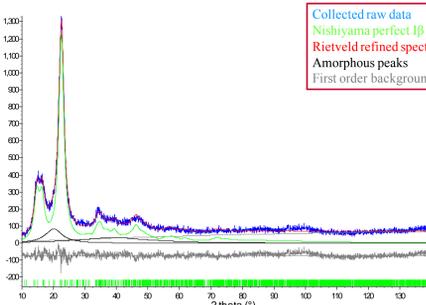
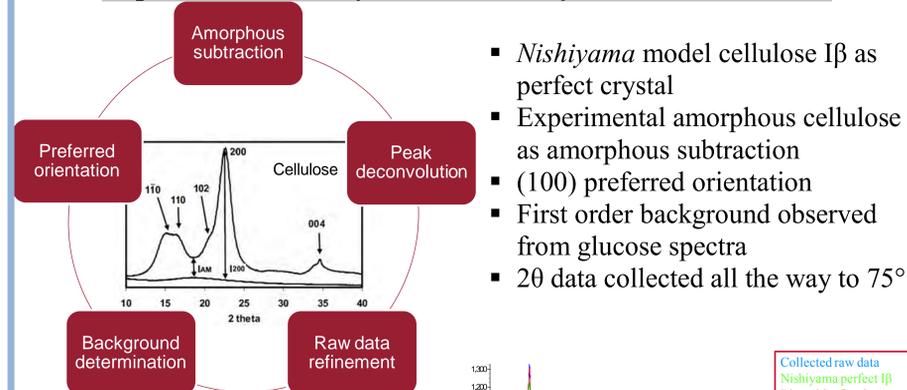
Results and Discussion

Characterization of NCC

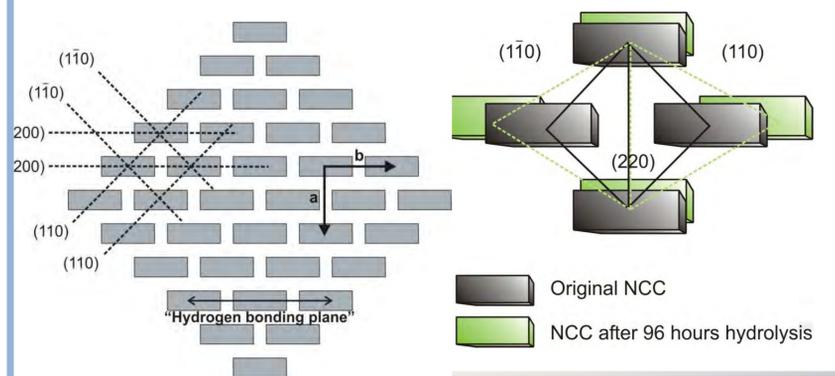


- ❖ NCC: particle size ~3-5 nm in width, ~200 nm in length
- ❖ NCC degree of polymerization: 90-96

Improvement of X-ray diffraction analysis for CrI calculation



Proposed Mechanism of NCC decrystallization



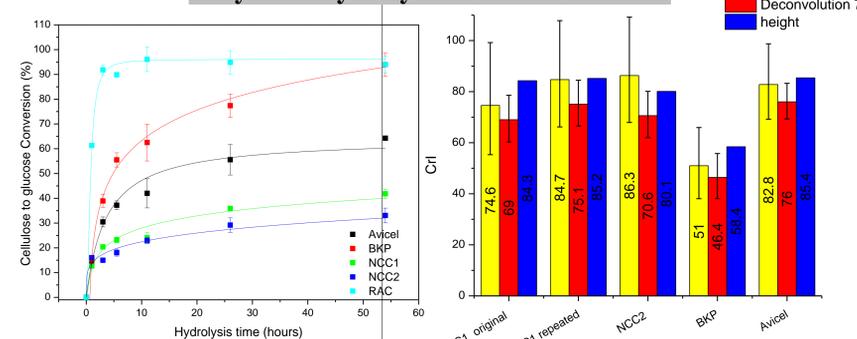
Summary

- ❖ An improved X-ray diffraction (XRD) analysis was developed to examine the crystalline structural changes of NCC during enzymatic hydrolysis.
- ❖ Results showed that NCC was attacked from (110) and (1 $\bar{1}$ 0) plane where there was a shift between layers that resulted to 2% elongation along both directions.
- ❖ Proposed mechanism of decrystallization indicated a decreased size of crystallite by chain opening structure instead of hydrogen bond breakage.

Acknowledgement

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Enzymatic hydrolysis of Model Cellulose



Enzymatic decrystallization of NCC analyzed by XRD and NMR

Samples	d-spacing (nm)		Crystallite size (nm)				CrI (%)		
	(1 $\bar{1}$ 0)	(110)	(200)	(004)	(200)	(004)	CrI	CrI	
Cellulose I- β (Nishiyama)	0.58	0.53	0.39	0.26	4.02	5.15	5.58	4.01	75.11 \pm 9.38
NCC	0.58	0.53	0.39	0.26	4.02	5.15	5.58	4.01	75.11 \pm 9.38
NCC after 96 hour hydrolysis	0.59	0.54	0.39	0.26	2.60	2.80	4.57	3.62	91.70 \pm 6.76