NC STATE UNIVERSITY

2013 DOE Bioenergy Technologies Office (BETO) Project Peer Review

WBS: 7.1.4.1

Integrated Biomass Refining Institute at North Carolina State University

May 23, 2013

Technology Area Review: Biochemical Conversion
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Organization: North Carolina State University

NC STATE achieve! Innovation in Action

Goal Statement

- Biomass Pretreatment and Reactivity of the Resulting Sugars
  - How does residual lignin effect enzymatic hydrolysis?
  - How can residual lignin and LCC be characterized?
  - How does residual hemicellulose affect enzymatic hydrolysis?
  - Are coupled mechanical/enzymatic pretreatment/hydrolysis scenarios effective?
  - How can complex fermentations be analyzed on-line and subsequently modeled?

- Focus on feedstocks and conversion system of interest across the Southeastern US.

Quad Chart Overview

Timeline

- Project Initiated: 4/22/09
- Project end date 3/31/13
- 100% complete

Barriers Addressed

- Bt-C. Biomass Recalcitrance: Critical physical and chemical properties that determine the susceptibility of cellulosic substrates to hydrolysis
- Bt-D. Pretreatment Chemistry: Role that lignin and other pretreatment products play in impeding access to cellulose on a molecular level
- Bt-J. Catalyst Development: Improvement in the robustness of catalysts and their ability to perform in hydrolysate broths can significantly lower capital costs

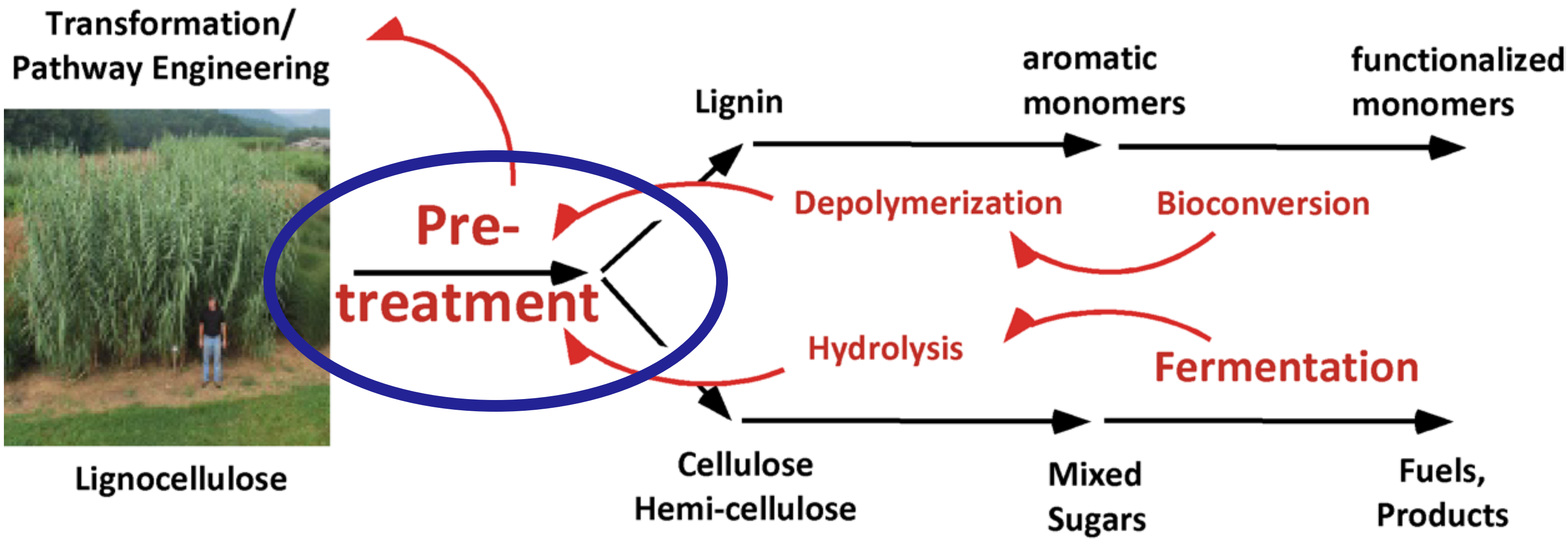
Budget

Funding for FY09 ($984,000 / $247,370)
Funding for FY10 ($1,208,405 / $302,102)
Funding for FY11 ($1,000,000 / $250,000)
Years the project has been funded: 3
/average annual funding: $1,064,135

Partners

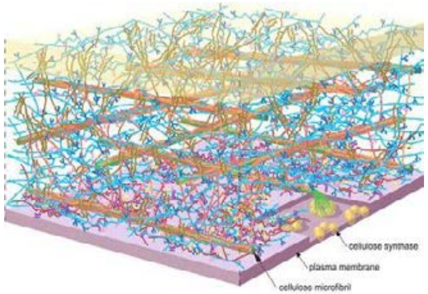
- Novozymes - Benchmark pretreatment technologies for different biomass vis-a-vis ethanol production

Project Overview



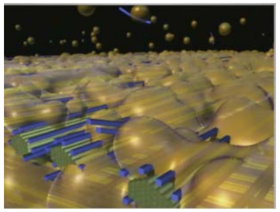
- Pretreatment informs the ultimate fates of sugars and lignin; it is the central processing step linking biomass composition with biomass utilization. Optimal processing effectiveness suggests the establishment of feedback loops whereby downstream performance and capabilities inform the development of upstream catalysts and processes, whether one is considering sugar or lignin processing. Our emphasis is on sugars at this time, and we are working to effectively bridge pretreatment technologies with fermentative capabilities.

Biomass Reactivity



Lignocellulosic biomass

Pretreatment

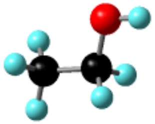


Pretreated biomass

Enzyme hydrolysis

Monomeric sugar

Fermentation



Ethanol

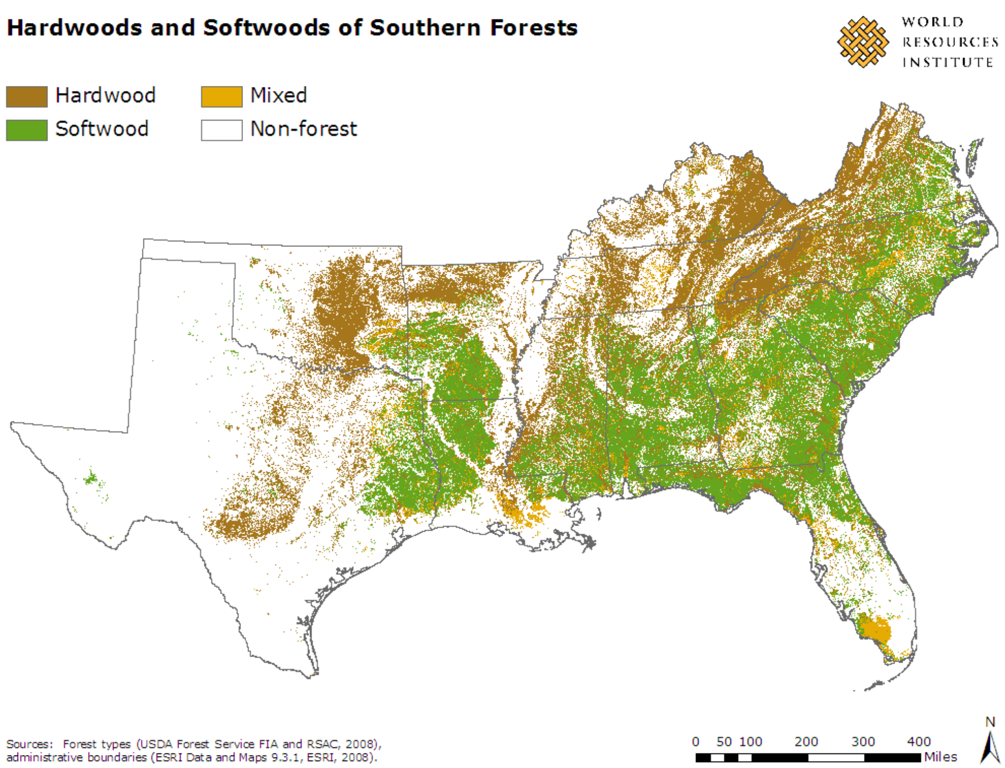
Biomass

- Lignin content
- Lignin structure/ Lignin-carbohydrate complex
- Distribution of lignin and hemicellulose (xylan)
- Cellulose crystalline structure/ Crystallinity index
- Cellulose degree of polymerization
- Pore volume and structure
- Enzyme penetration

Enzyme

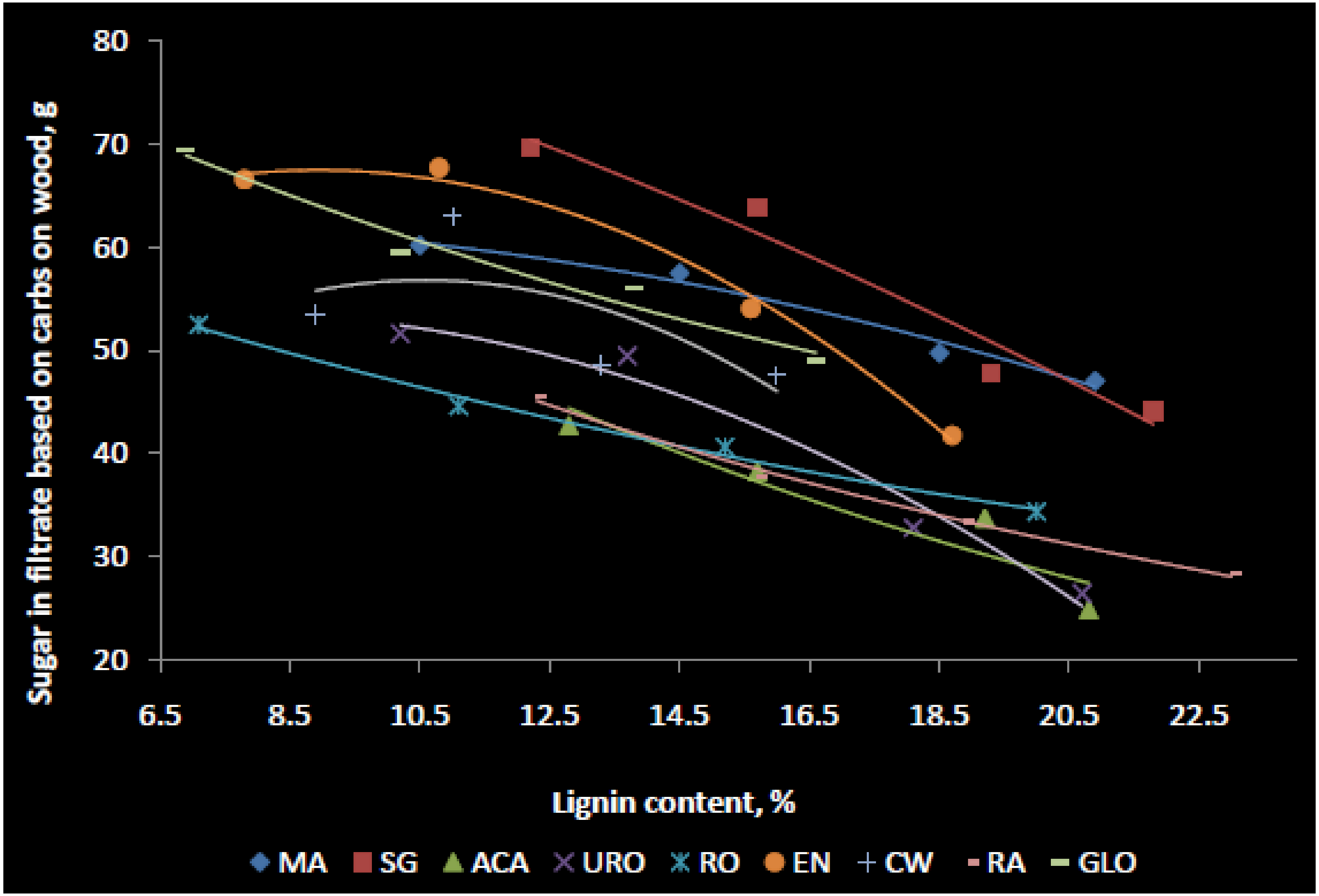
- Components
- Synergism
- Adsorption
- End-product inhibition
- Enzyme inactivation over time

Softwood: Dominant Biomass in Southeast



- Softwood, such as loblolly pine, accounts for 80% of planted forests in the southeast of America.
- 30 million acres of plantation pine on low cost land with high-density

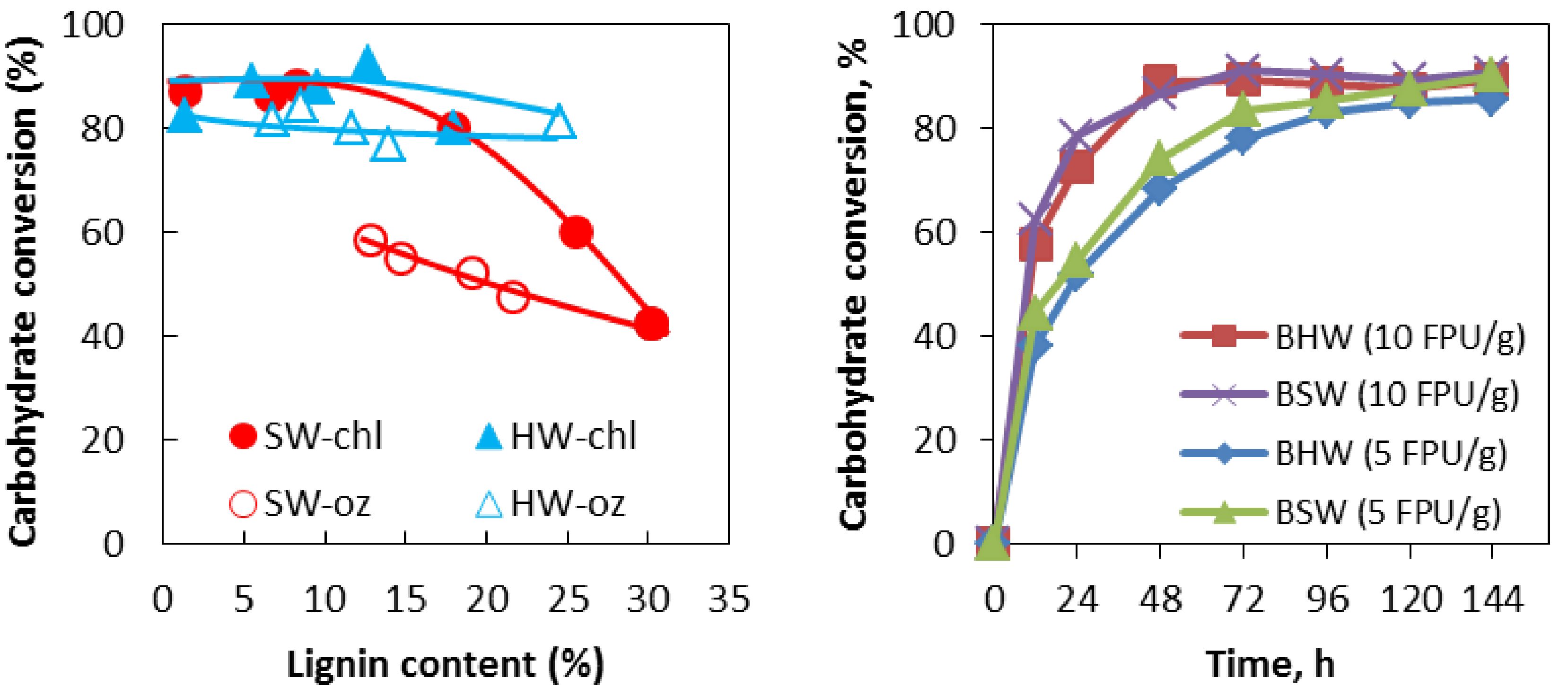
Hardwood Reactivity after Kraft Pulping



Red maple, sweet gum, acacia, *E. urograndis,* red oak, *E. nitens,* cottonwood, red alder, *E. globulus*

Hardwood vs. Softwood in Enzymatic Hydrolysis

BHW: fully bleached hardwood pulp
BSW: fully bleached softwood pulp

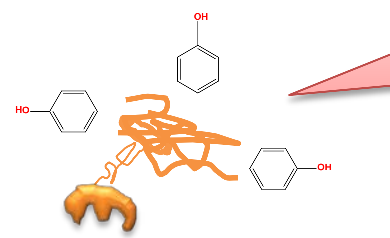


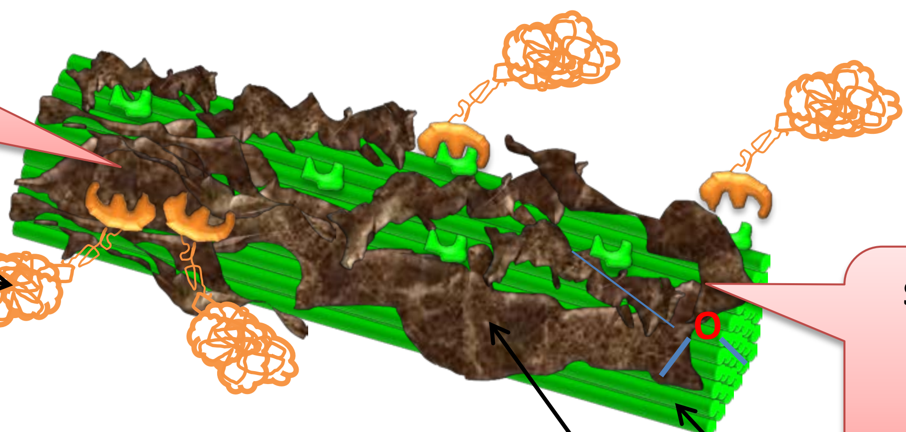
- **Softwood is more resistant to enzymatic hydrolysis than hardwood even at the same lignin content basis.**
- **The hydrolysis of bleached hardwood and softwood has no difference.**

The difference in <u>lignin chemistry and structure</u> might be an important factor determining the digestibility

Suggested Mechanisms of Lignin-induced Inhibition

- ❖ Non-productive binding
- ❖ Surface blockage (physical, chemical)
- ❖ Enzyme inhibition by soluble lignin
- ❖ Lignin spatial distribution





However, the exact mechanisms how cellulases interact with lignin during enzymatic hydrolysis have yet to be fully resolved.

Preparation of Milled Wood Lignins (MWLs)

Wood Substrates

❖ Untreated wood

- ✓ *Eucalyptus globulus*
- ✓ Red maple
- ✓ Loblolly pine

❖ Pretreated wood

- ✓ Auto-hydrolysis hardwood (Au-HW)
- ✓ Auto-hydrolysis softwood (Au-SW)

Wood chips of 600 od g were loaded in each batch. The water to solid ratio was 4:1 (v/w). The pretreatment was conducted at 180 °C for 1h.

- ✓ Green liquor hardwood (GL-HW)
- ✓ Green liquor softwood (GL-SW)

Wood chips of 800 od g was mixed with GL with a sulfidity of 40%. GL to wood ratio was 4:1 (v/w). The TTA charge was 16% and 20% for hardwood and softwood, respectively. The pretreatment conditions for softwood and mixed hardwood were H-factor of 800 at 170°C and H-factor of 400 at 160 °C, respectively.

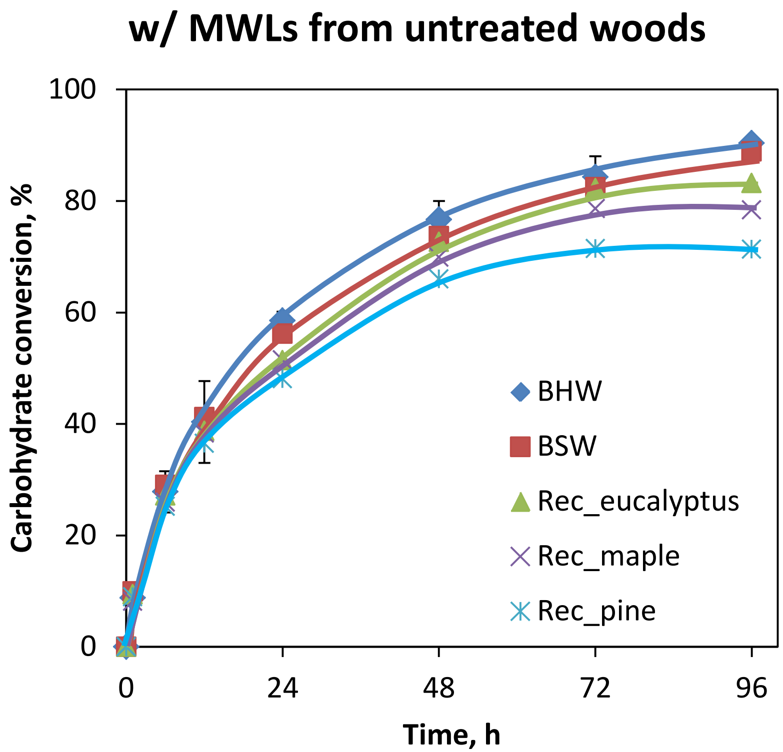
Wood substrate

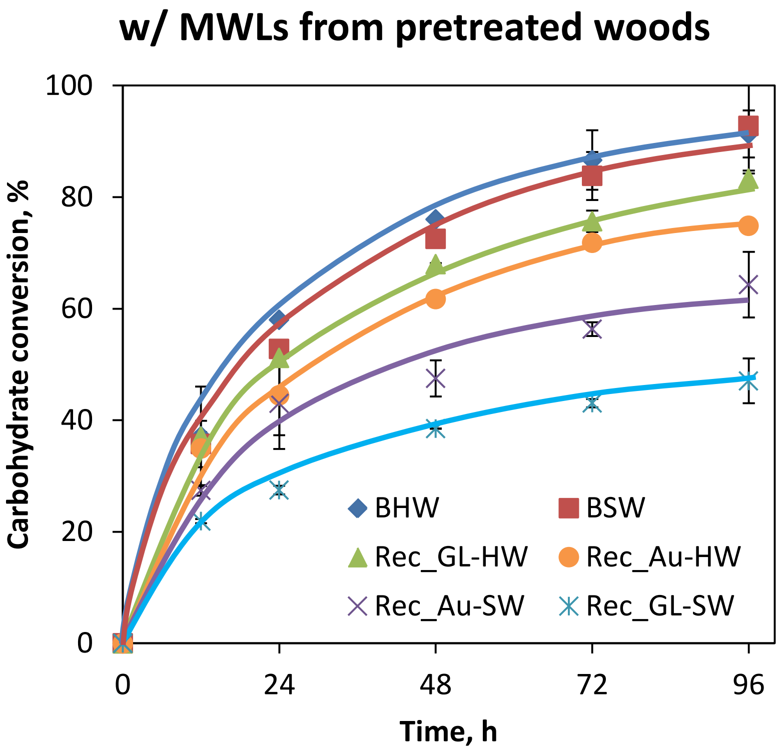
1. Wiley mill milling → **Wiley wood**
2. Extraction w/ benzene : ethanol (2:1 v/v) for 24 h → **Extractive free sawdust**
3. Ball milling 600 rpm, (30 min operation, 15 min suspension in each cycle) → **Ball milled wood**
4. Extraction with 96% dioxane for 3 times, 24 h each time → **Soluble** / **Residue**
5. Soluble: Freeze dry → **Crude MWL**
6. Purification w/ 90% acetic acid → **MWL**

Time of ball milling:

6 h for untreated HW
8 h for untreated SW
4 h for pretreated HW
6 h for pretreated SW

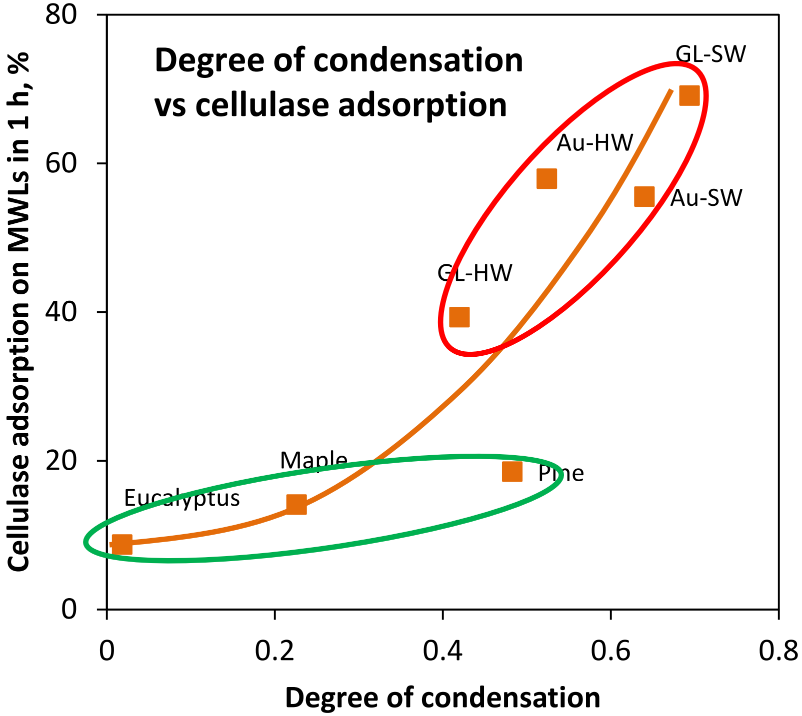
Enzymatic Hydrolysis: Reconstructed Substrates

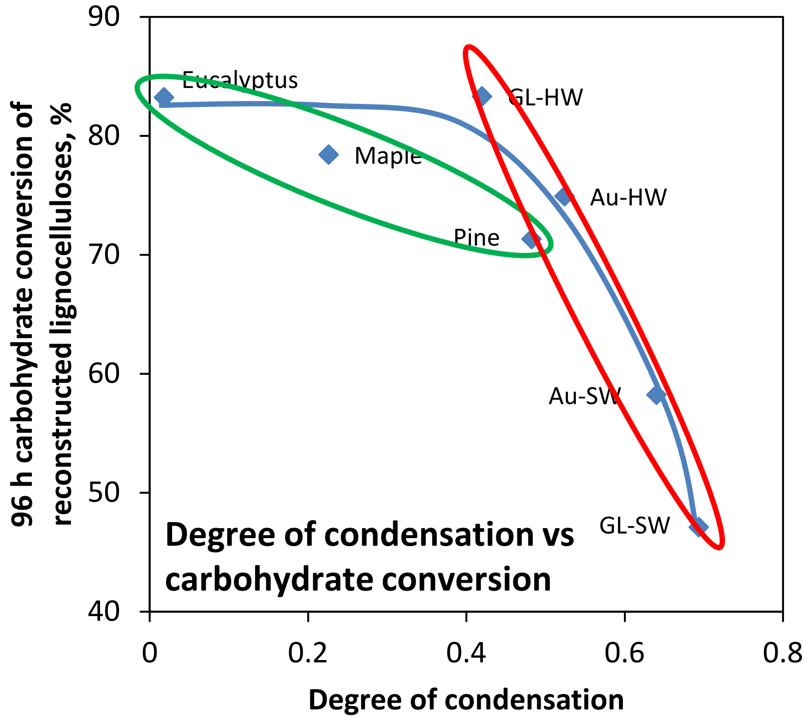




- w/ MWLs from untreated woods
  : BHW ≈ BSW > Rec_eucalyptus > Rec_maple > Rec_pine
- w/ MWLs from pretreated woods
  : BHW ≈ BSW > Rec_GL-HW > Rec_Au-HW > Rec_Au-SW > Rec_GL-SW

Degree of Condensation vs. Cellulase Adsorption/ Carbohydrate Conversion



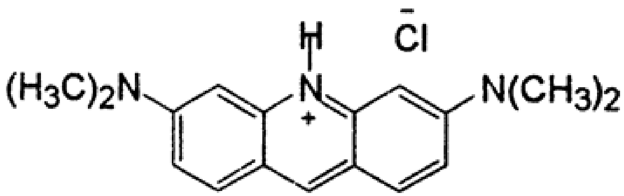


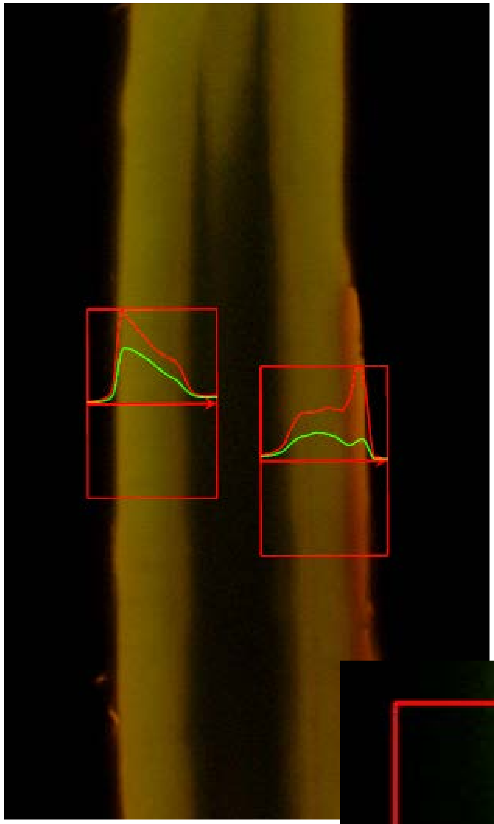
❖ **Degree of condensation of lignin showed a negative impact on cellulase adsorption and enzymatic hydrolysis.**

Confocal Microscopy: Lignin Distribution

- Acridine Orange (AO) Dye
  - $1.25x10^{-5}$ M in DI H_2O

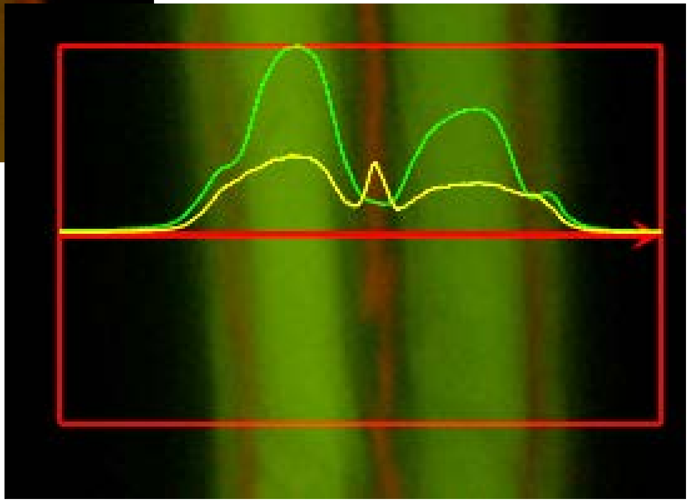
- **AO has emission shift when interacting with lignin: Green → Red**
  - Emission between 515 nm and 540 nm = Green
  - Emission above 590 nm = Red
  - **Lignin concentration is correlated to red/green intensities**



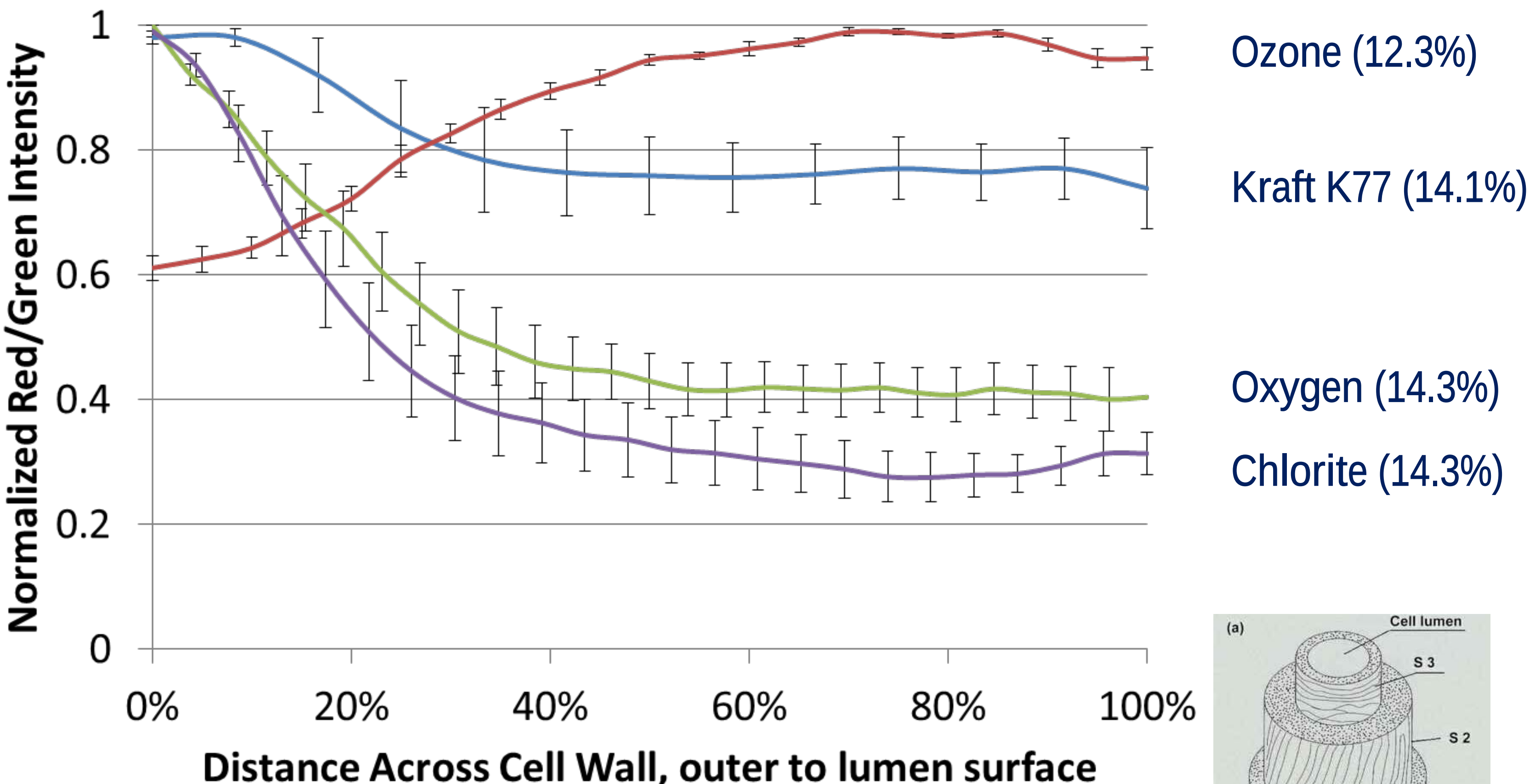


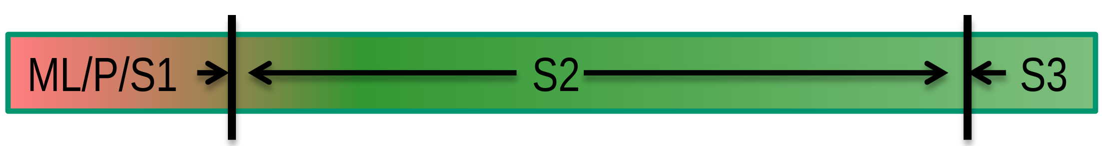
Alkaline pretreatment & oxygen delignification

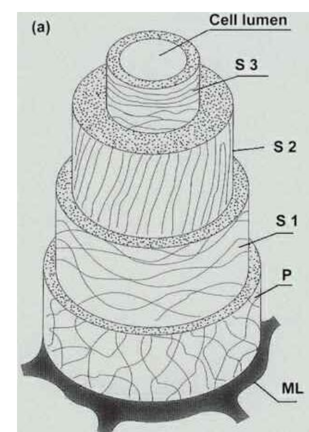
Reconstructed BSW with isolated pine lignin



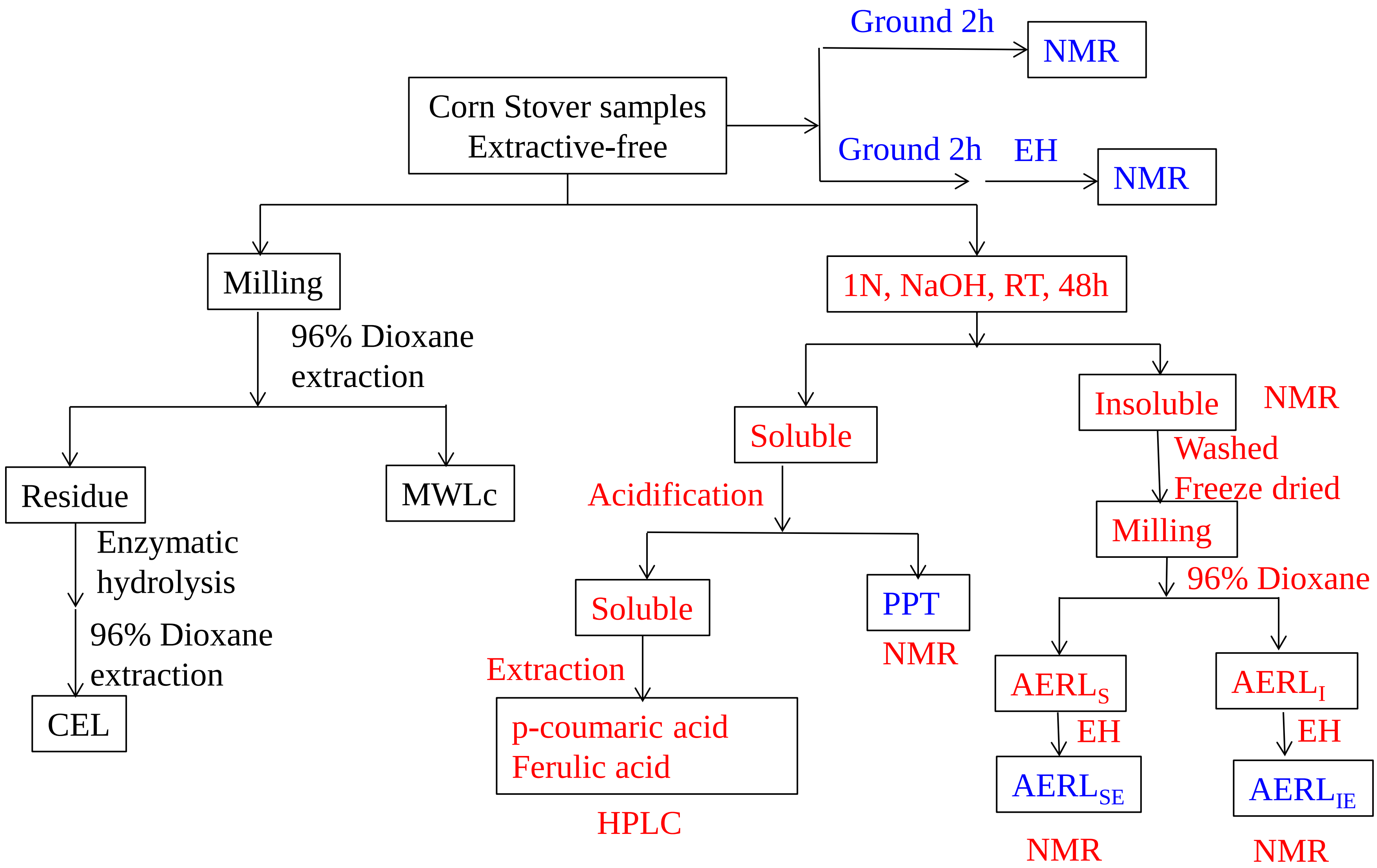
Lignin Distribution Across Cell Wall







Characterization of Lignin in Stem, Cob and Leaf of Corn Stover



Response to Alkaline Extraction

Composition of the extractive-free samples

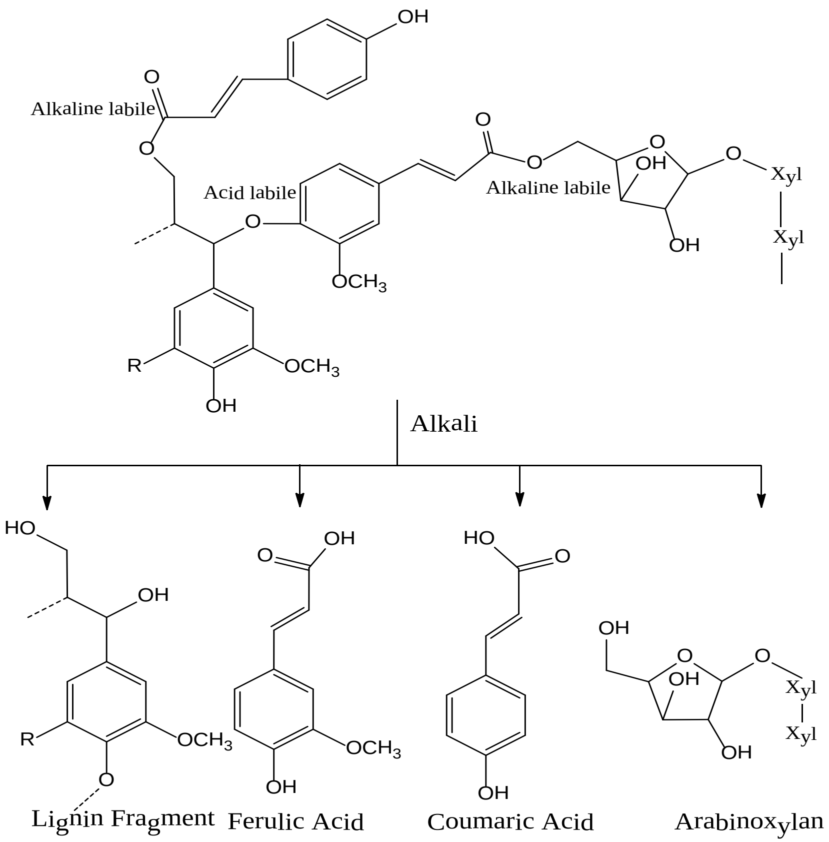
No.	%Ara	%Glu	%Xyl	%TC	%TL	%TB	%Ash
Stem	**2.0±0.08**	**36.1±0.36**	**20.8±0.12**	**59.7±0.60**	**22.3±0.24**	**82.1±0.36**	**7.8**
Cob	**2.9±0.09**	**32.4±0.66**	**28.9±0.51**	**65.2±1.24**	**17.4±1.86**	**82.6±3.10**	**2.0**
Leaf	**4.3±0.01**	**36.4±0.46**	**23.9±1.18**	**66.2±1.68**	**20.6±0.16**	**86.8±1.84**	**5.4**

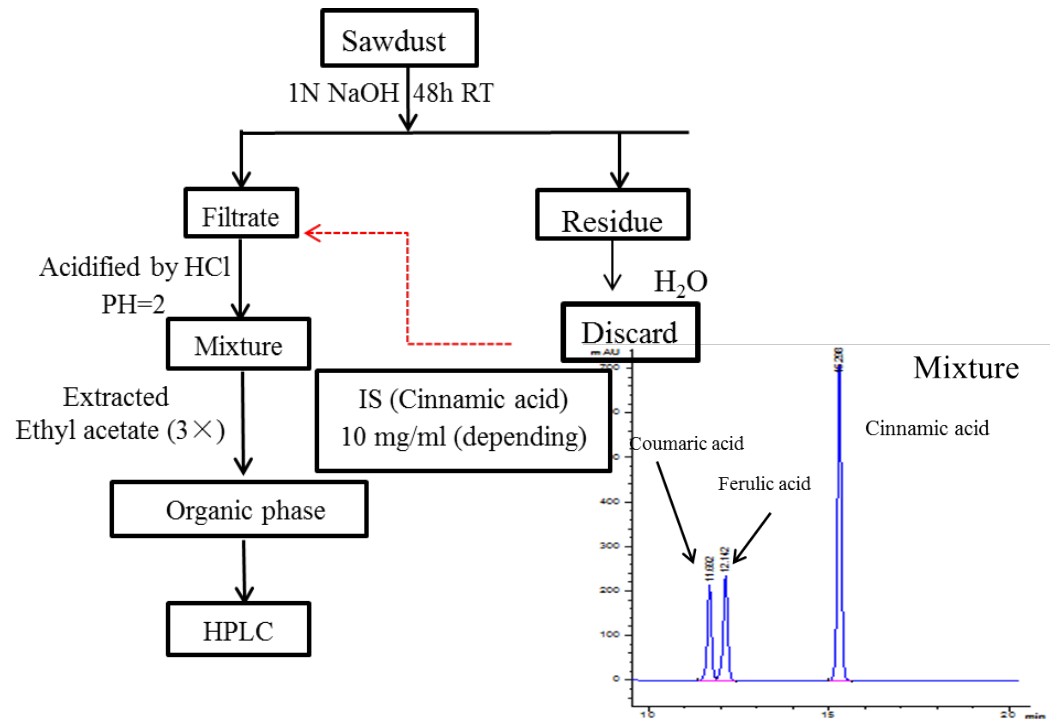
Composition of the alkaline extracted samples

No.	%Glu	%Xylan	%TC	%TL
Stem	**36.6±0.02**	**6.3±0.01 (30%)**	**44.1±0.02**	**5.3±0.01 (24%)**
Cob	**32.2±0.39**	**9.8±0.23 (34%)**	**43.6±0.09**	**3.4±0.01 (20%)**
Leaf	**33.0±0.24**	**6.4±0.01 (27%)**	**41.1±0.25**	**4.6±0.12 (22%)**

All values based on the extractive-free sample

Amount of *p*-coumaric and Ferulic Acid from CS





p-coumaric and ferulic acids quantification

Sample	Lignin(g)	% based on lignin		
		%Coumaric	%Ferulic	%Total
stem	**1.96**	**7.12**	**1.15**	**8.27**
cob	**1.74**	**6.33**	**2.60**	**8.93**
leaf	**1.97**	**2.06**	**0.66**	**2.72**

Note: % is based on lignin

^{13}C NMR of MWLc and CEL

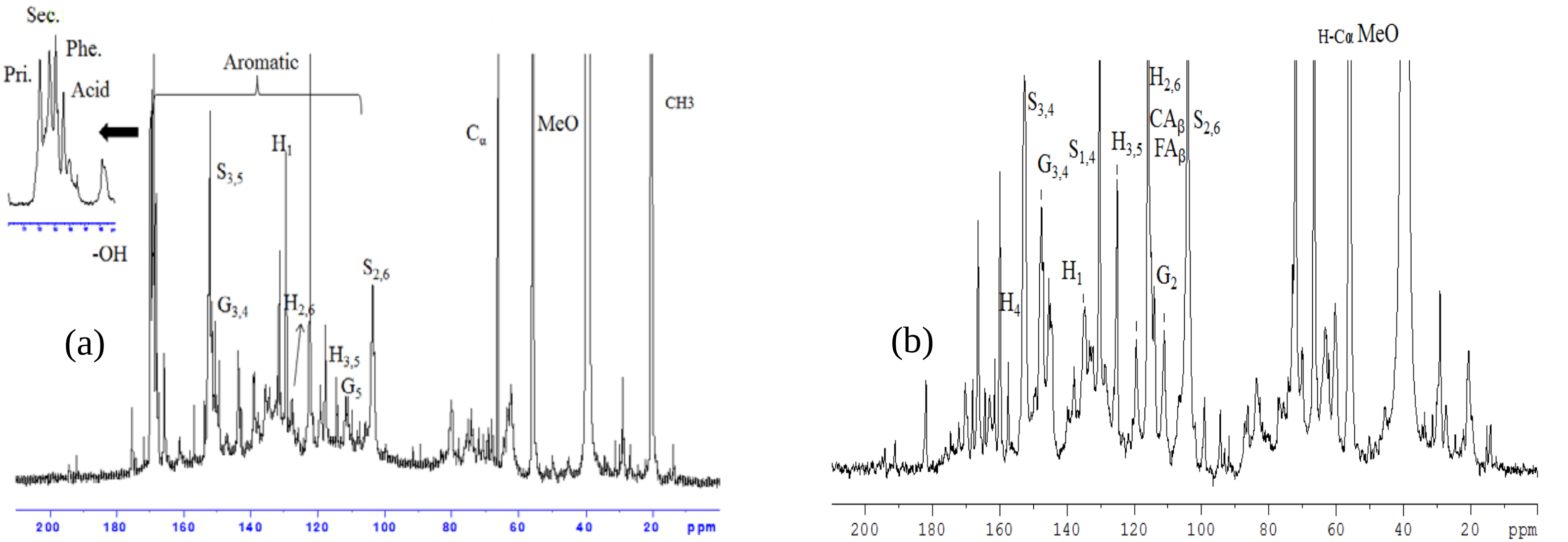


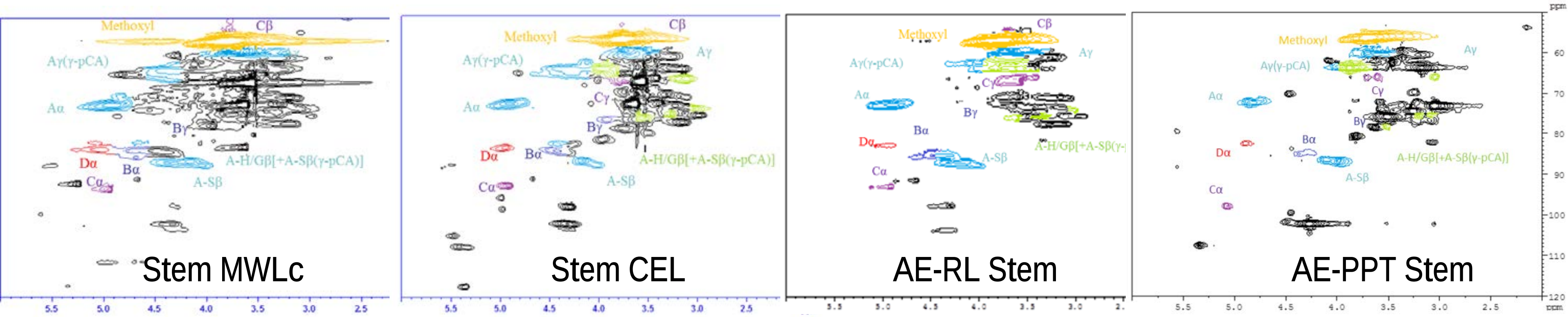
Figure 1. ^{13}C NMR spectra: (a) acetylated stem MWLc; (b) non-acetylated stem MWLc

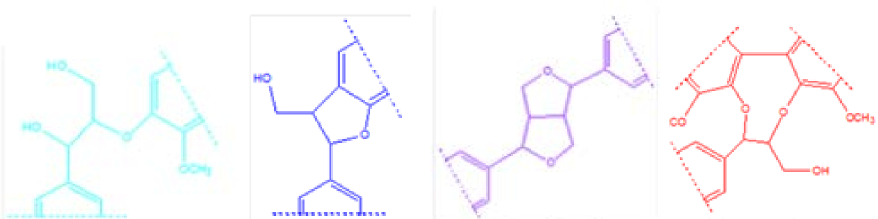
Table 4. Units calculation based on ^{13}C of acetylated and non-acetylated samples

Units	MWLc						CEL					
	Leaf ac	Leaf non	Cob ac	Cob non	Stem ac	Stem non	Leaf ac	Leaf non	Cob ac	Cob non	Stem ac	Stem non
G_2/C_9	3.2	4.2	2.4	4.4	2.6	4.3	2.7	3.8	5.6	5.3	3.1	4.8
$S_{2,6}/C_9$	6.7	8.2	5.8	9.5	10.5	17	7.5	11	6.4	7.9	11.3	18.6
$I_{90\text{-}83}/C_9$	3.8	6.0	3.7	6.5	3.9	7.9	3.5	6.1	4.4	6.8	4.8	8.6
S/G	1.1	1.0	1.2	1.1	2	2	1.4	1.5	0.6	0.7	1.8	1.9
β-O-4	13.4		16.7		24.4		12.9		14.6		23.1	
MeO/C_9	0.6	0.5	0.8	0.7	1.2	1.2	0.6	0.7	0.5	0.6	1.2	1.3
H:S:G	47:27:26		30:38:33		10:60:30		46:32:22		31:27:42		8:58:34	

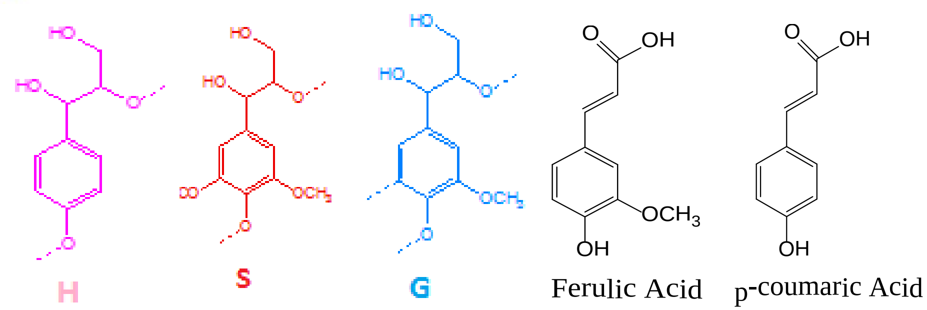
^{1}H-^{13}C HSQC NMR: MWLc, CEL96 and AE Samples

Side chain



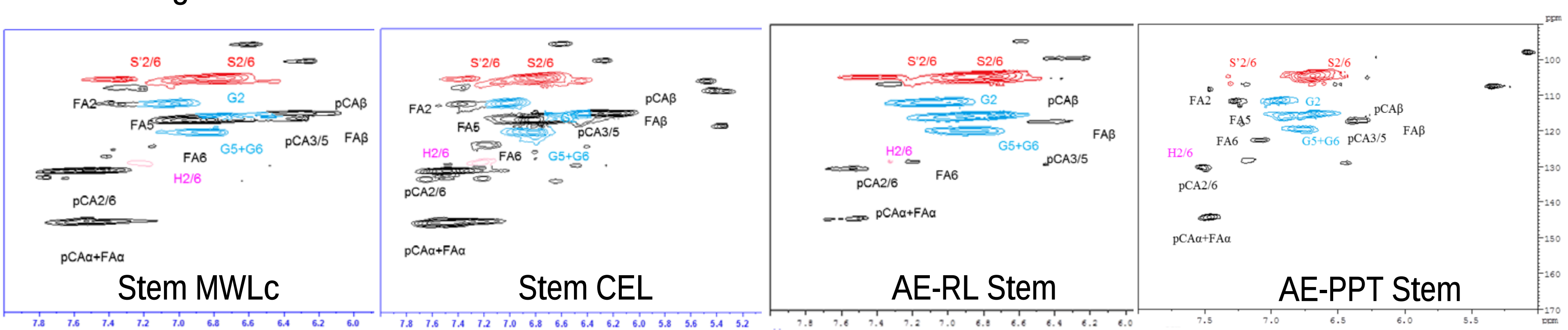


A: β-aryl ether (β-O-4) | B: phenylcoumaran (β-5) | C: resinol (β-β) | D: dibenzodioxcin (5-5/ β-O-4, α-O-4)



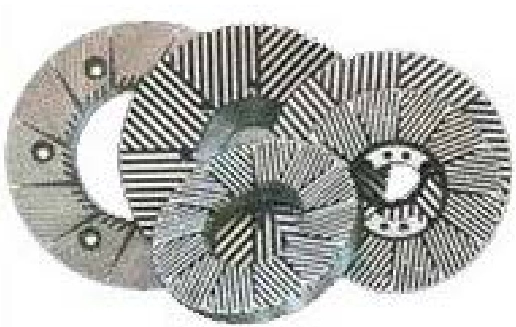
Ferulic Acid | p-coumaric Acid

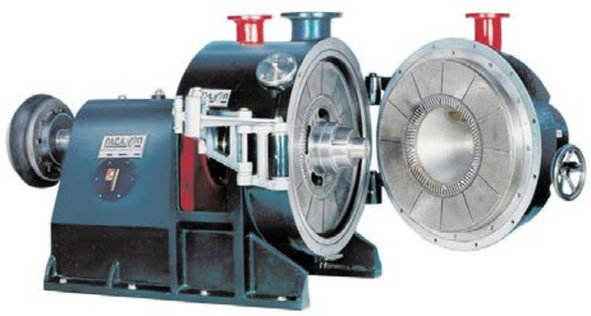
Aromatic region

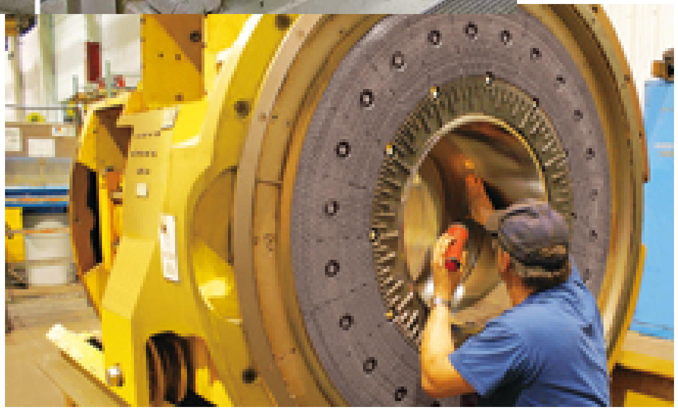


Refining for Pulp/Paper Production

- Industrial disc refiner
  - Fiber slurry enters through the center and passes between rotating discs
  - Refiner plates can be customize and optimized for specific applications
  - Slurry is forced through the refiner towards the outlet by centrifugal force



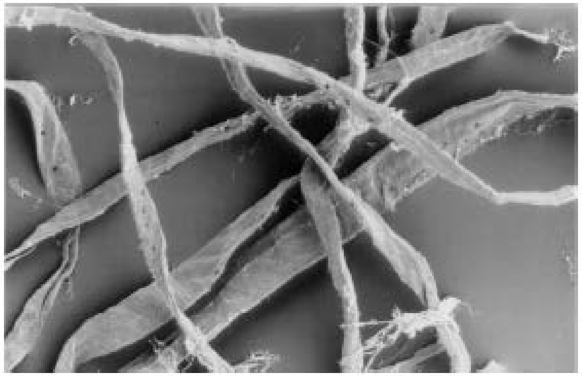
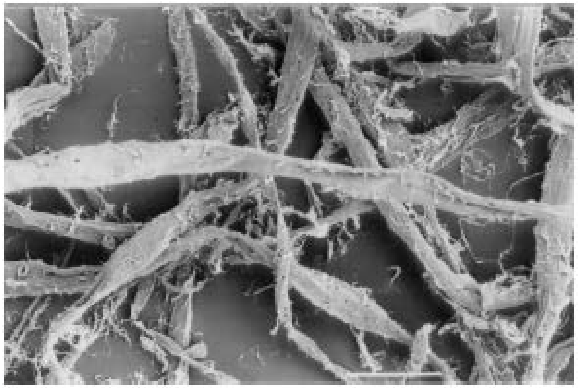
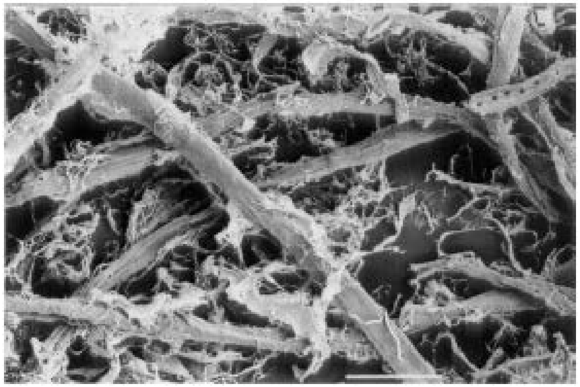




Refining for Pulp/Paper Production

- Refining objectives
  - **Fiberization** of cooked chips: especially in high yield semi-chemical or linerboard operations to break-up chips and fiber bundles (“de-shive refining” or “blow-line refining”)
  - **Strength development** by fibrillation of fibers in water to increase surface area, flexibility and promote bonding when dried to increase paper strength (“fiber refining” or “papermachine refining”)

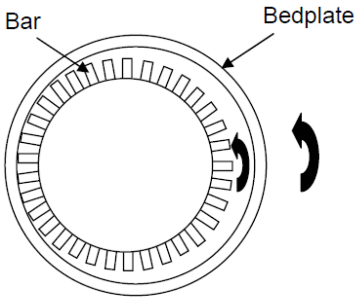
- Refining mechanisms
  - **External fibrillation** – Creating fibrils on fiber surface
  - **Internal fibrillation** – Delaminating and loosening of internal structures
  - **Fiber cutting** – Fiber shortening due to shearing action

(a)  (b)  (c) 

Refining for Biofuels Production

- Refining objectives
  - Opening up biomass structure (**fiberization, fibrilation, delamination**) for effective enzymatic hydrolysis
  - **Reduction of enzyme cost** (enzyme dosage)

- Potential refiner location
  - Refining can be carried out at the blow line of a continuous digester
  - Refining can be carried out after a blow tank
  - Or, any place prior to enzymatic hydrolysis



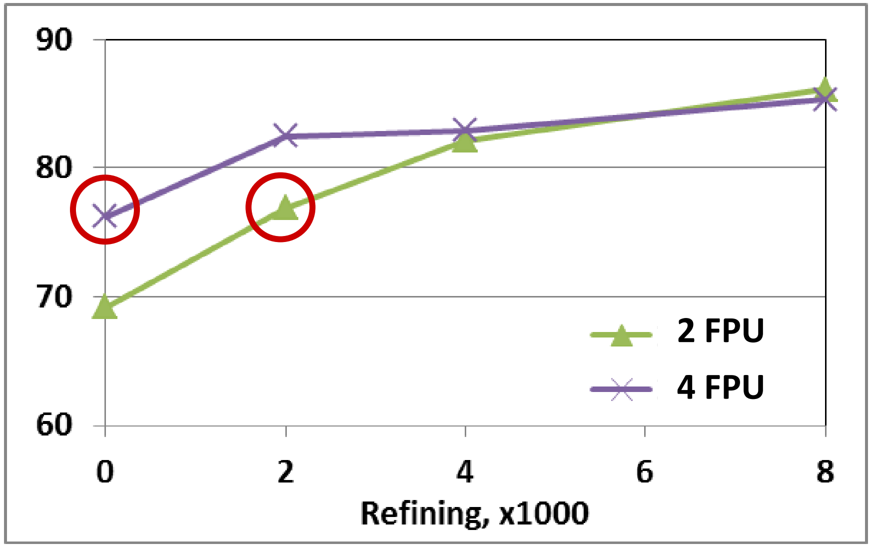
Lab-scale PFI Refining

- Rotation speed and gap distance constant
- Vary the number of revolutions
- Batch system

Refining Effect on Different Pulps

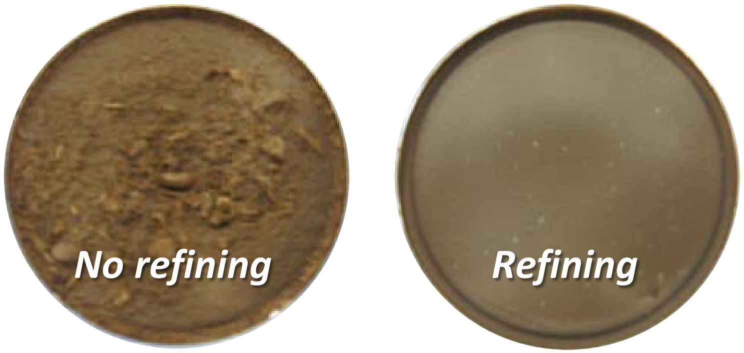
Dilute-acid Pretreatment, Corn Stover

96 hr total carbohydrate conversion based on total carbo. in pretreated CS, %



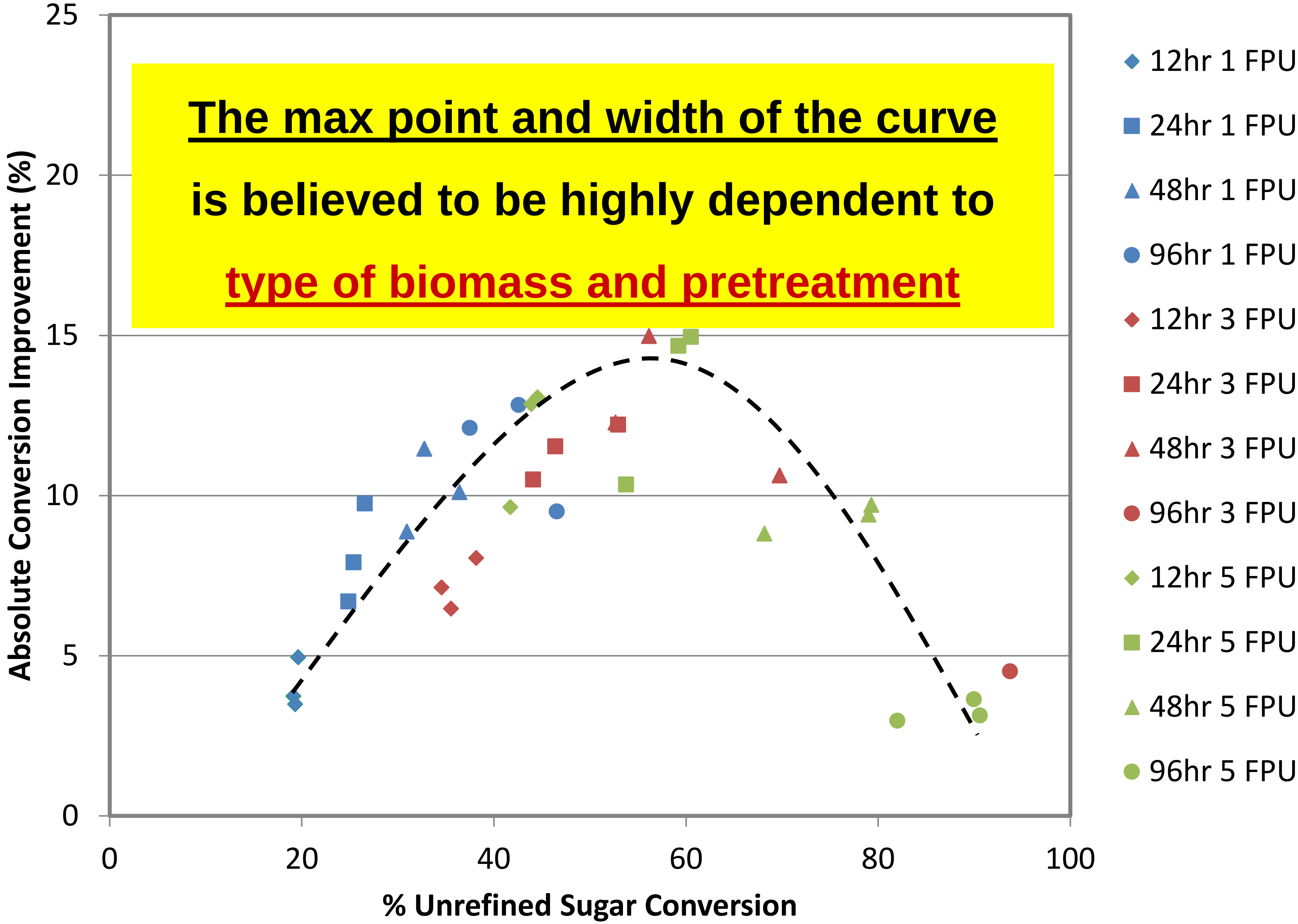
Dilute acid pretreated CS

From Melvin Tucker and Xiaowen Chen, NREL



- With PFI refining at 2K revolution, enzyme dosage reduced by half (4 → 2)
- Severe refining is not necessary

Refining Efficiency for Kraft Hardwood Pulps

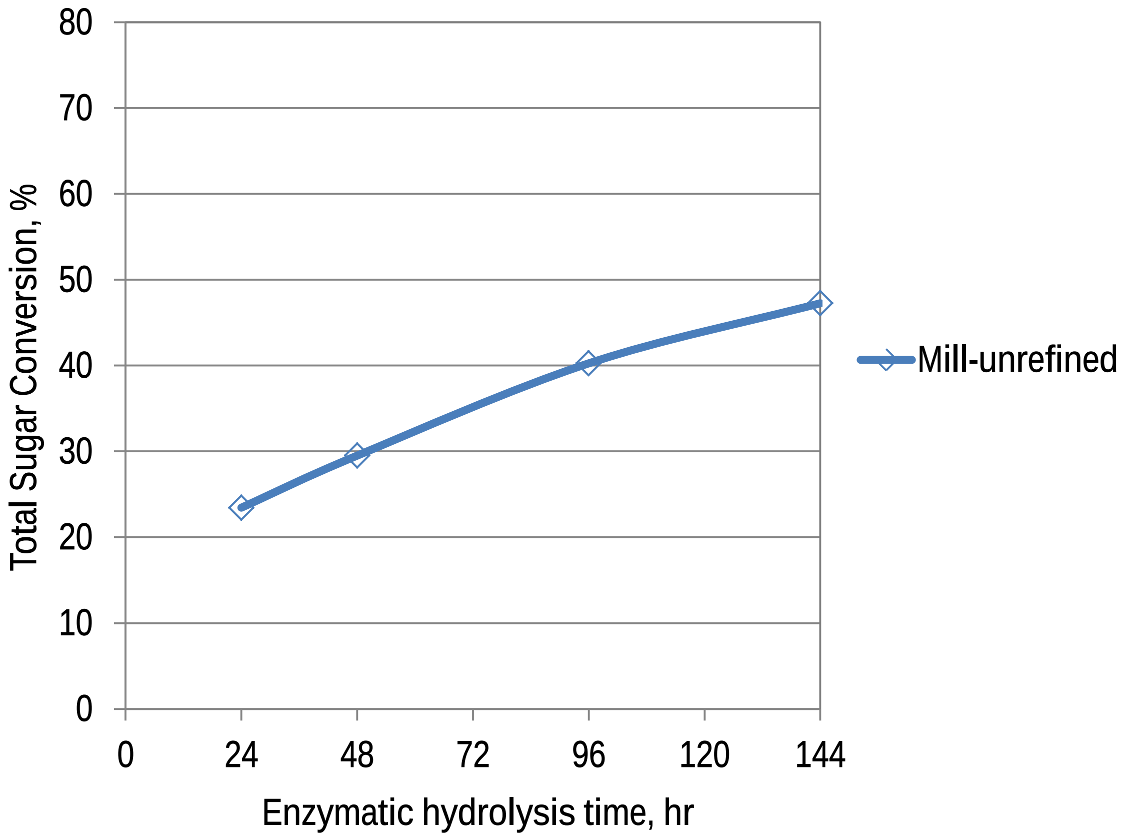


Industrial Refining: Commercial Pulps

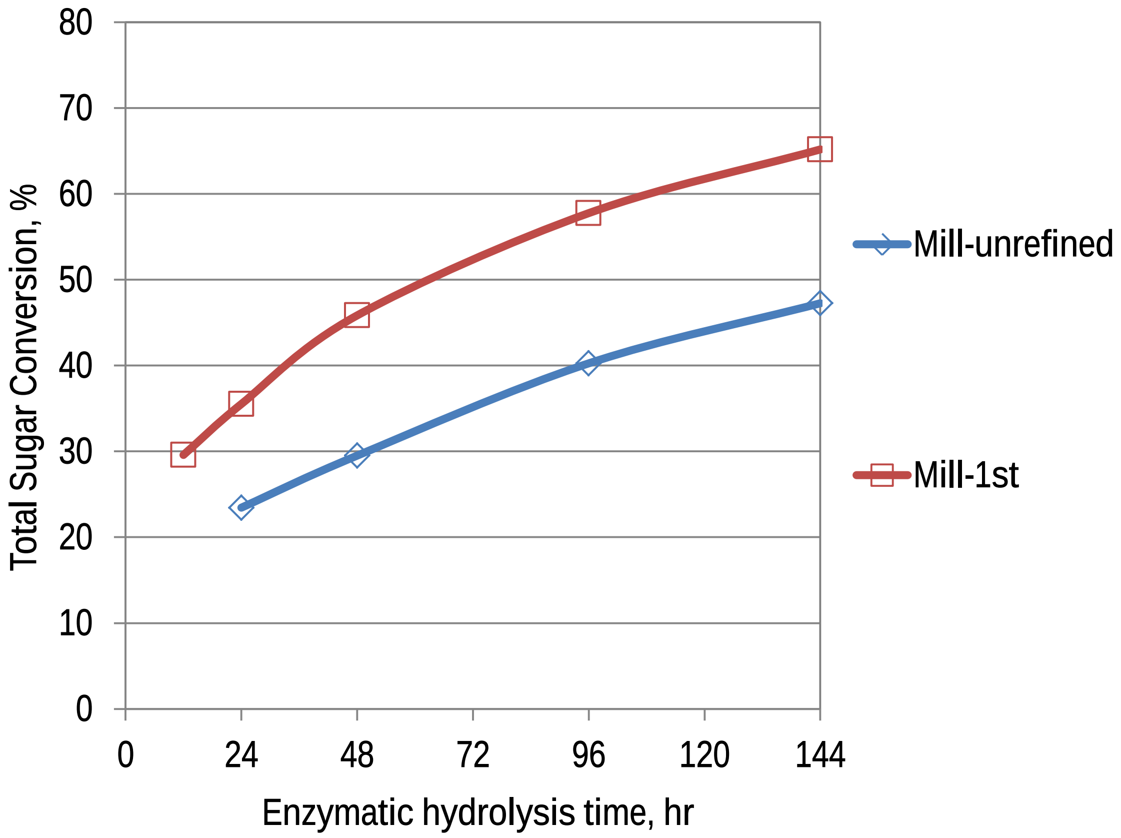
Sample	Freeness	WRV, g/g	Fiber length, mm	Fines, wt %
Mill - Unrefined	730	1.205	1.106	12.3
Mill - Primary refined	710	1.543	1.117	9.3
Mill - Secondary refined	610	1.708	0.961	9.6
NCSU pilot refiner at 30°C	-	1.921	0.925	10.7

- Enzymatic hydrolysis
  - 5 FPU/OD gram biomass
  - Enzyme charge: Novozymes CTec2:HTec2 = 1:9 by volume

Industrial Refining: Commercial Pulps

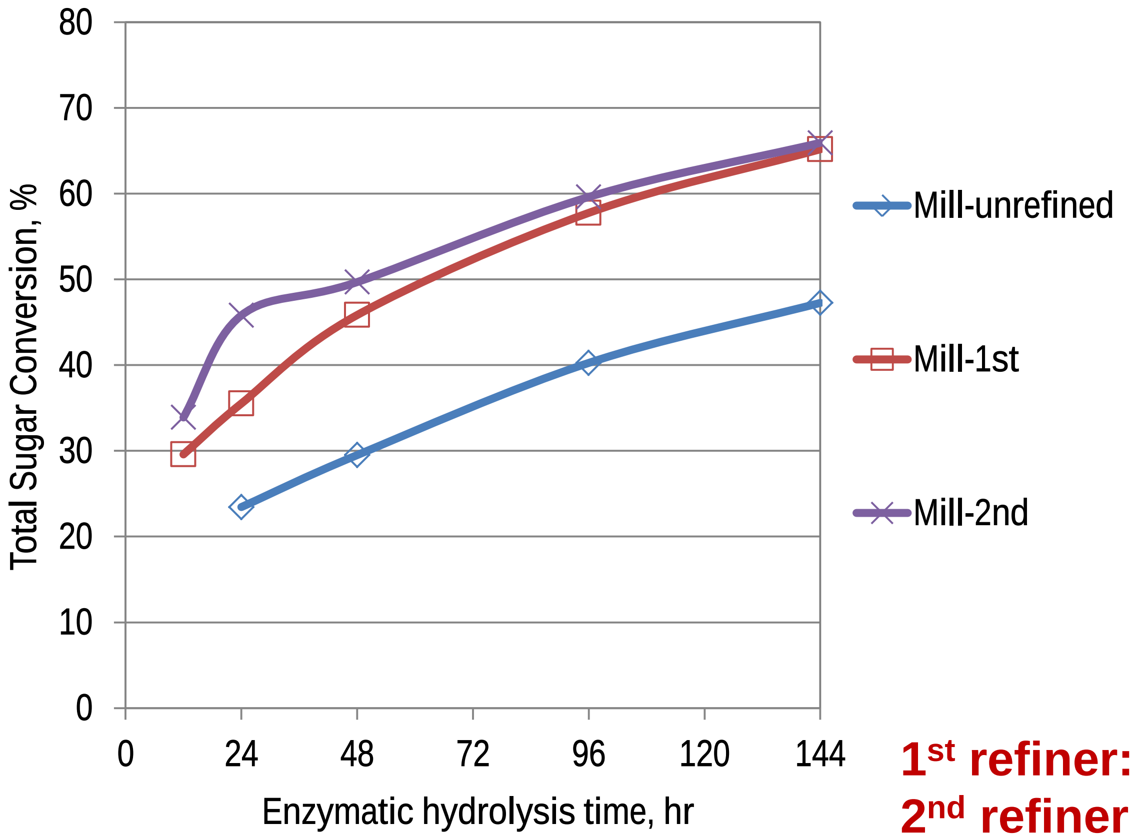


Industrial Refining: Commercial Pulps



20% improvement by 1st refiner

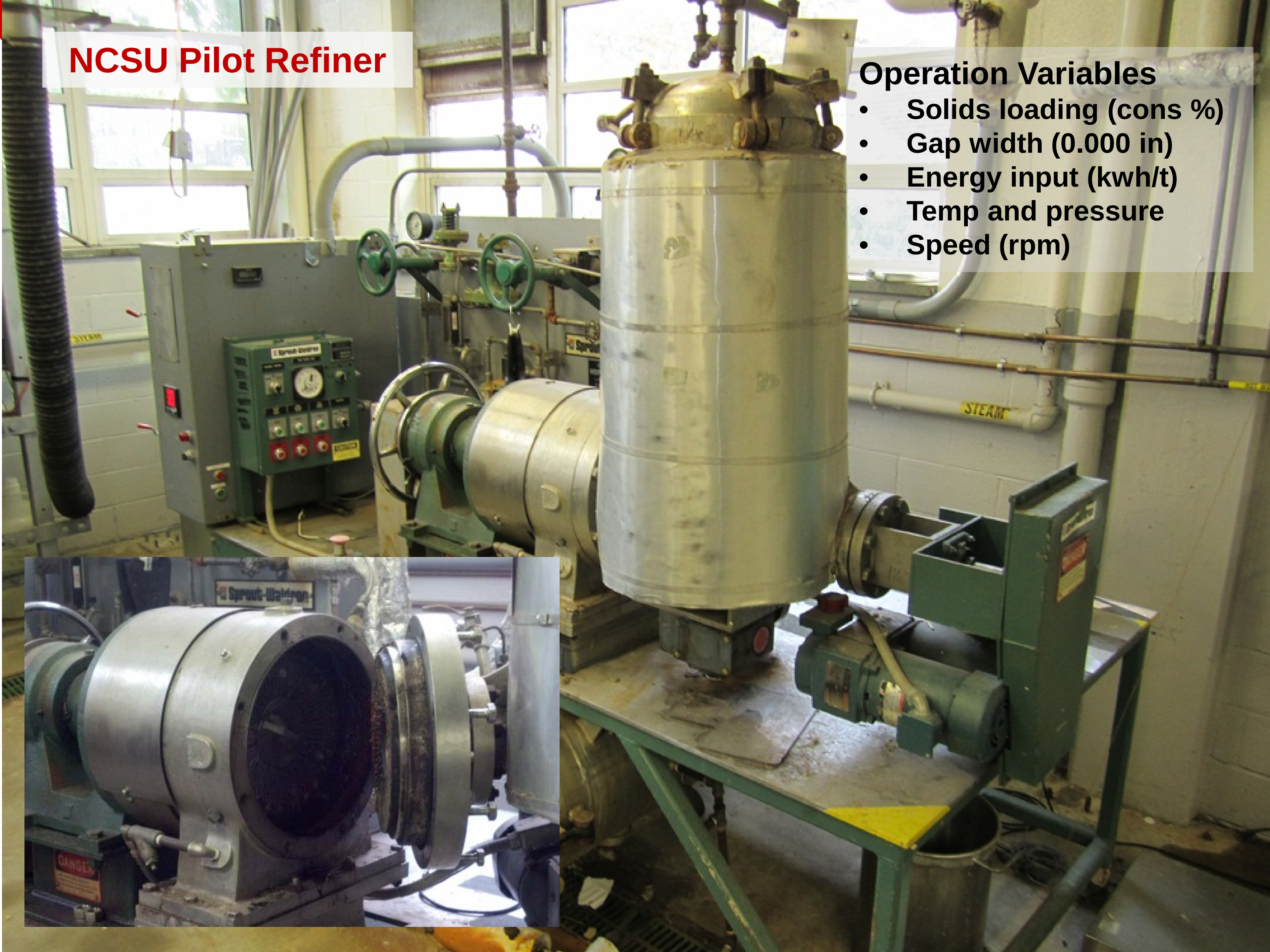
Industrial Refining: Commercial Pulps



1st refiner: 67.0 kwh/mt
2nd refiner: 79.5 kwh/mt

Improvement by 2nd refiner is marginal
2nd refiner might be not necessary for biofuels application

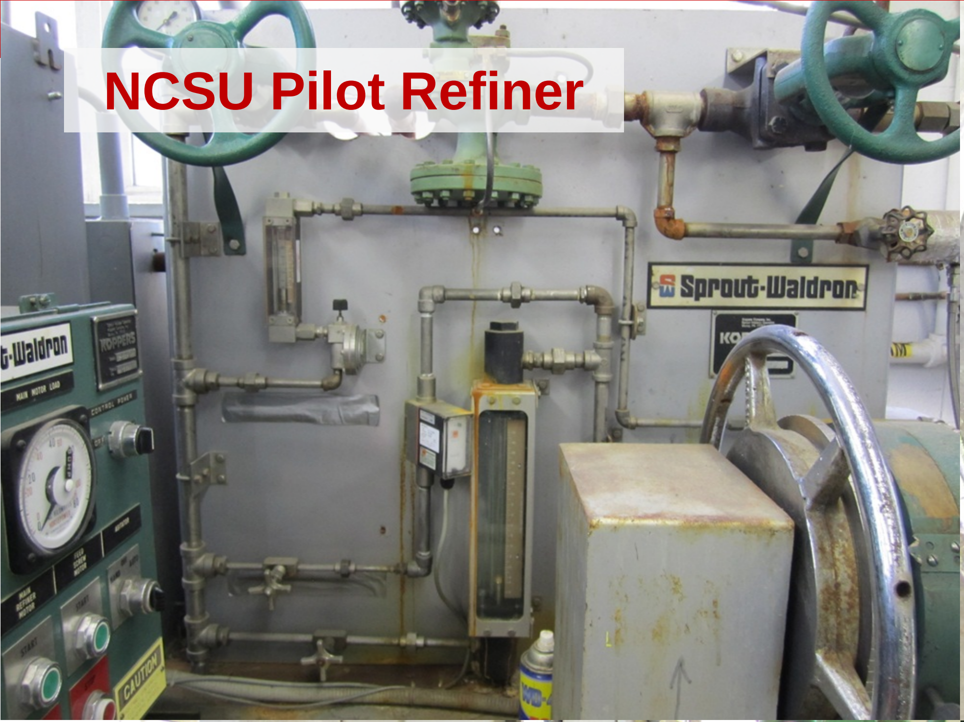
NCSU Pilot Refiner



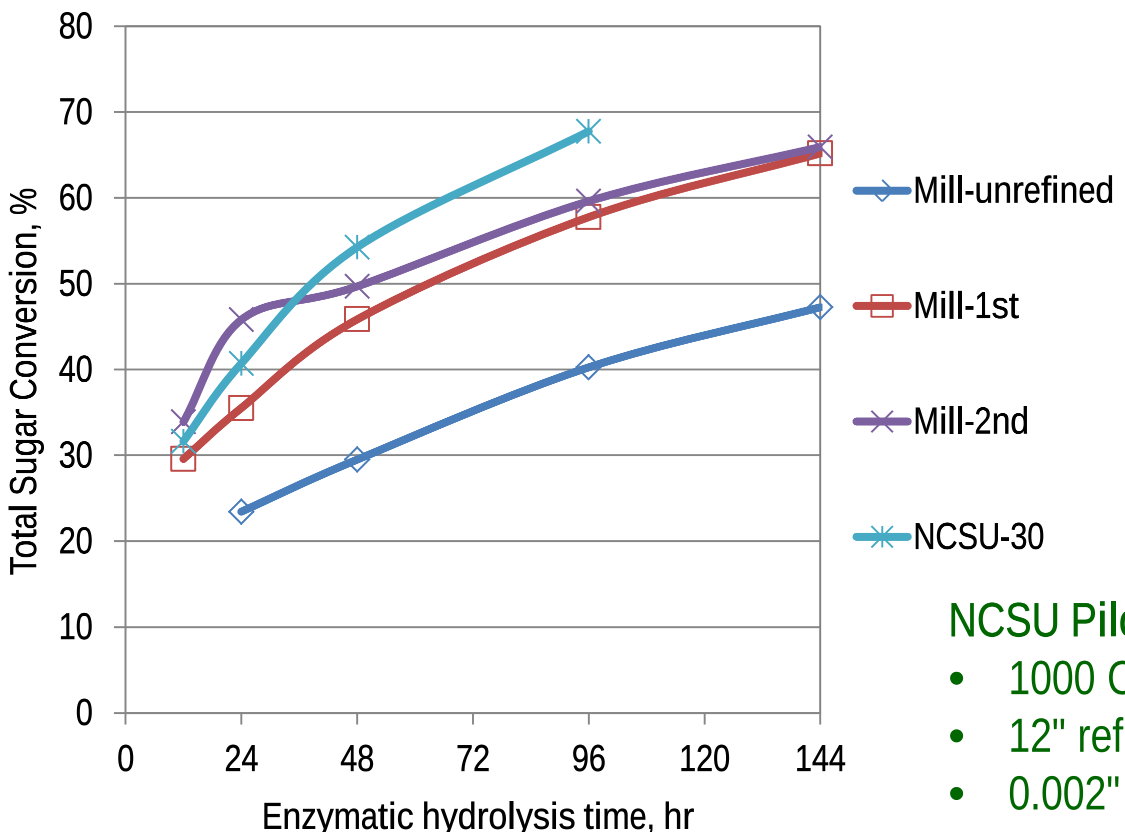
Operation Variables

- Solids loading (cons %)
- Gap width (0.000 in)
- Energy input (kwh/t)
- Temp and pressure
- Speed (rpm)

NCSU Pilot Refiner



Industrial Refining: Commercial Pulps



NCSU Pilot refiner operation

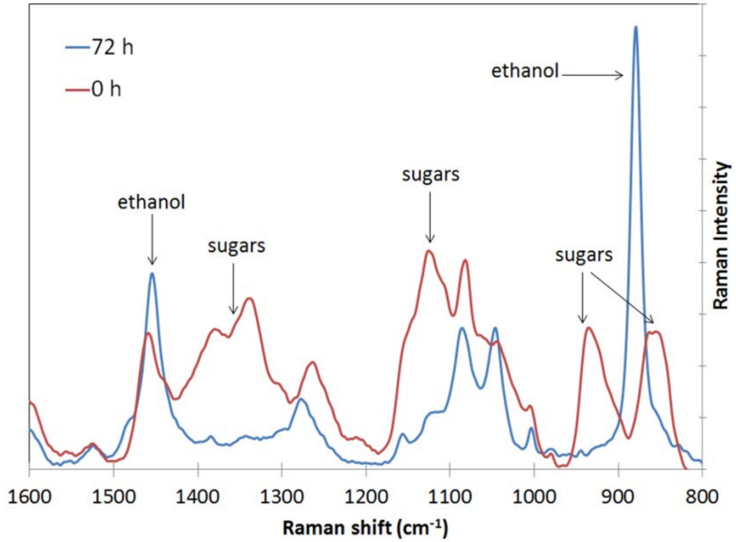
- 1000 OD g batch
- 12" refiner plate
- 0.002" gap size
- 30.0% consistency

~30% improvement by NCSU pilot refiner at 30°C

Real-Time Monitoring of High-Gravity Corn Mash Fermentation using *In Situ* Raman Spectroscopy

- Raman advantages
  - Sharp, component-specific bands
  - Complementary to mid-IR
  - Non-destructive
  - Real-time, on-line monitoring using fiber-optic probes

6 components (analytes)
- Dextrin (DP4+)
- Maltotriose
- Maltose
- Starch sum
- Glucose
- Ethanol



16 samples/batch
- HPLC values
- Multivariate PLS
- Cross-validation
- Preprocessing
  - Mean centering
  - Gap 2nd derivative

Partial Least Squares modeling results

Algorithm: PLS1 Region: 800-1600cm^{-1}

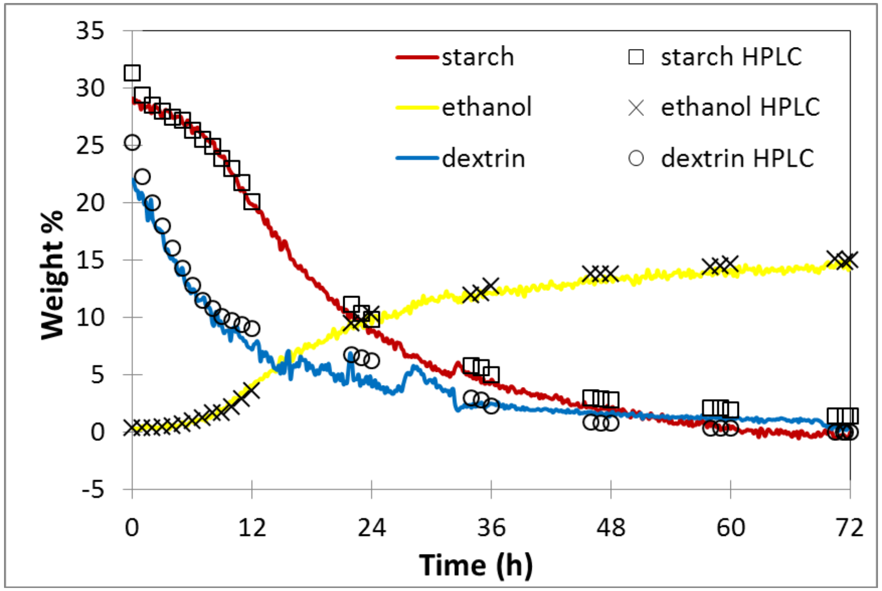
Preprocessing: Mean Center, Gap 2nd derivative - 15 point smoothing

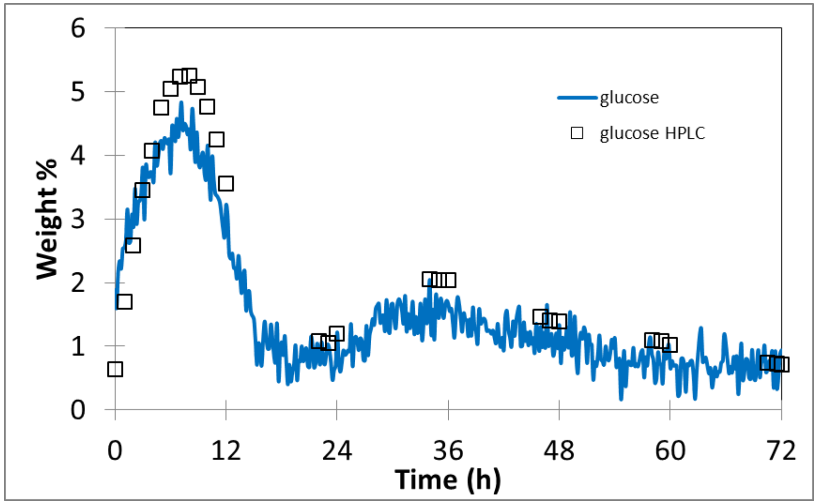
Component	R^2	SEC	calibration sets	points	factors
Starch	(0.969, 0.979)	(0.784, 0.716)	2	(88, 79)	(4,3)
Dextrin	(0.973, 0.910)	(0.792, 0.690)	2	(93, 73)	(9,5)
Maltotriose	0.968	0.302	1	170	8
Maltose	0.941	0.618	1	174	6
Glucose	0.955	0.383	1	160	5
Ethanol	(0.962, 0.971)	(0.569, 0.217)	2	(78, 85)	(5,5)

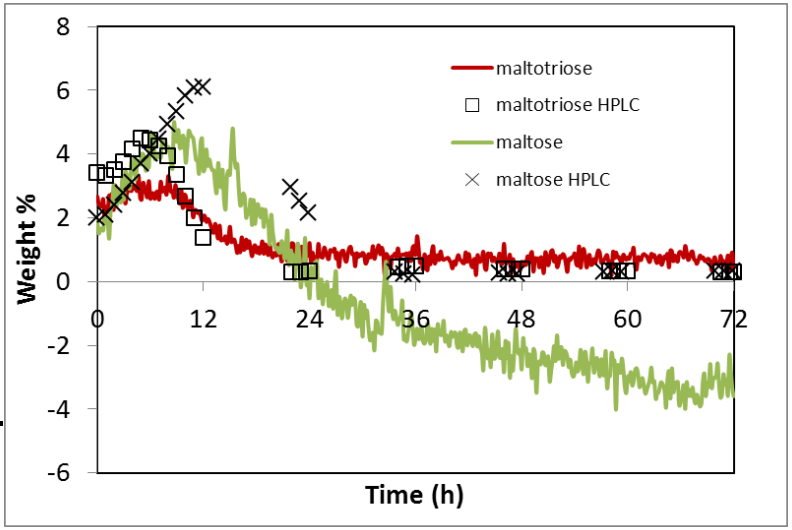
- PLS models based on 8 VHG corn mash fermentations.
- Excellent fits for all components with reasonable number of factors.
- Two-set calibration models provided better predictive capability for starch, dextrin, and ethanol.

SEC – standard error of calibration (cross validation)

Model validation: PLS model predictions for 9th fermentation run (not included in modeling)

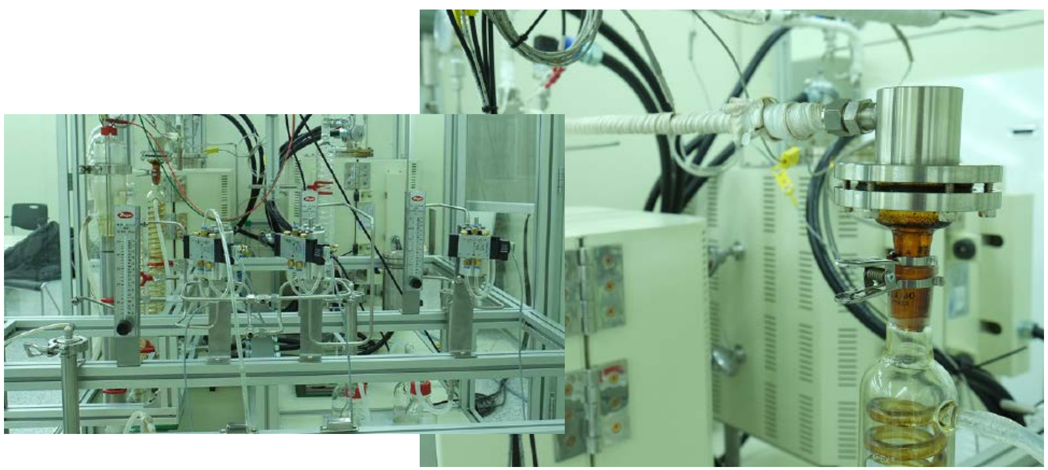


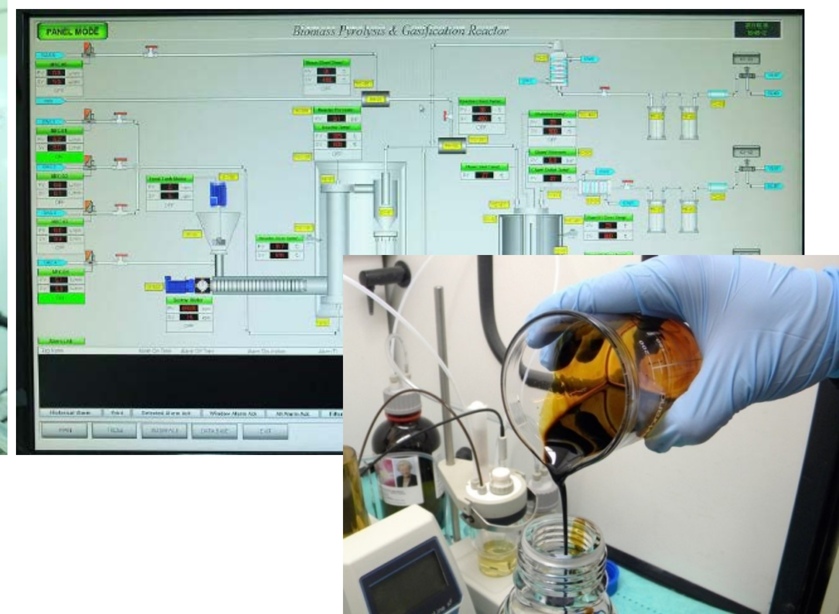




S. R. Gray, S. W. Peretti, and H. H. Lamb, *Biotechnol. Bioeng.*, **110**, (2013) 1654 – 62 .

Fluidized-bed Fast Pyrolysis Reactor

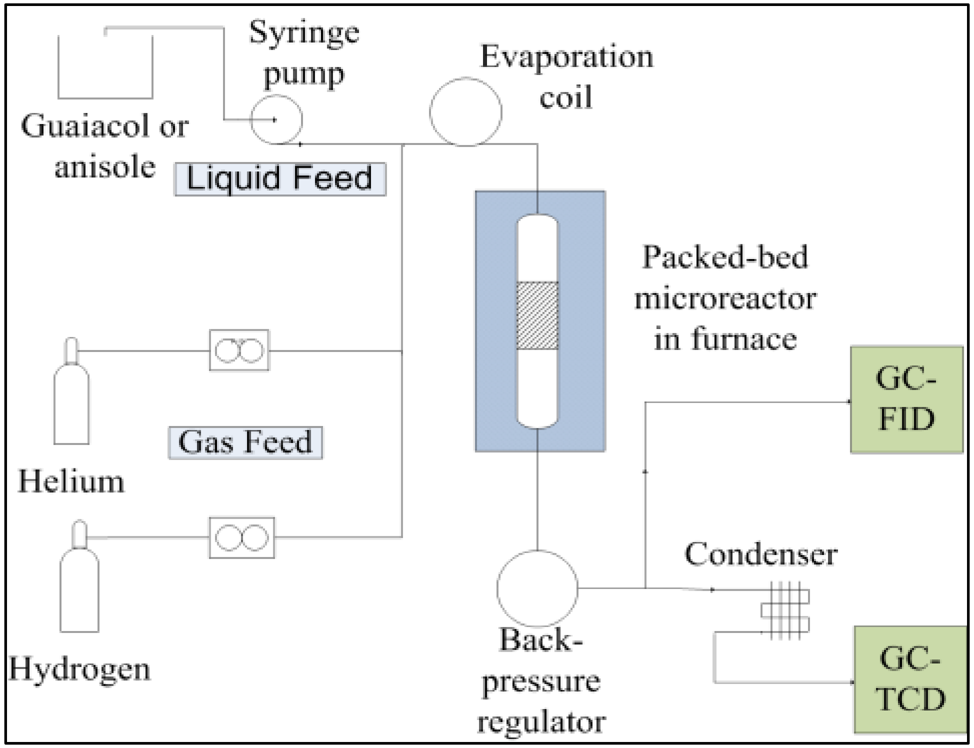




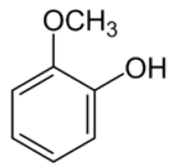
Deoxygenation of guaiacol and anisole over carbon-supported Pd and Re catalysts

Hypothesis

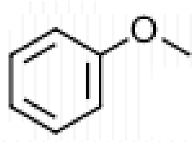
- A bimetallic PdRe/C catalyst will have improved HDO activity due to the hydrogenation activity of Pd combined with the ability of Re to break C-O bonds.
- This catalyst will also have water-gas shift activity, **eliminating more oxygen per carbon.**



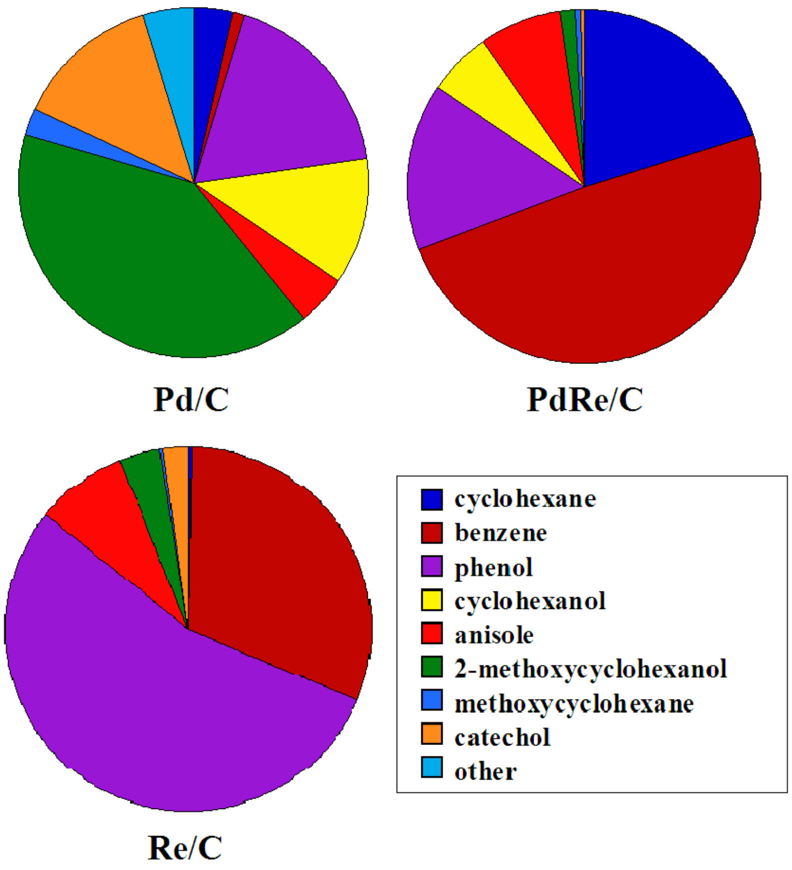
C_6 and C_7 products

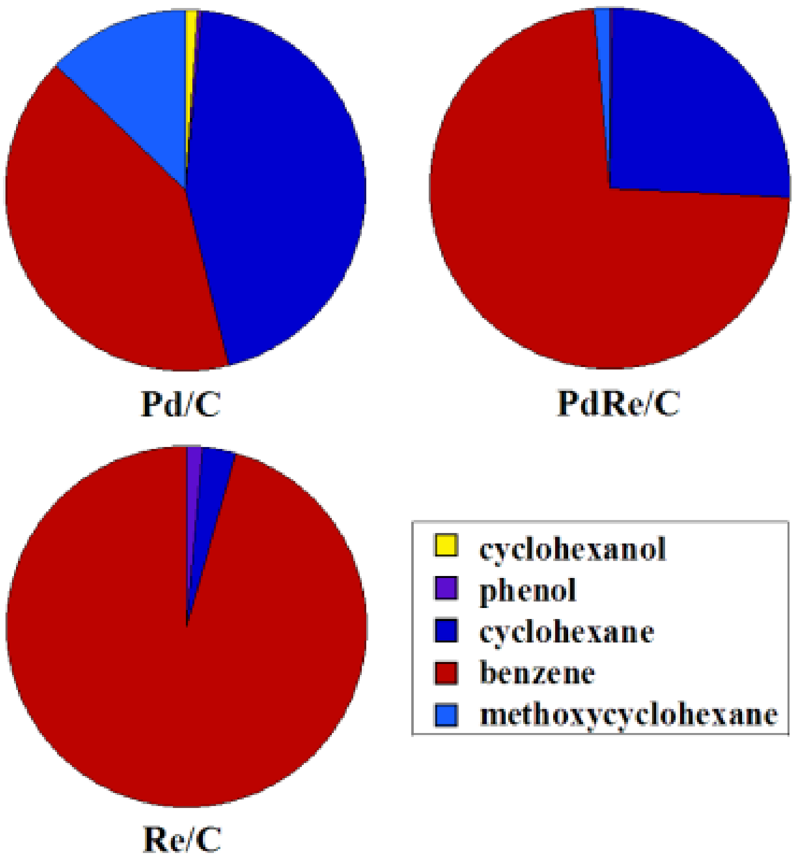


guaiacol



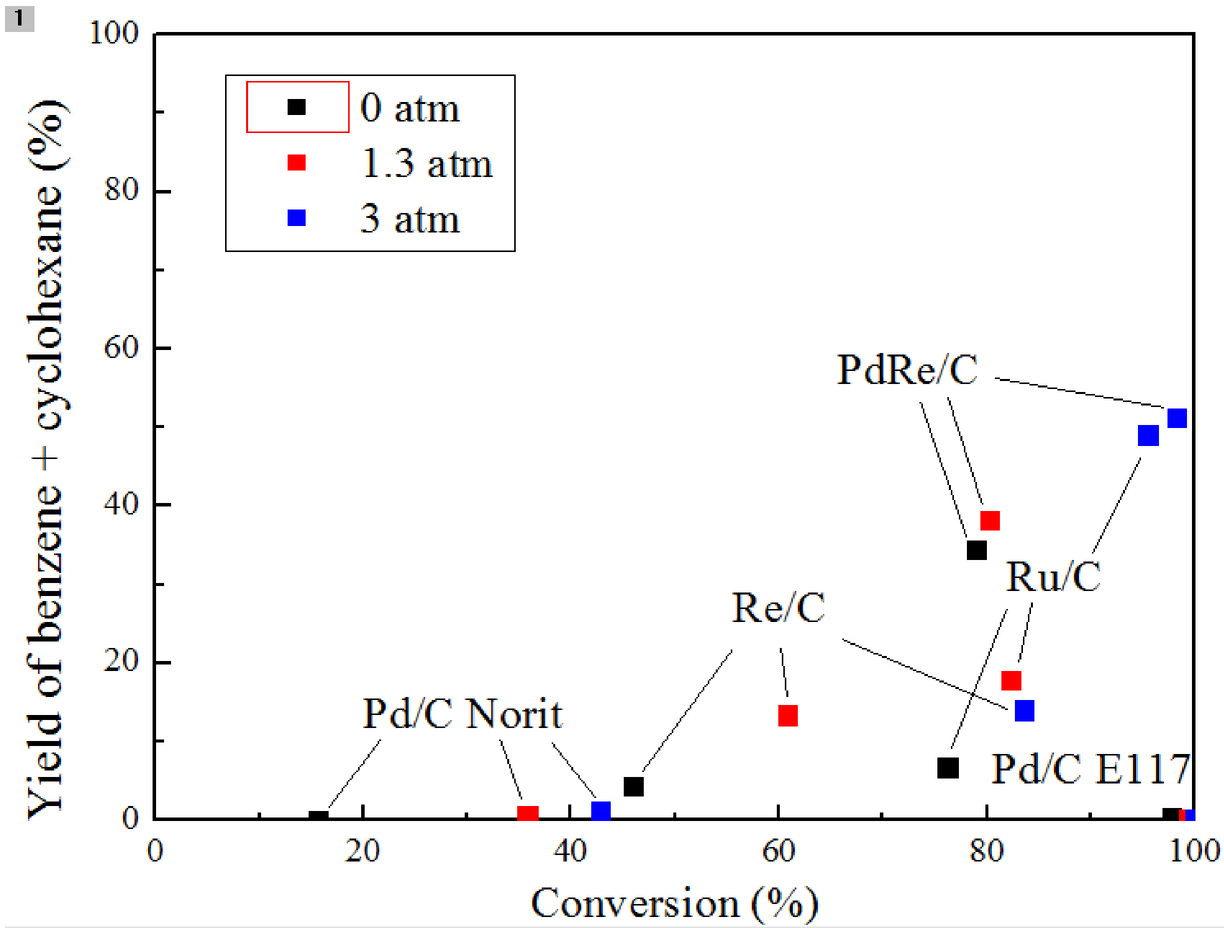
anisole





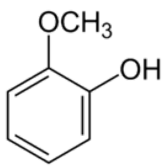
Conditions: 300°C, ambient pressure, WHSV ~1 h^{-1}, 100 sccm H_2

Guaiacol HDO Summary (C basis)

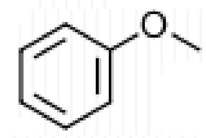


Conditions: 300°C, WHSV ~1 h^{-1}, 100 sccm H_2

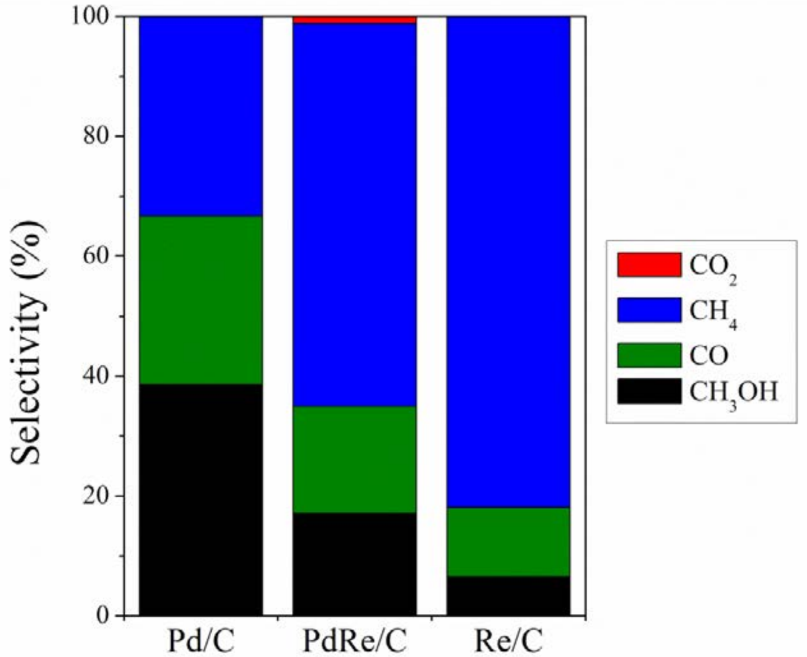
C_1 products

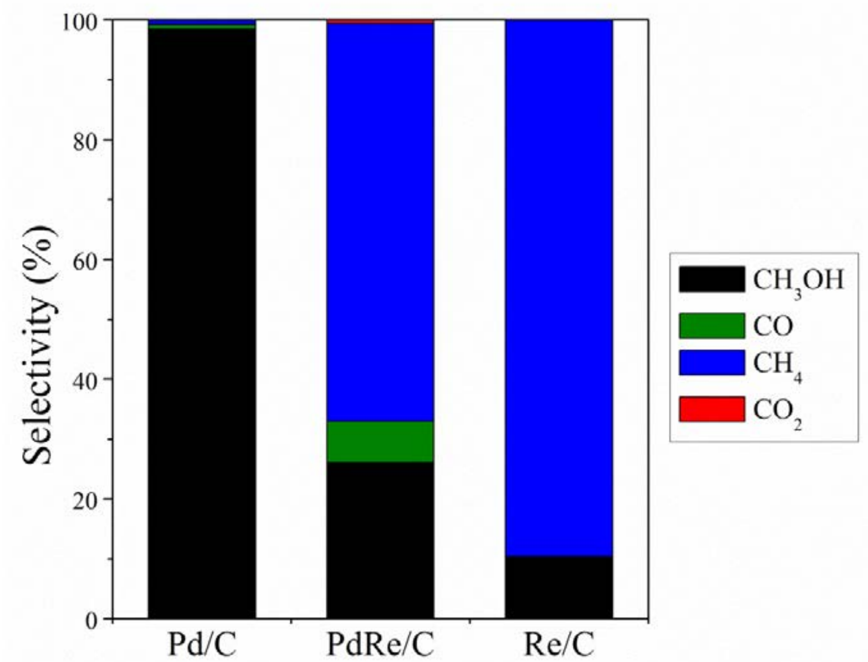


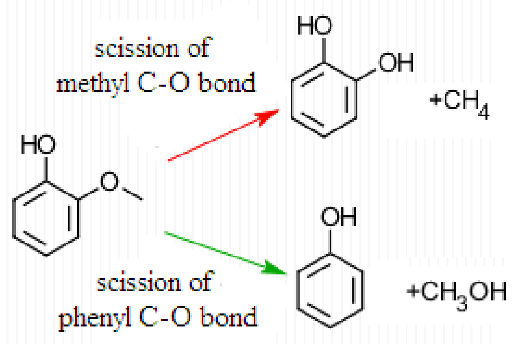
guaiacol

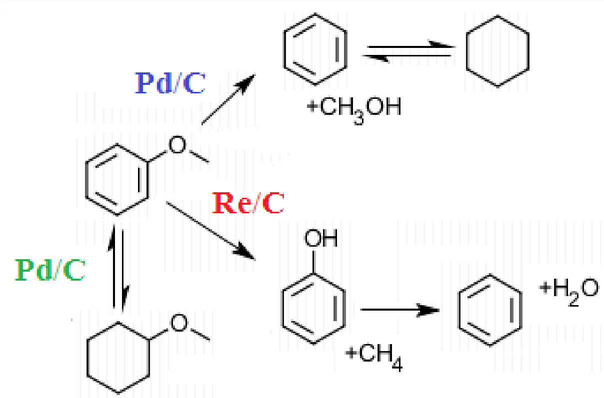


anisole









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Thank you for your attention