A PERSPECTIVE ON PRETREATMENT CHEMISTRY: Recent Advances and Future Challenges

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USA "30 by 30" goal

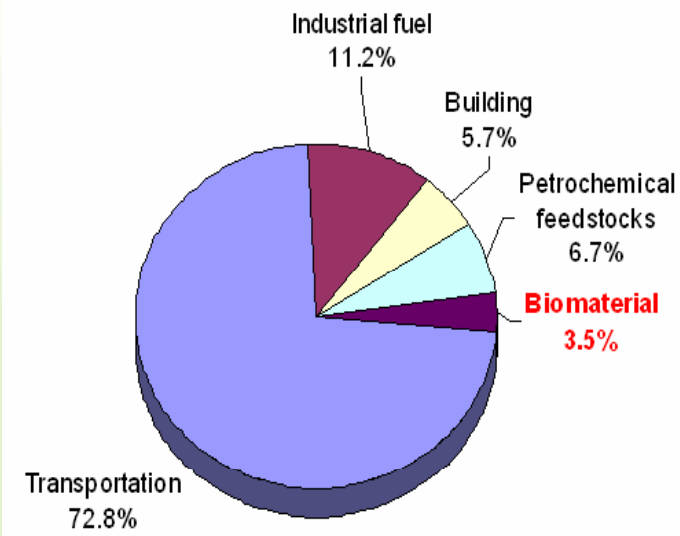
- US petroleum consumption
  - 329 billion gallons/year
  - >1000 gallons/year per person
- Goal set by Congress: Replace 30% gas and diesel consumption with biofuels by the 2030
- Requires approximately one billion dry tons of biomass for 60 billion gallons using current technology

"20 in 10" goal from 2007

- Decrease U.S. petroleum consumption by 20% in 10 years —"20 in 10"
- Grow production of renewable fuels to 35 billion gallons per year

Energy Independence and Security Act

2008 Farm Bill



Predicted Petroleum Use in 2025-2030

Source: Winning the Oil Endgame, RMI 2005, and *NRC 2000

Decreasing U.S. Petroleum Consumption

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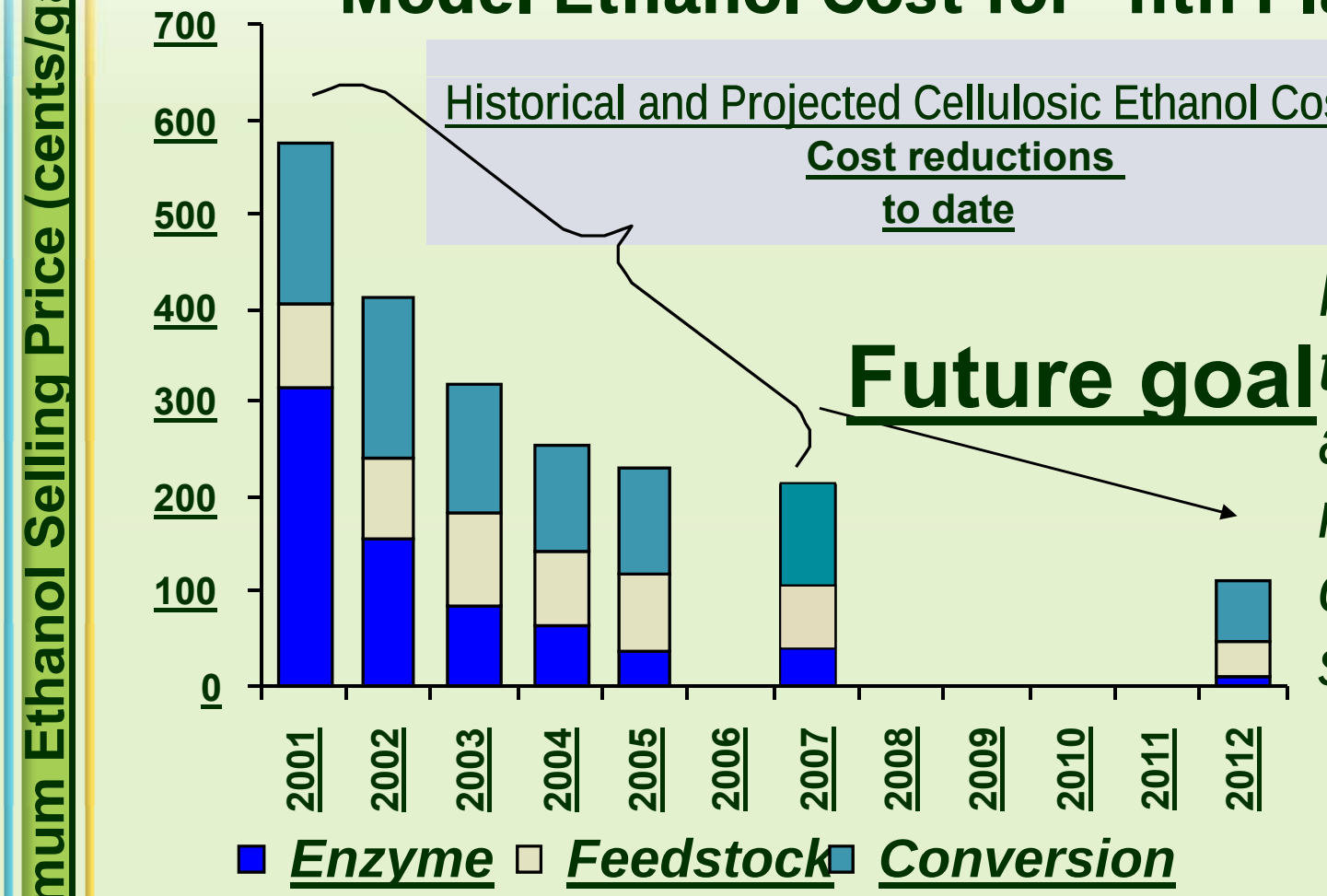
Reducing Cost of Cellulosic Ethanol
Model Ethanol Cost for "nth Plant"



In order for biofuels to succeed in the US and world-wide, they must be both cost-competitive and sustainable.

Cost-competitive in the blend market by 2012

Decreasing U.S. Petroleum Consumption

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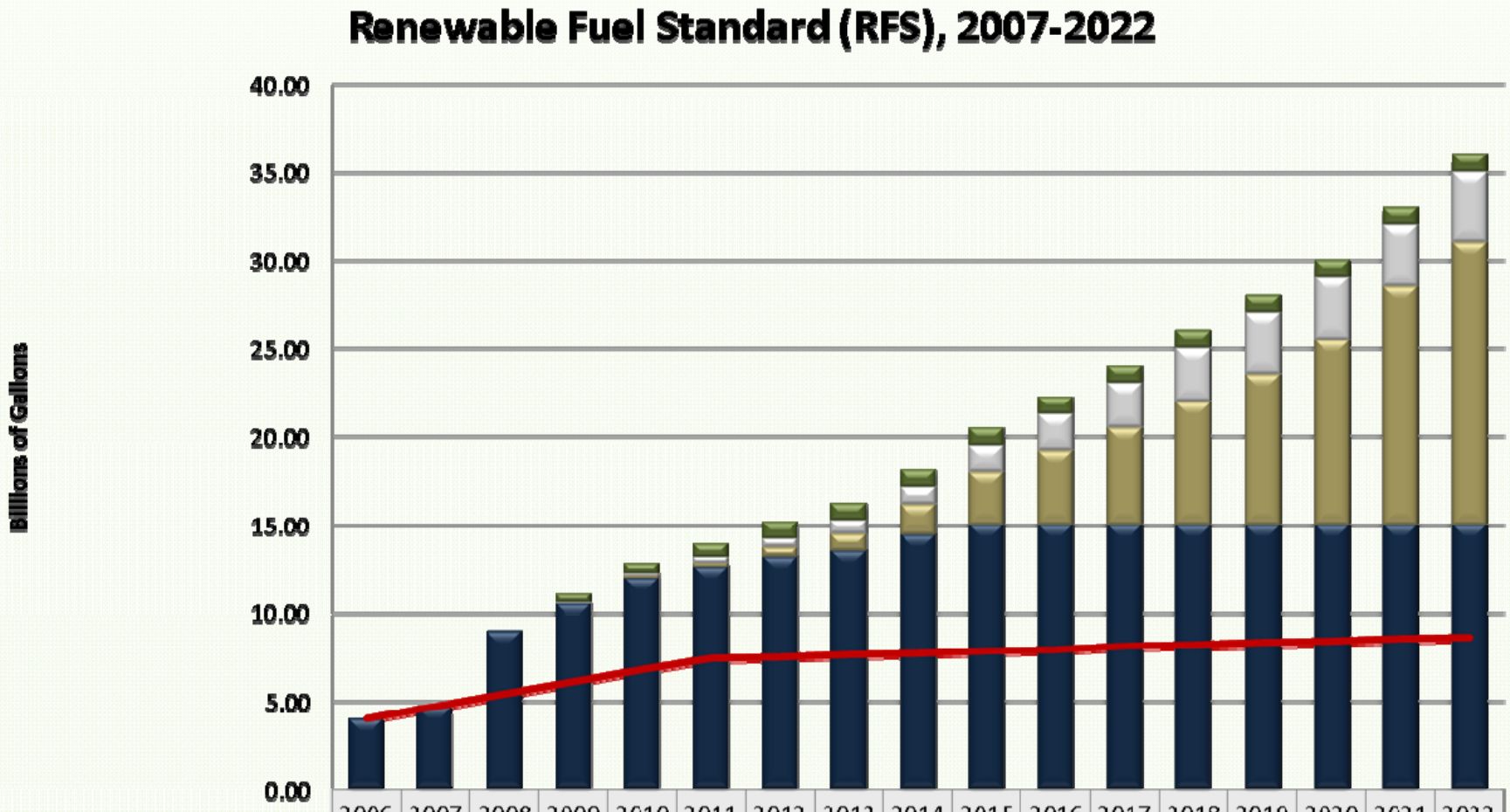
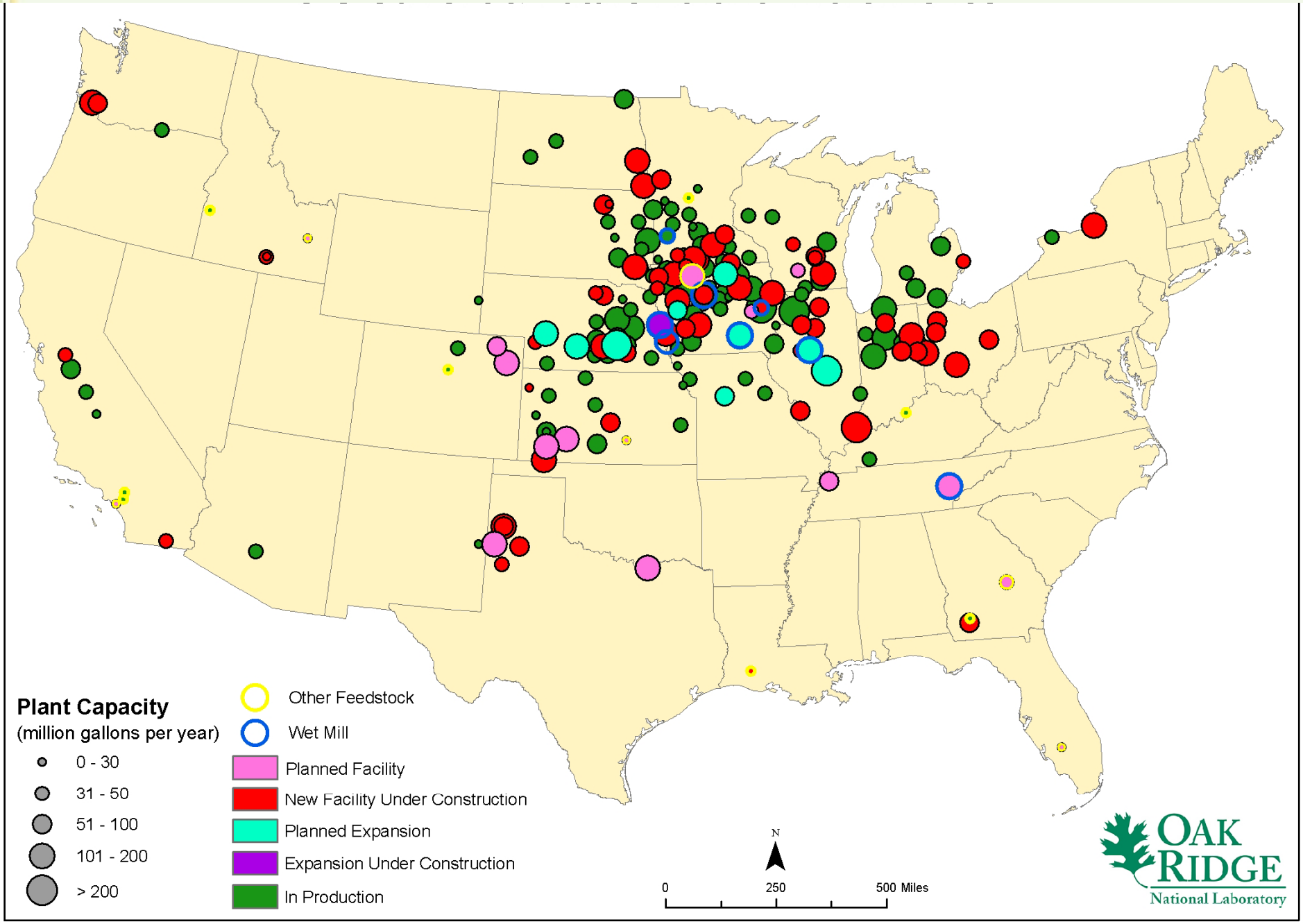


	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Biomass-based Diesel				0.50	0.55	0.80	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Non-cellulosic Advanced				0.10	0.20	0.30	0.50	0.75	1.00	1.50	2.00	2.50	3.00	3.50	3.50	3.50	4.00
Cellulosic Advanced					0.10	0.25	0.50	1.00	1.75	3.00	4.25	5.50	7.00	8.50	10.50	13.50	16.00
Conventional Biofuels	4.00	4.70	9.00	10.50	12.00	12.60	13.20	13.50	14.40	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Current RFS PL 109-55	4.00	4.70	5.40	6.10	6.80	7.40	7.50	7.60	7.70	7.80	7.90	8.10	8.20	8.30	8.40	8.50	8.60

Source: Hart Energy Consulting, Government Affairs, 2007

Current and Future Ethanol Biorefineries December 2007



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Major DOE Biofuels Project Locations

Geographic, Feedstock, and Technology Diversity

Potential Biomass Resource and Refinery Capacity in 2012: Logging Residues, Crop Residues, Switchgrass

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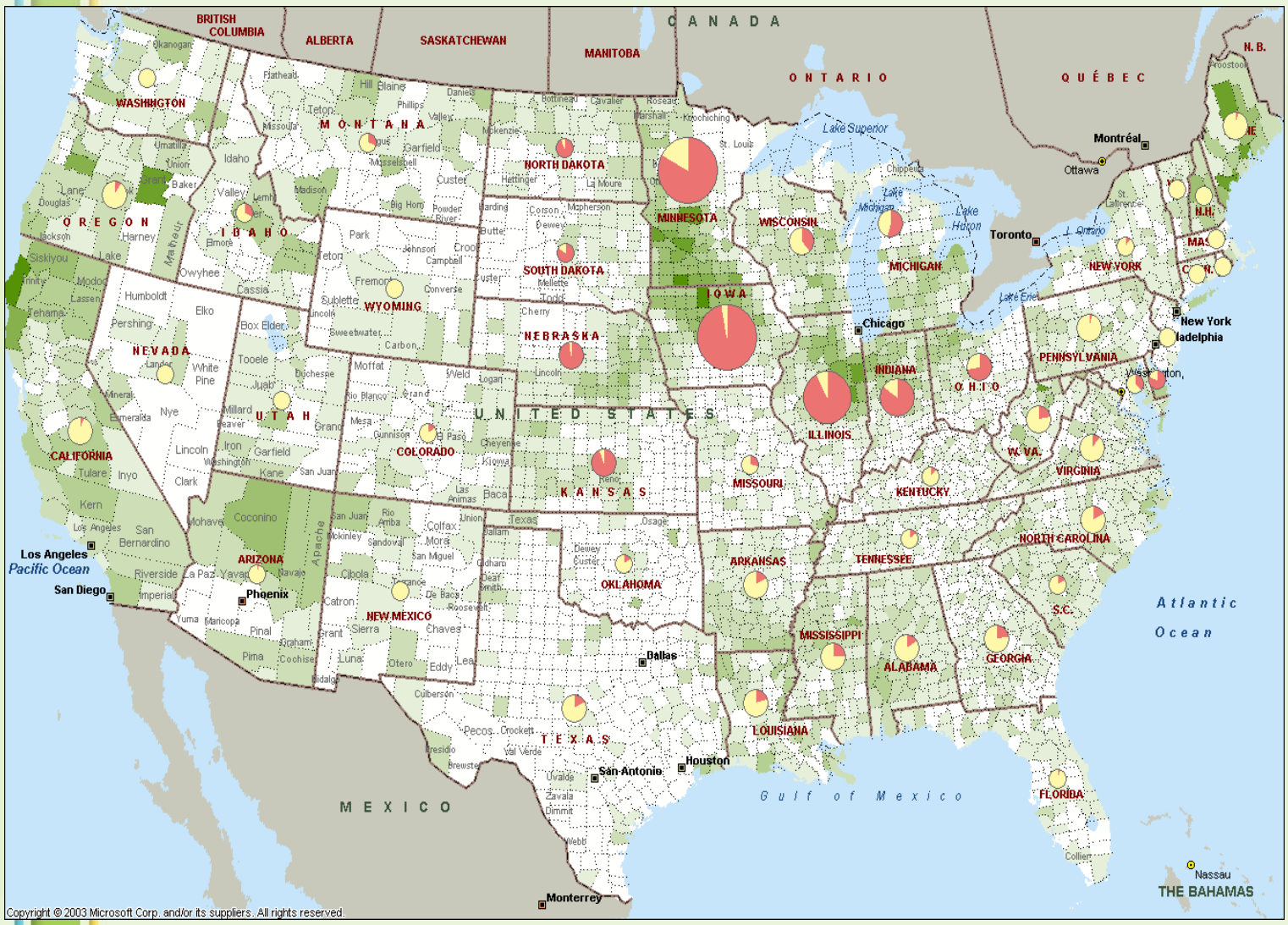

Total Number of 1 Million Dry Ton Capacity Refineries

- 14.4 - 18.0
- 10.8 - 14.3
- 7.2 - 10.7
- 3.6 - 7.1
- 0.0 - 3.5

- Biochemical Conversion Potential
- Thermochemical Conversion Potential

Total Residue in Dry Tons

- 750,001 to 900,000
- 600,001 to 750,000
- 450,001 to 600,000
- 300,001 to 450,000
- 150,001 to 300,000
- 75,001 to 150,000
- 25,001 to 75,000
- 0 to 25,000

Data do not include pulp & paper assets in the Southeast

Potential Biomass Resource and Refinery Capacity in 2030: Forest Residues (all), Crop Residues, Switchgrass

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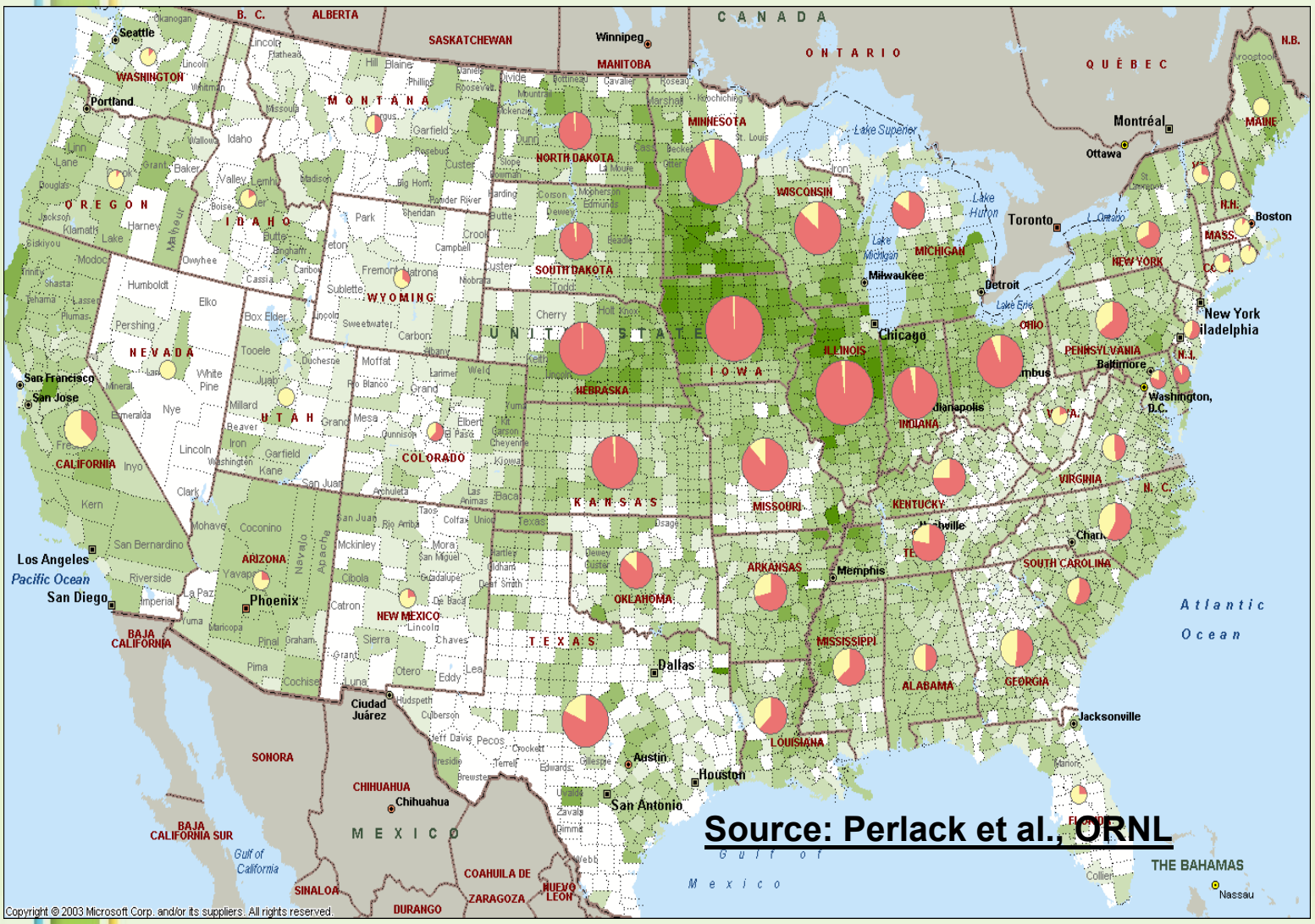


1-Million Ton Refineries by State

- 50 - 90
- 20 - 49
- 10 - 19
- 5 - 9
- 0 - 4

- Biochemical Refineries
- Thermochemical Refineries

Biomass Total by County

- 1,500,001 to 2,500,000
- 1,000,001 to 1,500,000
- 700,001 to 1,000,000
- 400,001 to 700,000
- 100,001 to 400,000
- 50,001 to 100,000
- 25,001 to 50,000
- 0 to 25,000

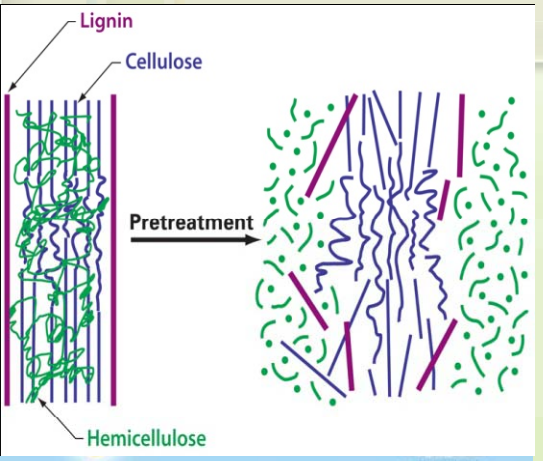
Data do not include pulp & paper assets in the Southeast

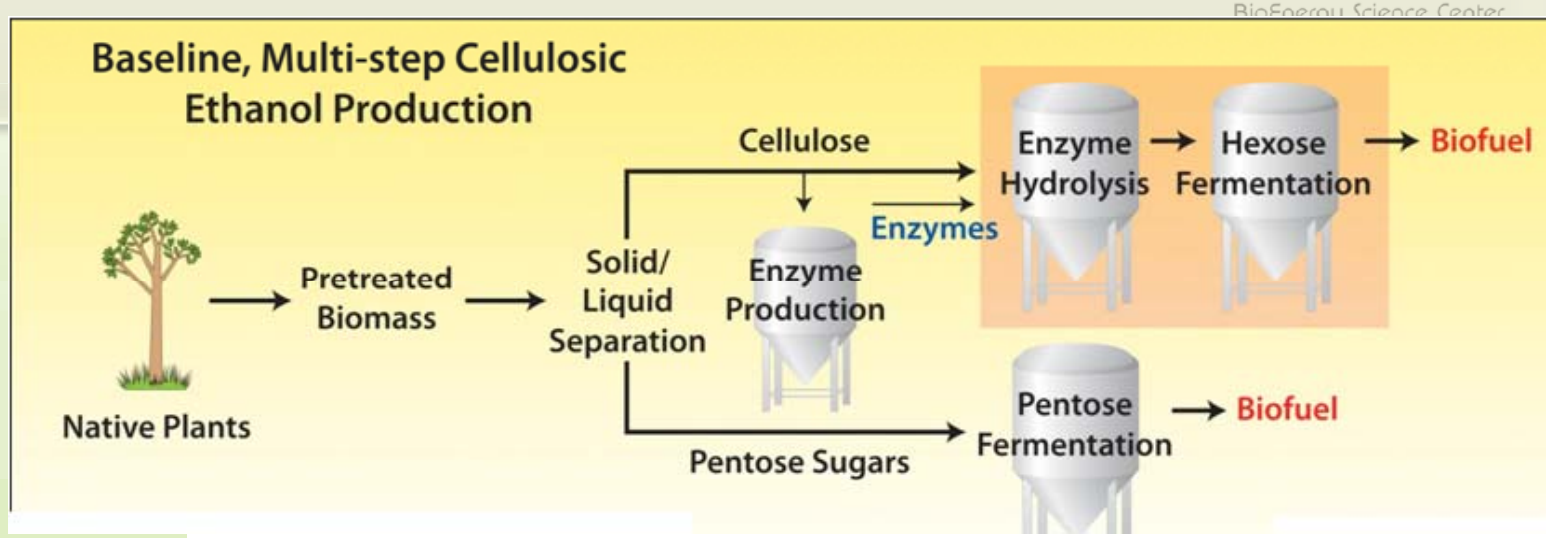
The Southeast and Midwest will be the sources for US biomass

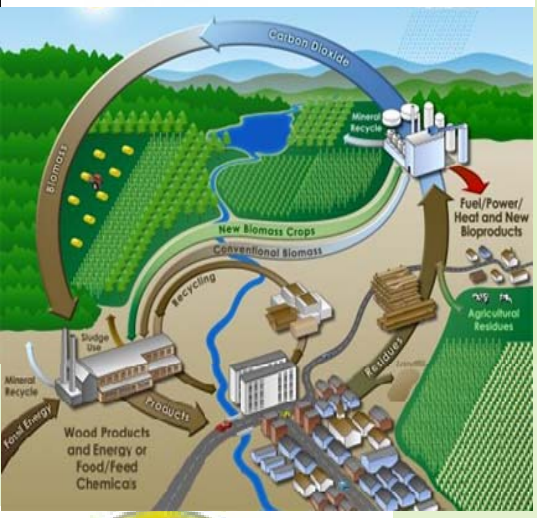
Science

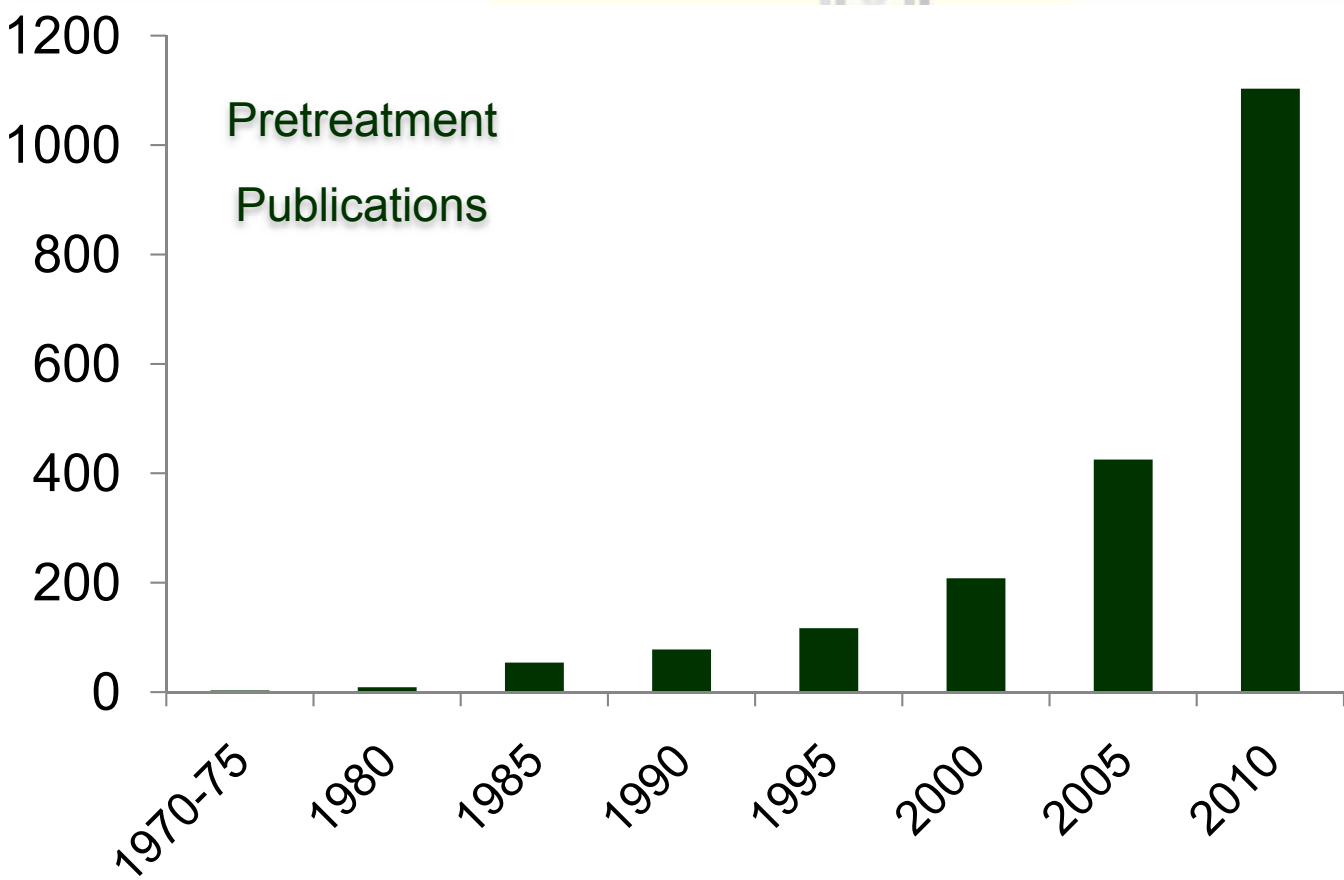
PRETREATMENT

History









Recalcitrance/lignin?

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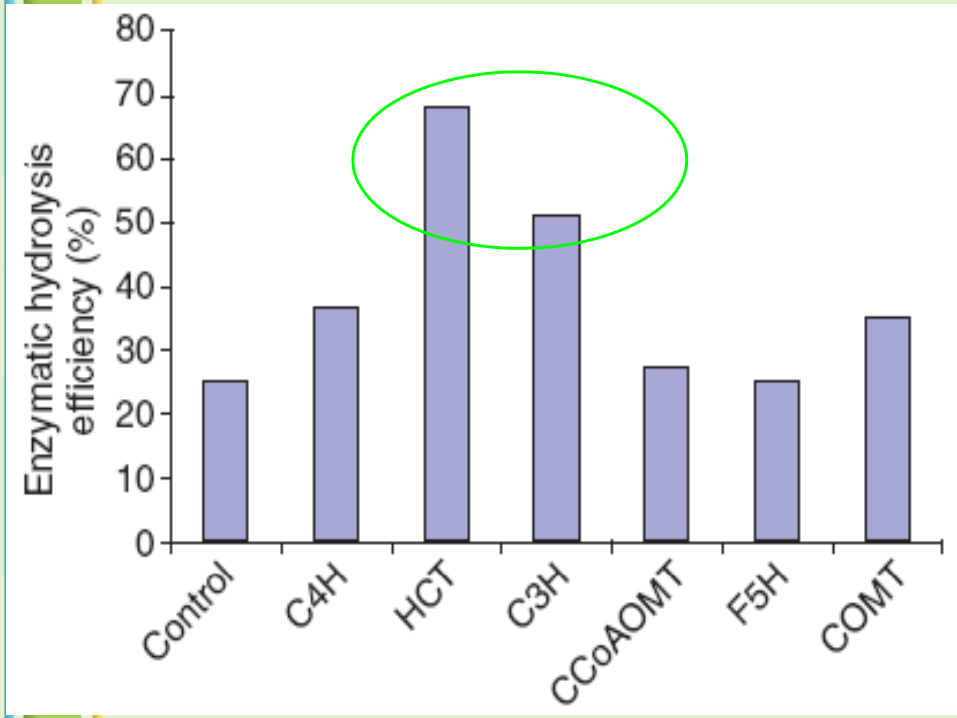
- Lignin is considered the most recalcitrant and as major obstacle in processing of plant biomass to biofuels
- Genetic engineering provides great potential to decrease the lignocellulosics recalcitrance

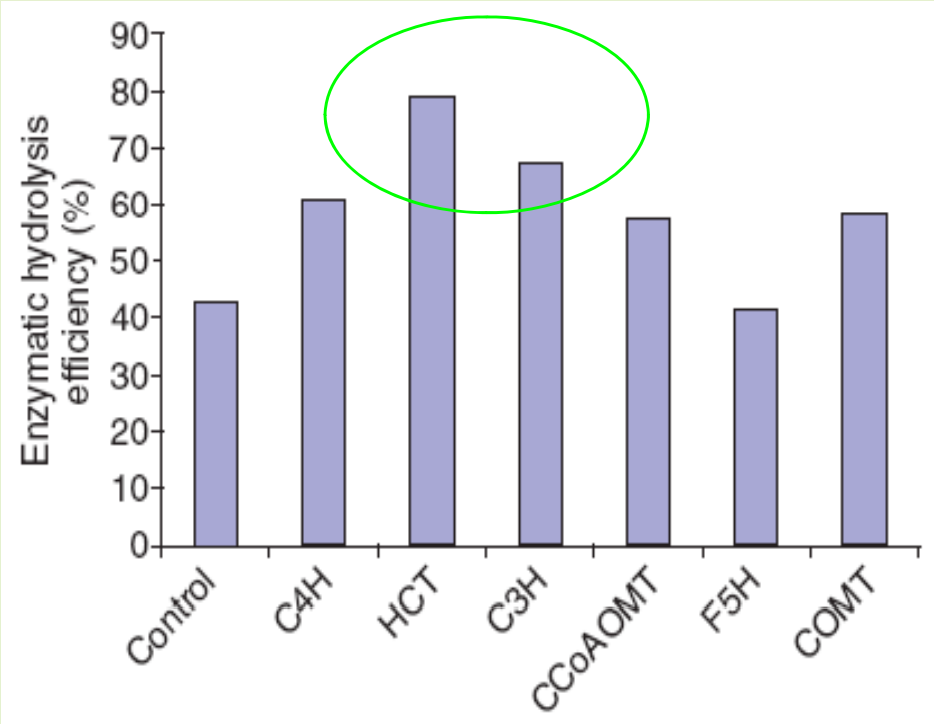
  - improving pulping process efficiency: O'Connel et al.
  - improving forages digestibility: Reddy et al.
  - improving fermentable sugar yields of alfalfa

Alfalfa Lignin Modification

- Anti-sense gene down-regulation of alfalfa lignin
- HCT and C3H lines have the prominent effects to improve saccharification - Transgenic alfalfas showed lower lignin content, less recalcitrance for enzymatic hydrolysis



Sugar release for alfalfa after enzymatic hydrolysis *without* acid pretreatment*



Sugar release for alfalfa after enzymatic hydrolysis *with* acid pretreatment

**Chen & Dickson Nature Biotechnology 2007,23 (7) 759-76.*

Alfalfa Cellulose/Lignin

Wild **C3H**

Alfalfa Samples

↓

Benzene/ethanol (v/v 2:1)

↓

Ball-milling

↓

Dioxane/water lignin extraction

↓

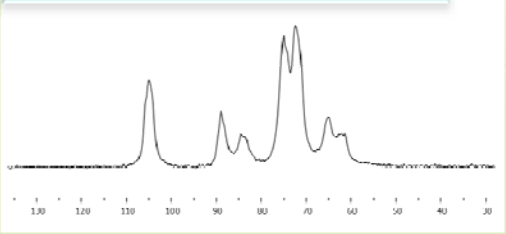
Purification

↓

Lignin analysis

Holocellulose/H^+

13C CP/MAS NMR



Alfalfa	CrI, %
CTR1	52.8
CTR49A	51.3
C3H9A	53.9
C3H4A	54.3
HCT3A	53.5
HCT30A	54.4

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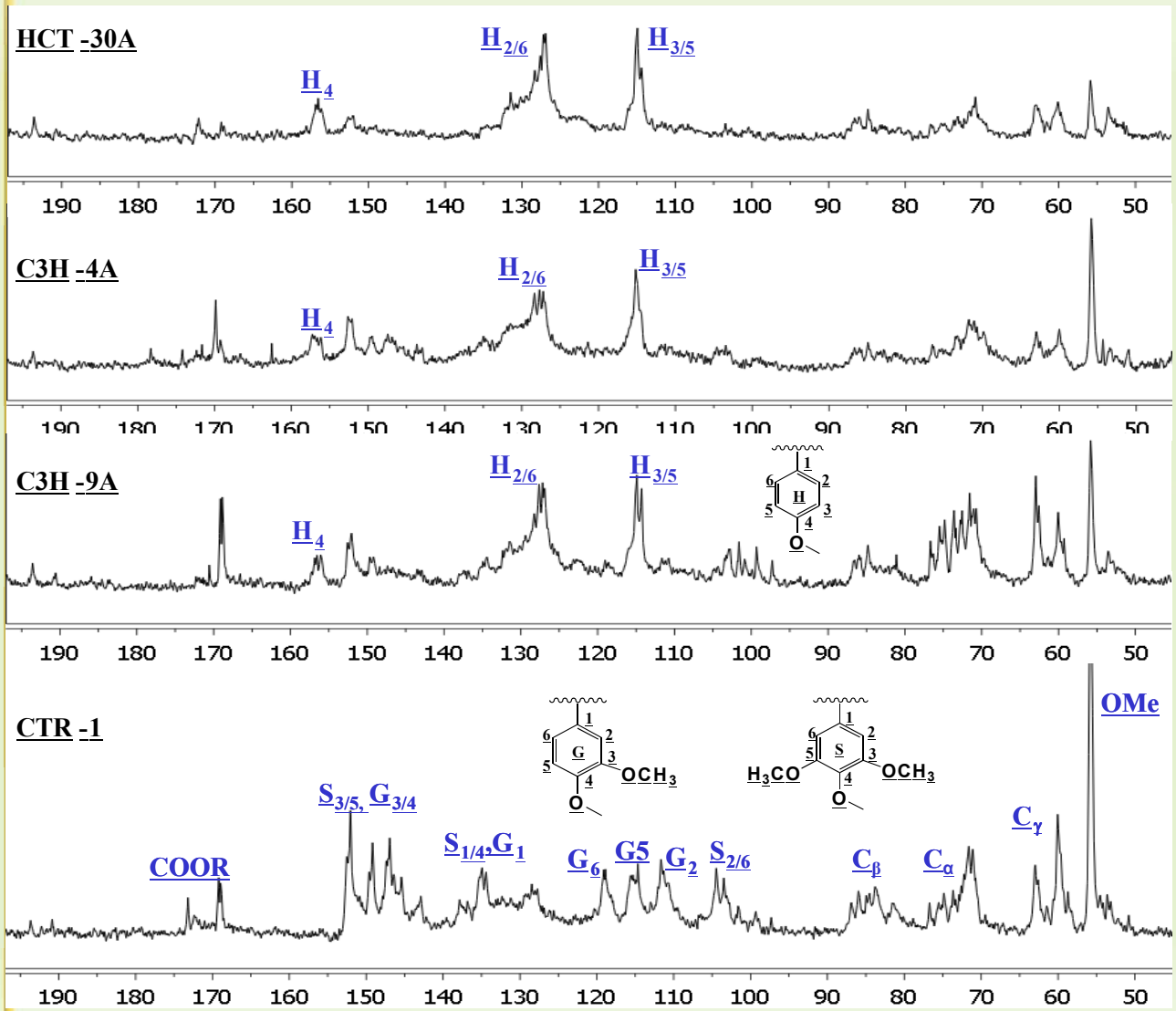
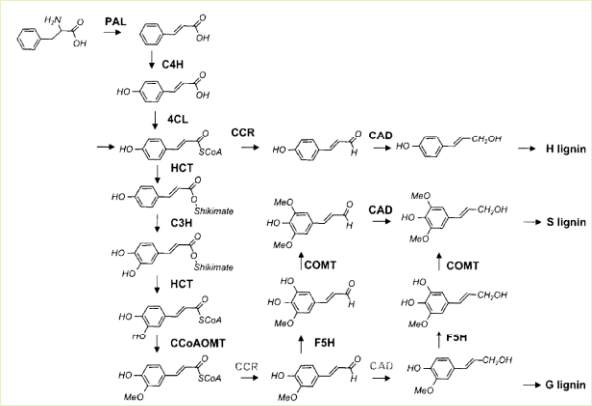
Quantitative ^{13}C NMR Characterization

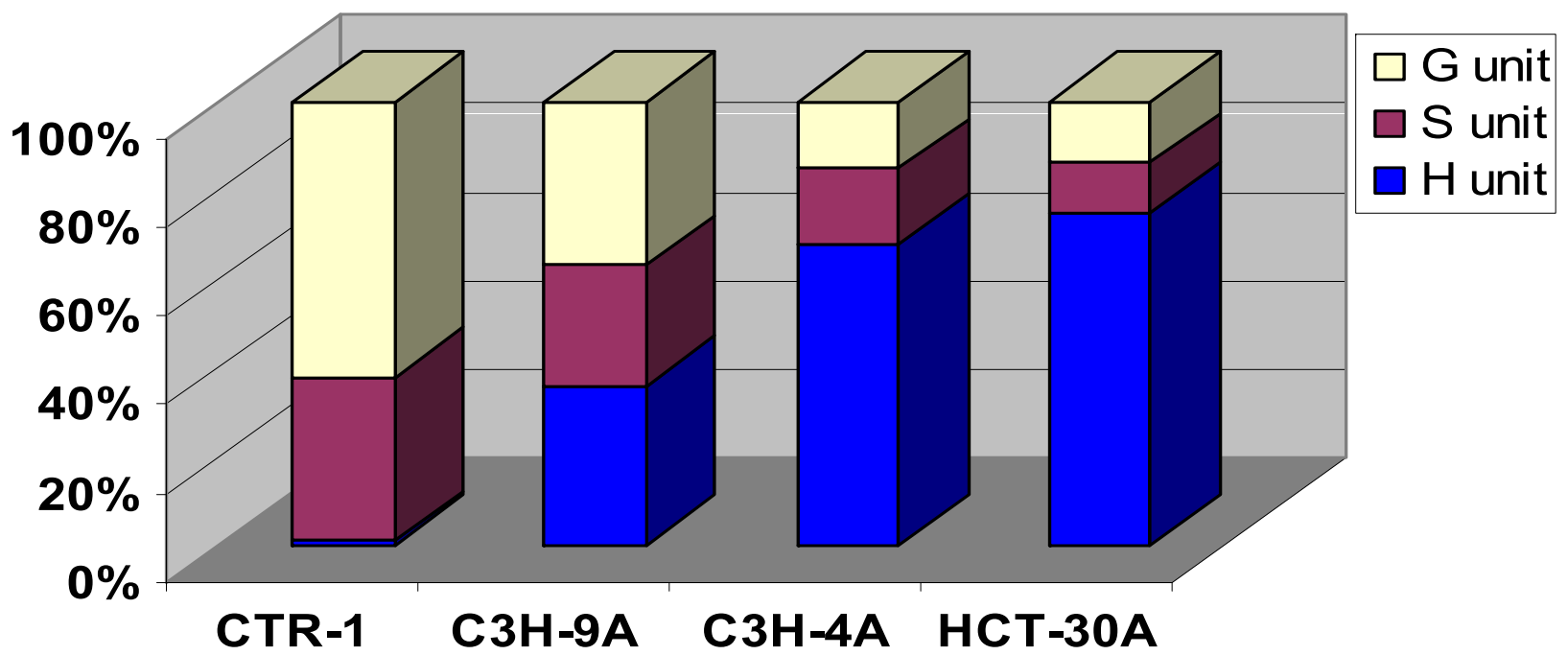


^{13}C quantitative NMR spectra of isolated alfalfa lignins

- DMSO as solvent
- Inverse gated pulse, 12 s delay, 50 °C
- 12288 scans



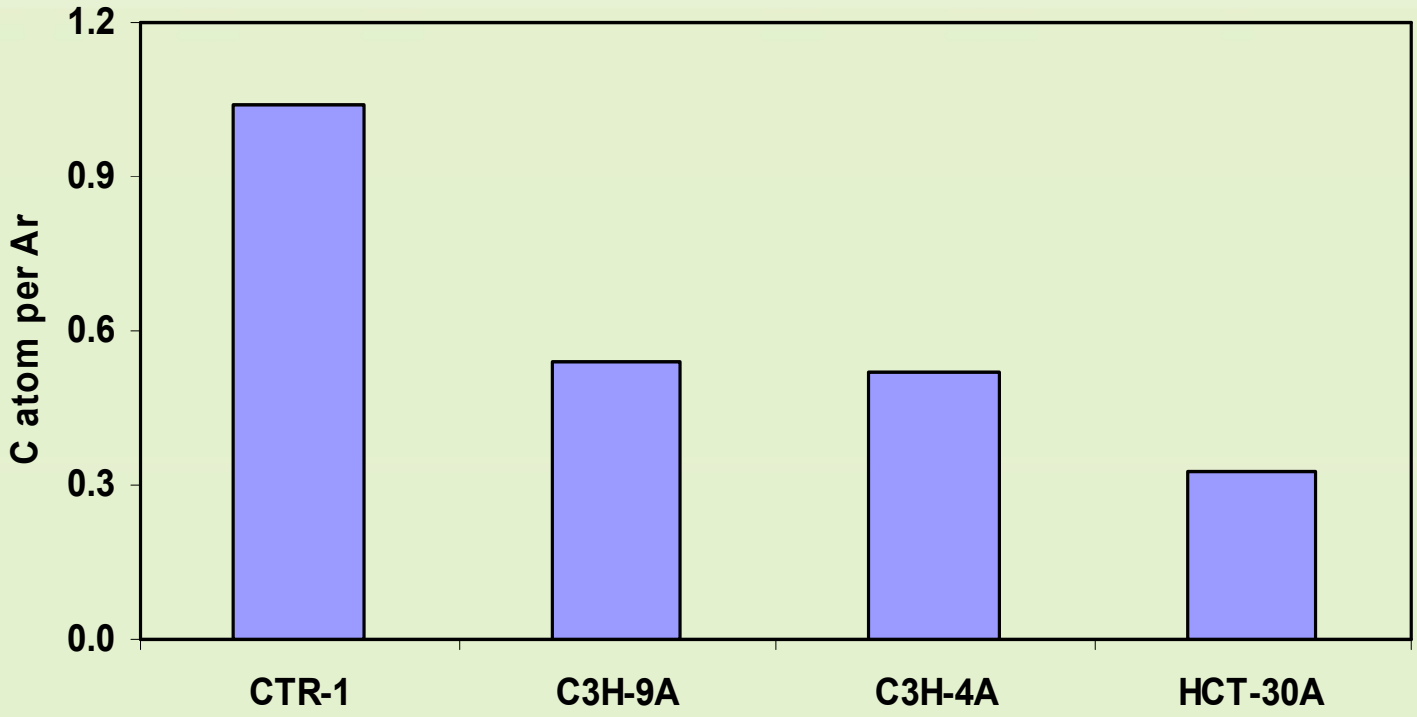
Quantitative ^{13}C NMR Characterization



H:S:G units composition of isolated alfalfa lignins

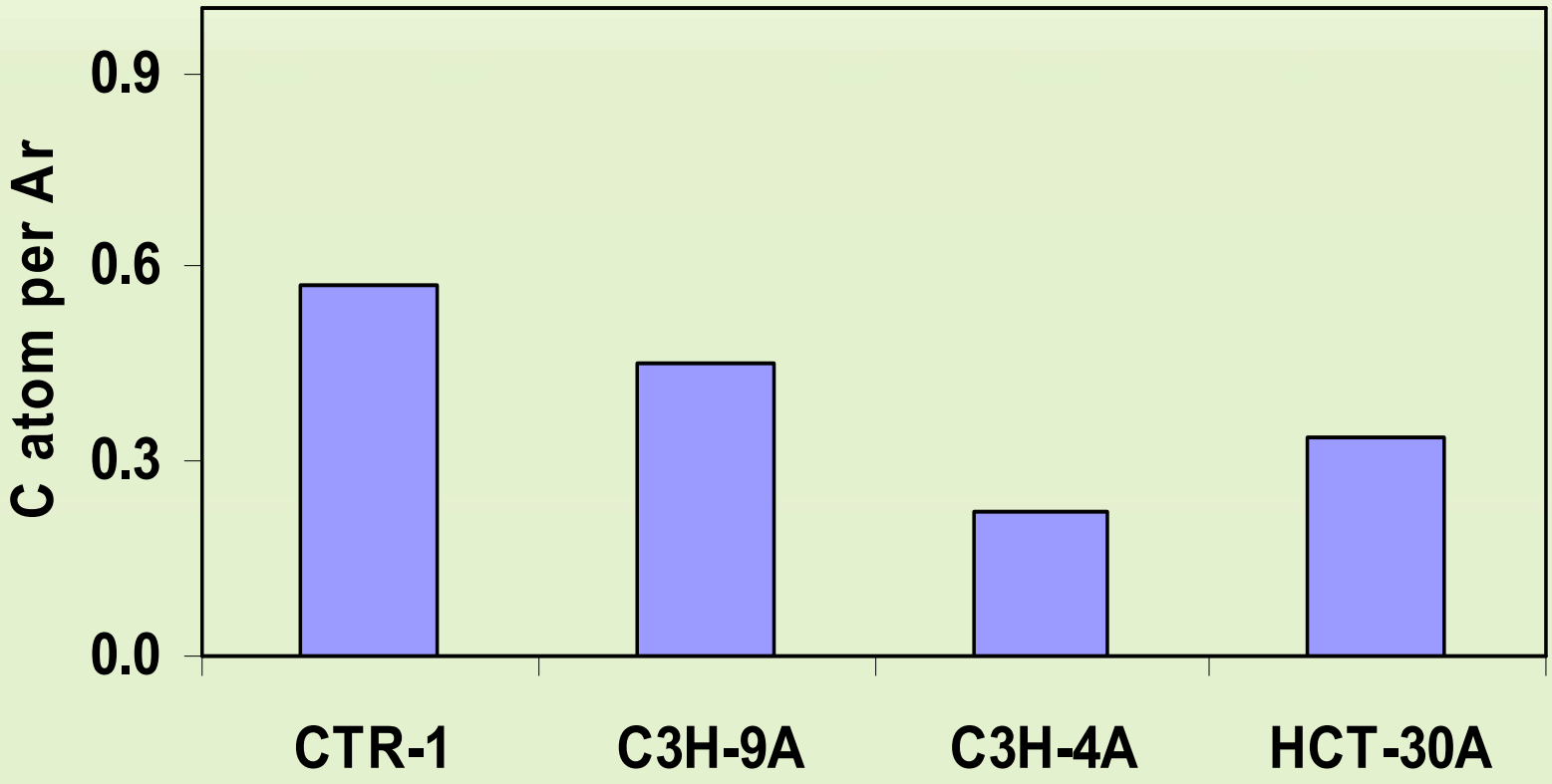
- H units increase from ~1% to 36~68% for C3H mutant, to ~75% for HCT transgenics.
- G units decrease by 36~75% for C3H mutant, ~77% for HCT transgenics
- S units decrease by 27~54% for C3H mutant, ~70% for HCT transgenics

Quantitative ^{13}C NMR Characterization



Methoxy group contents of isolated alfalfa lignins

Quantitative ^{13}C NMR Characterization



- β-O-4 linkage decrease ~by 61% for C3H, ~40% for HCT transgenics

Quantitative ^{13}C NMR Characterization

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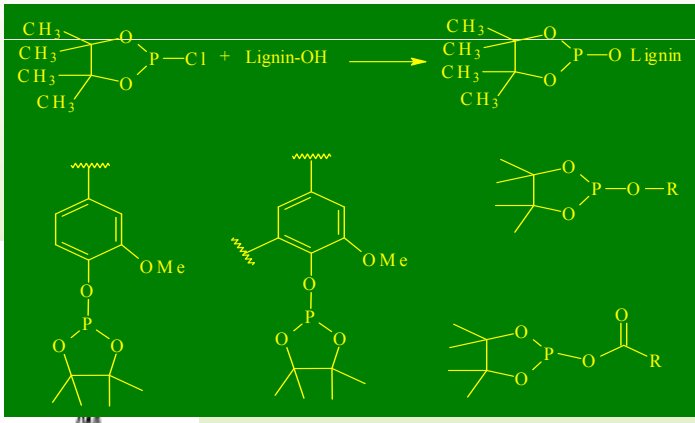
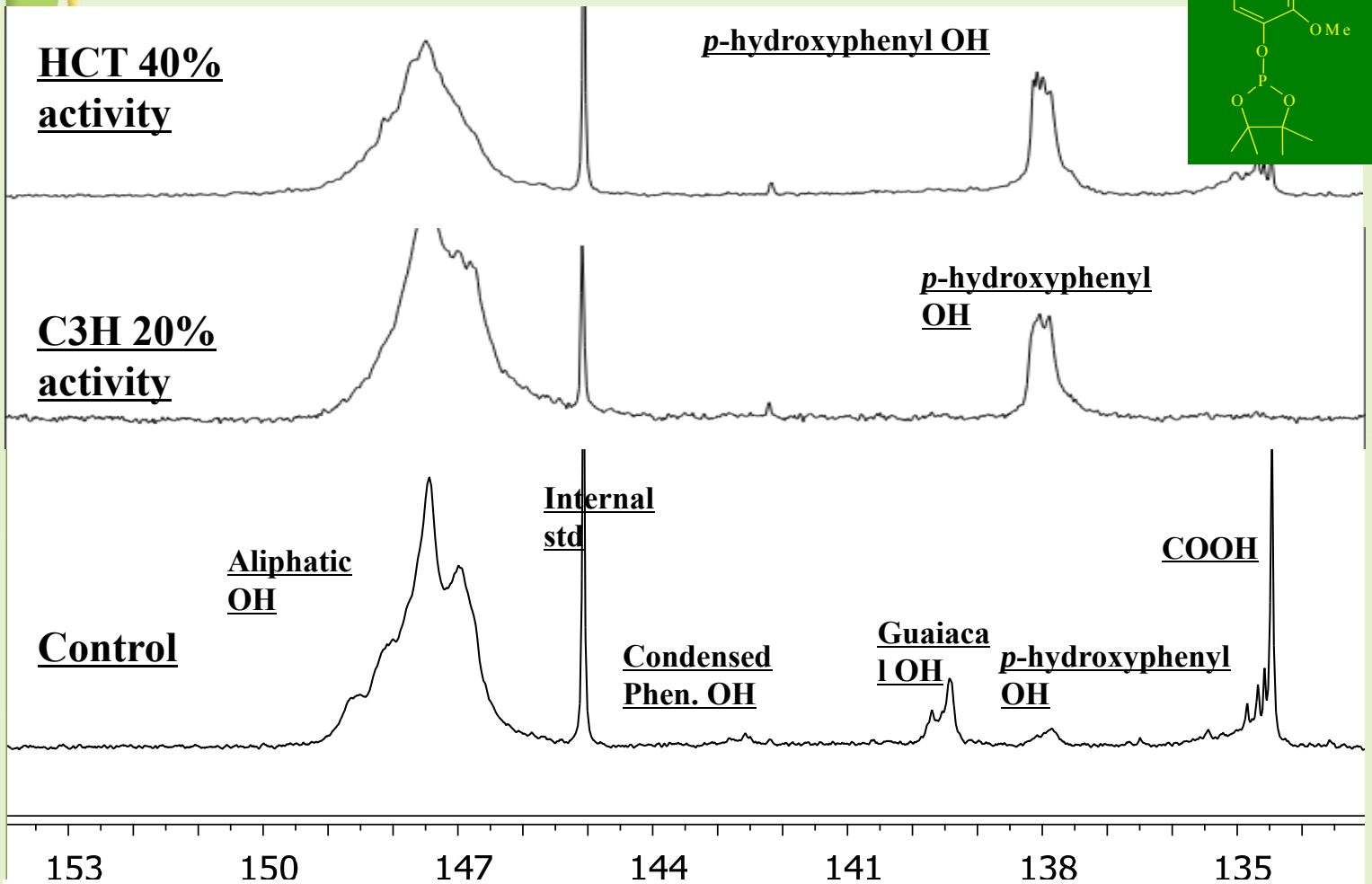
- H units increase from ~1% to 36~68% for C3H mutant, to ~75% for HCT transgenics.
- G units decrease by 36~75% for C3H mutant, ~77% for HCT transgenics
- S units decrease by 27~54% for C3H mutant, ~70% for HCT transgenics
- Methoxyl group content decrease by ~50% for C3H, ~68% for HCT transgenics
- β-O-4 linkage decrease ~by 61% for C3H, ~40% for HCT transgenics

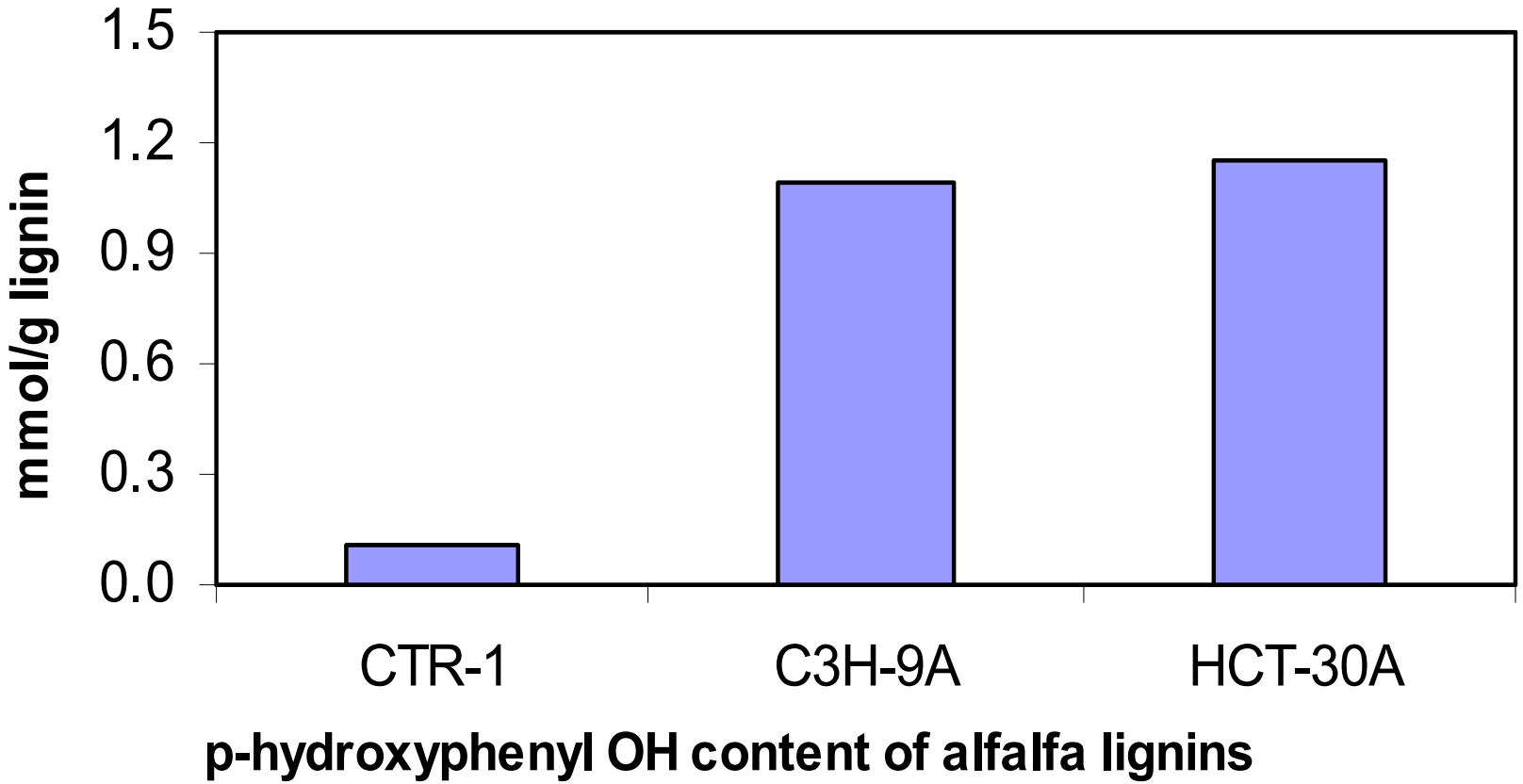
^{31}P NMR Characterization



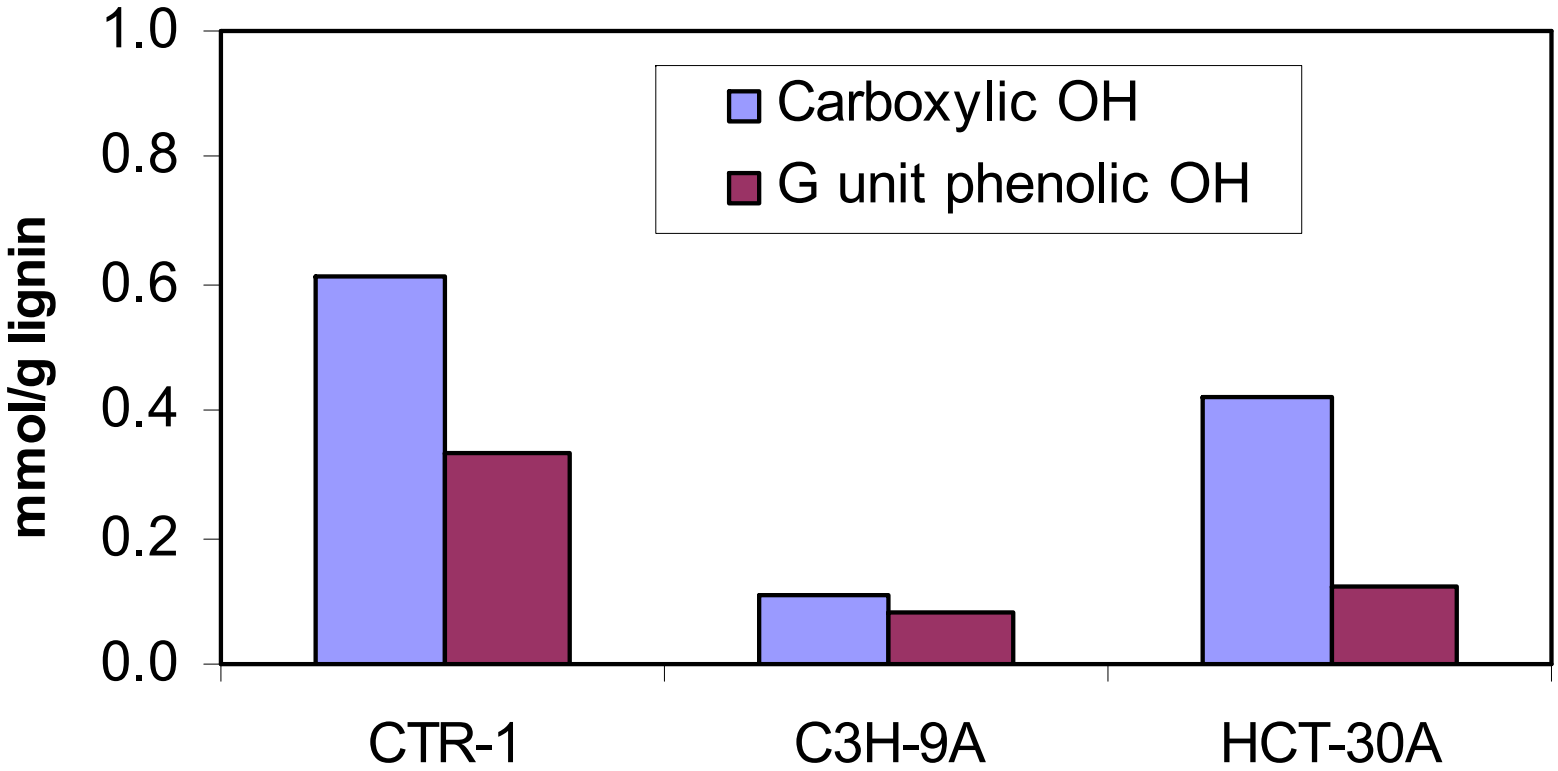


^{31}P NMR spectra of alfalfa lignins

^{31}P NMR Characterization



^{31}P NMR Characterization



Carboxylic OH and guaiacyl phenolic OH contents

^{31}P NMR Characterization

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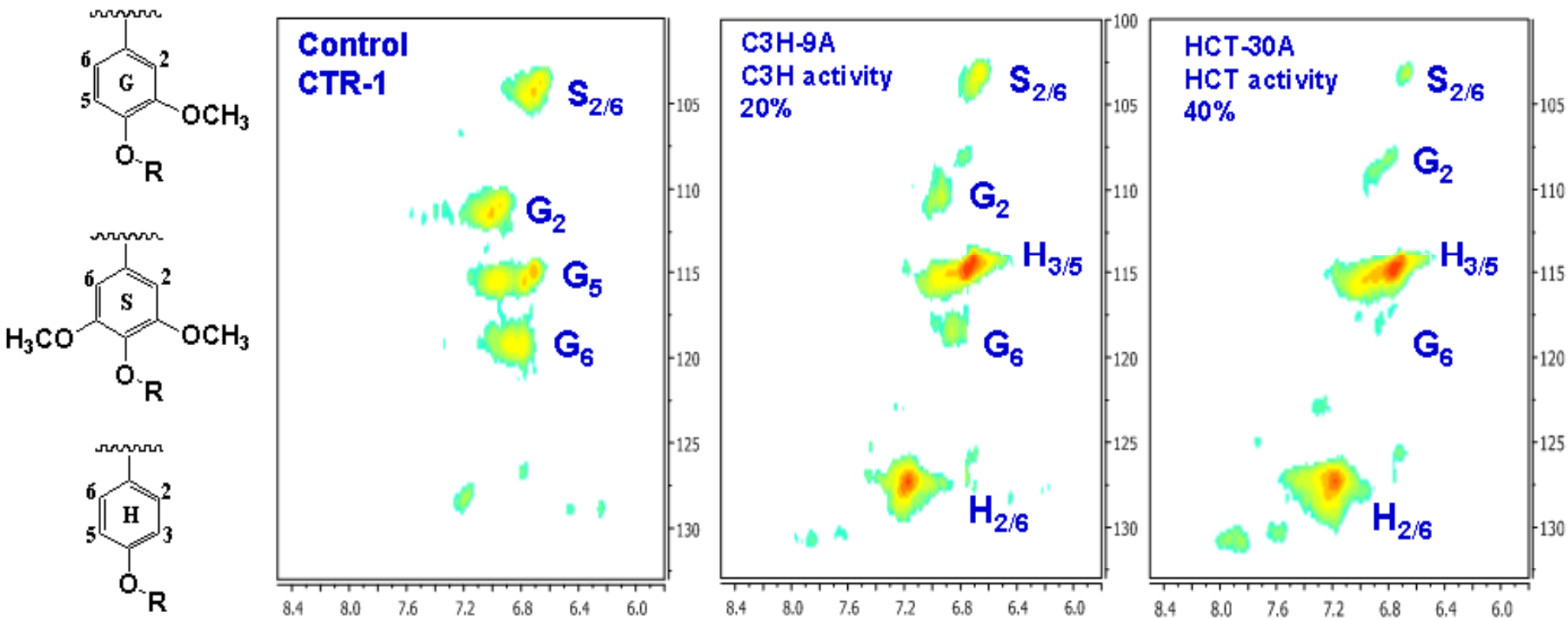
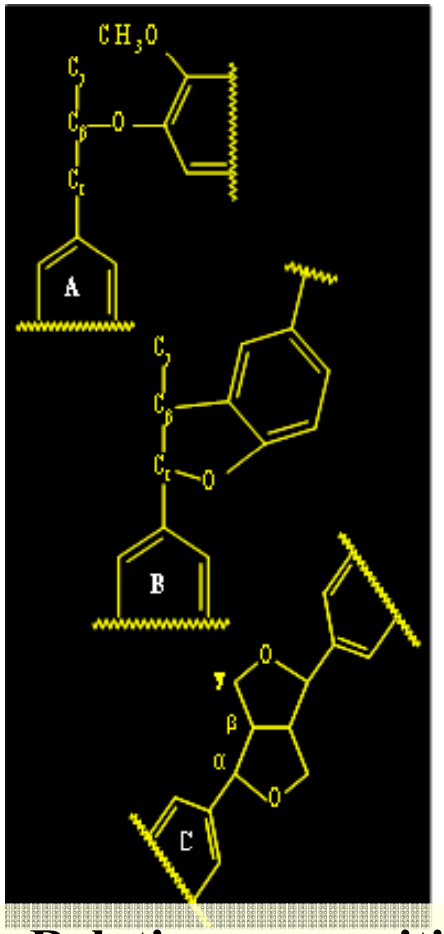
- p-Hydroxyphenyl OH increase by ~890% and 945% for C3H and HCT transgenics.
- Guaiacyl OH decrease by ~ by ~75% and 64% for C3H and HCT transgenics.
- Carboxylic OH decrease by ~ by ~81% and 31% for C3H and HCT transgenics.

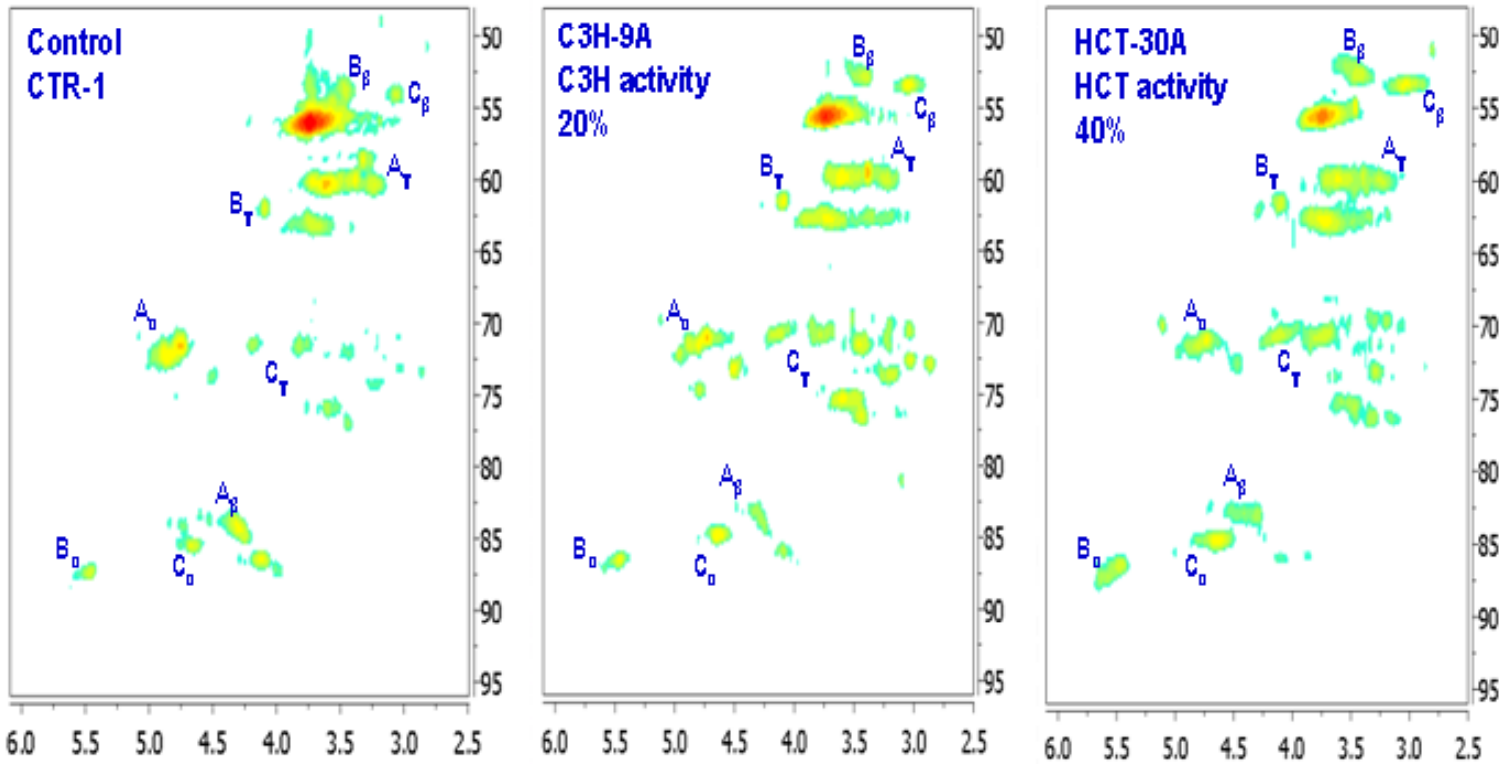
^{13}C-^{1}H HSQC 2D Correlation NMR characterization



Partial ^{13}C-^{1}H HSQC 2D correlation NMR (aromatic region) of alfalfa lignins

^{13}C-^{1}H HSQC 2D correlation NMR Characterization

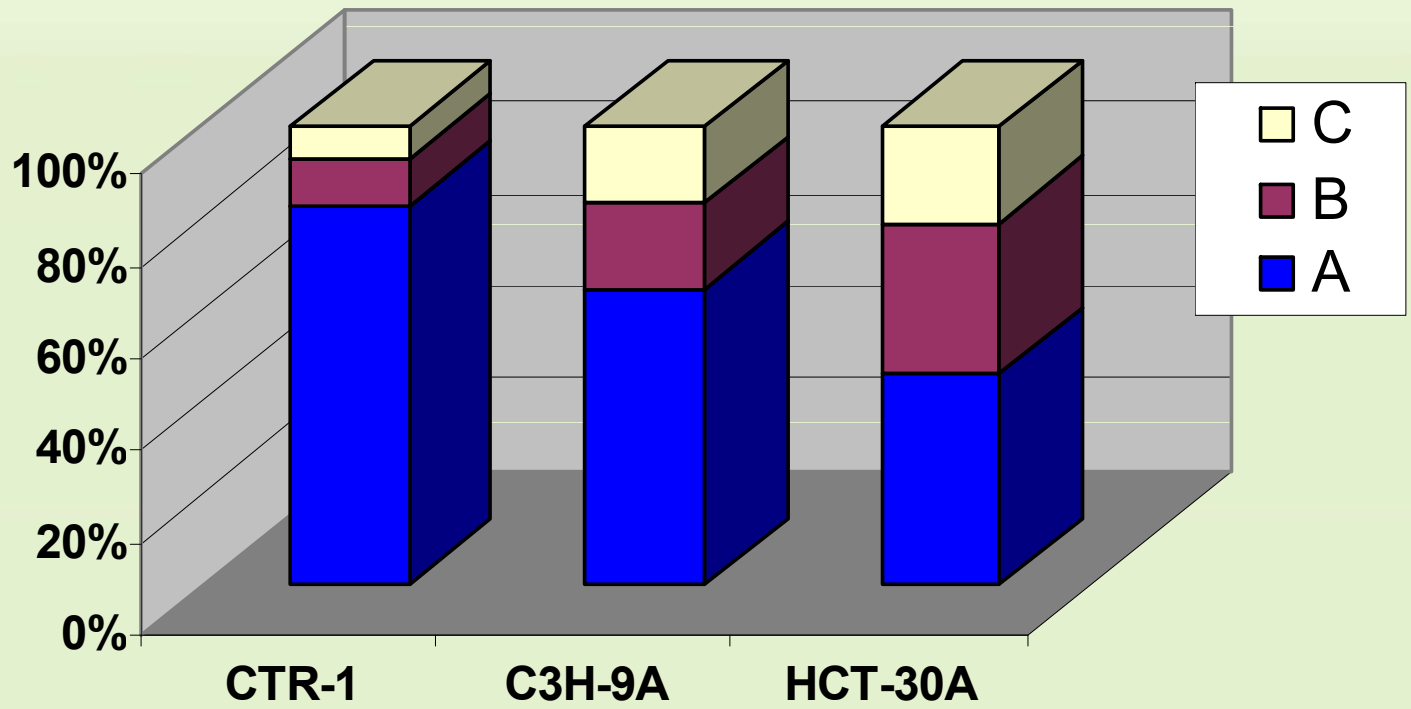




Partial ^{13}C-^{1}H HSQC 2D correlation NMR (aliphatic region) of alfalfa lignins

- Relative composition of β–O-4 decrease by ~21% and ~44% for C3H and HCT transgenics;
- Relative composition of phenylcoumaran (B) increase by ~85% and ~220% for C3H & HCT
- Resinol (C) increase by 53% and ~174% for C3H and HCT transgenics.

^{13}C-^{1}H HSQC 2D correlation NMR Characterization



Interunit linkages of alfalfa lignins

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- Relative composition of phenylcoumaran increase by ~85% and ~220% for C3H and HCT transgenics;
- Resinol increase by 53% and ~174% for C3H and HCT transgenics.

NMR Characterization

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- ^{31}P: p-Hydroxyphenyl OH increase by ~890% and 945% for C3H and HCT transgenics.
- ^{31}P: Guaiacyl OH decrease by ~ by ~75% and 64% for C3H and HCT transgenics.
- ^{31}P: Carboxylic OH decrease by ~ by ~81% and 31% for C3H and HCT transgenics.

- C3H and HCT gene down-regulation, lignin had significant increase of H unit with decrease of G and S units, as well as significant decrease of carboxylic group, methoxyl group and β-O-4 linkage contents.
- Interunit phenylcoumaran and resinol contents increase for C3H and HCT transgenic alfalfa

- *Impact on lignin DP under study*

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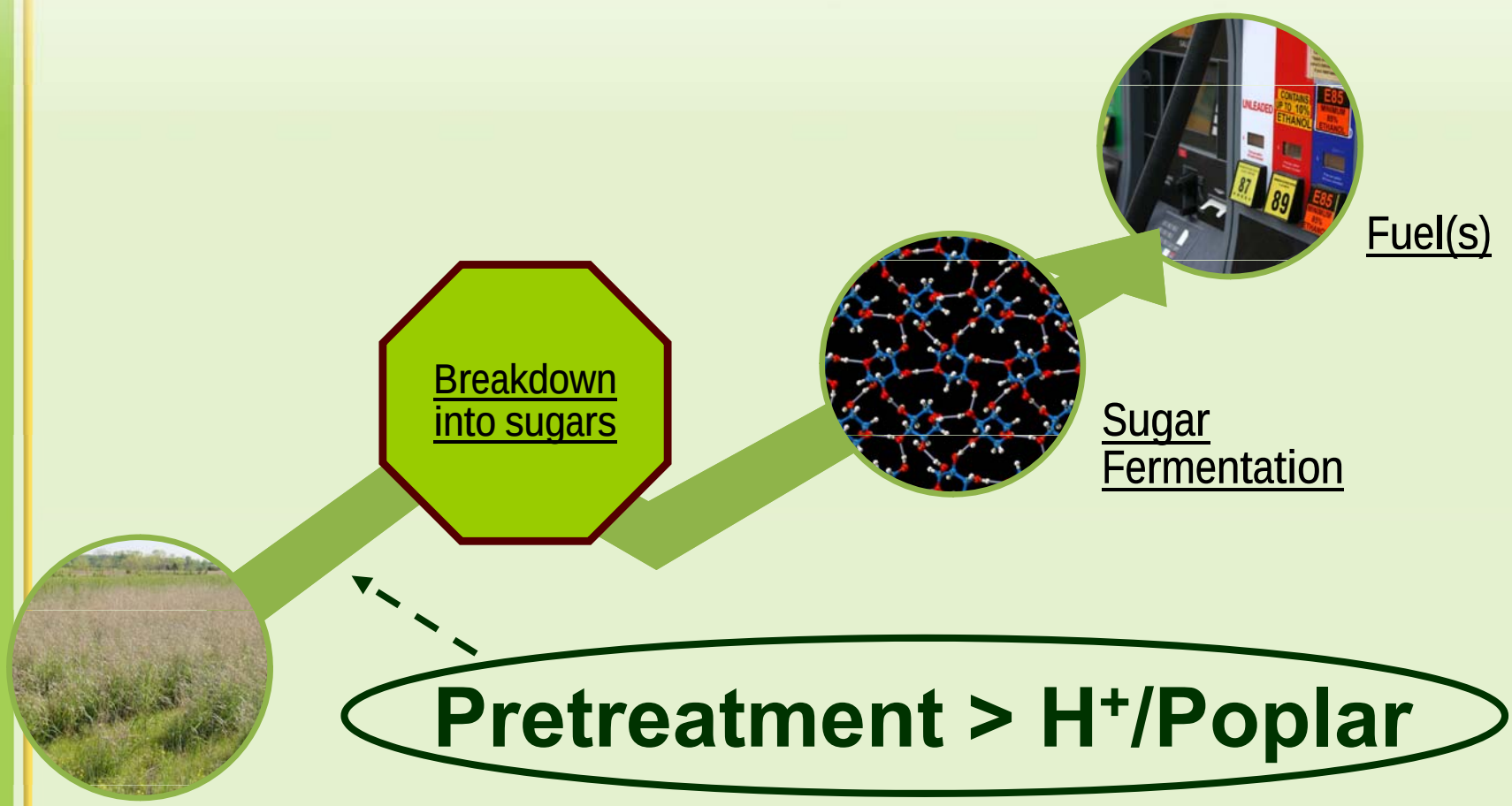
The Process of Bioethanol Production From Biomass



Pretreatment > H^+/Poplar

- Reduced recalcitrance of transgenic alfalfa
- No role for cellulose ultrastructure for samples studied
- Transgenics have lower lignin content, change in S:G:H, inter unit linkages, LCC & lignin is under review

Poplar Pretreated with Dilute Acid

Temp / ºC	Time /min	H_2SO_4 / % w/w
170	0.3	0.5
170	1.1	0.5
170	5.4	0.5
170	8.5	0.5
170	26.8	0.5

Composition of Poplar After Pretreatment

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Samples	Arabinose	Galactose	Glucose	Xylose	Mannose	Lignin
Control	0.4	0.8	57.9	13.5	2.8	24.6
Poplar_1	0	0.1	68.5	4.0	1.3	26.0
Poplar_2	0	0.0	70.2	3.6	1.2	25.0
Poplar_3	0	0.0	72.5	1.2	0.5	25.8
Poplar_4	0	0.0	74.2	1.0	0.4	24.4
Poplar_5	0	0.0	74.0	0.2	0.2	25.6

- **Xylan was removed during dilute acid pretreatment;**
- **Cellulose content increased as dilute acid proceeded;**
- **Overall klason lignin content gradually increases**

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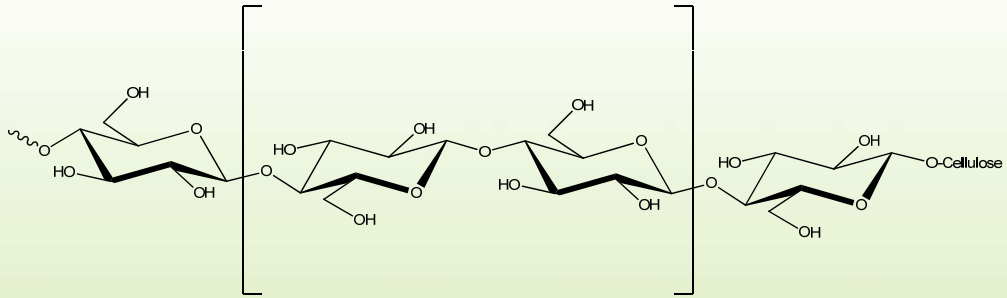
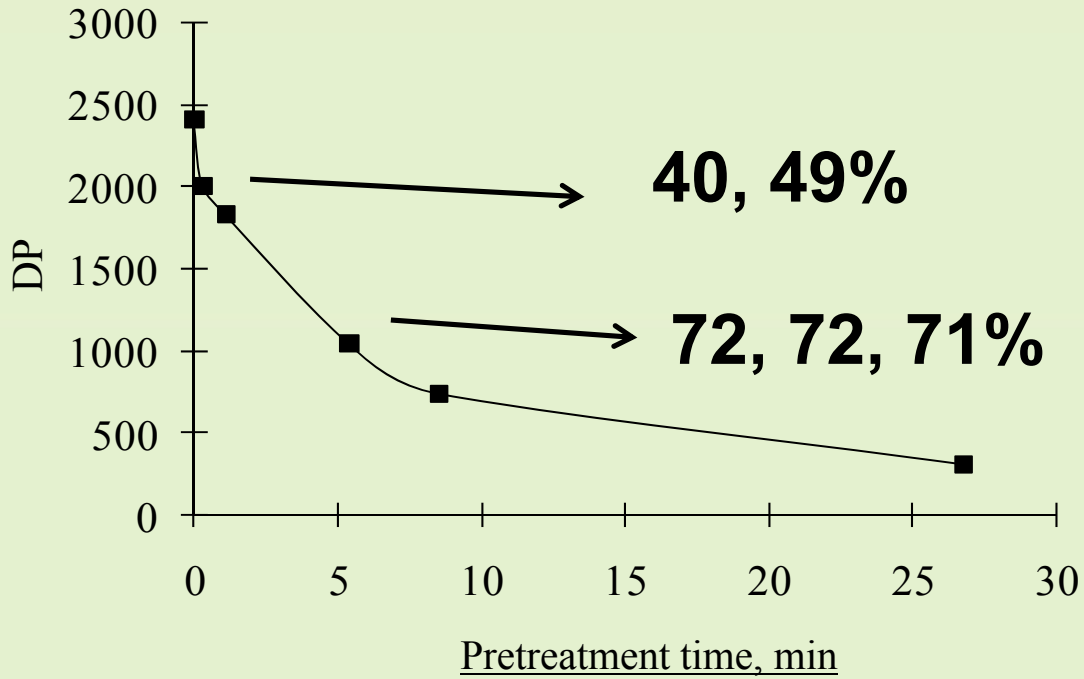
Cellulose: Molecular Weight Distribution

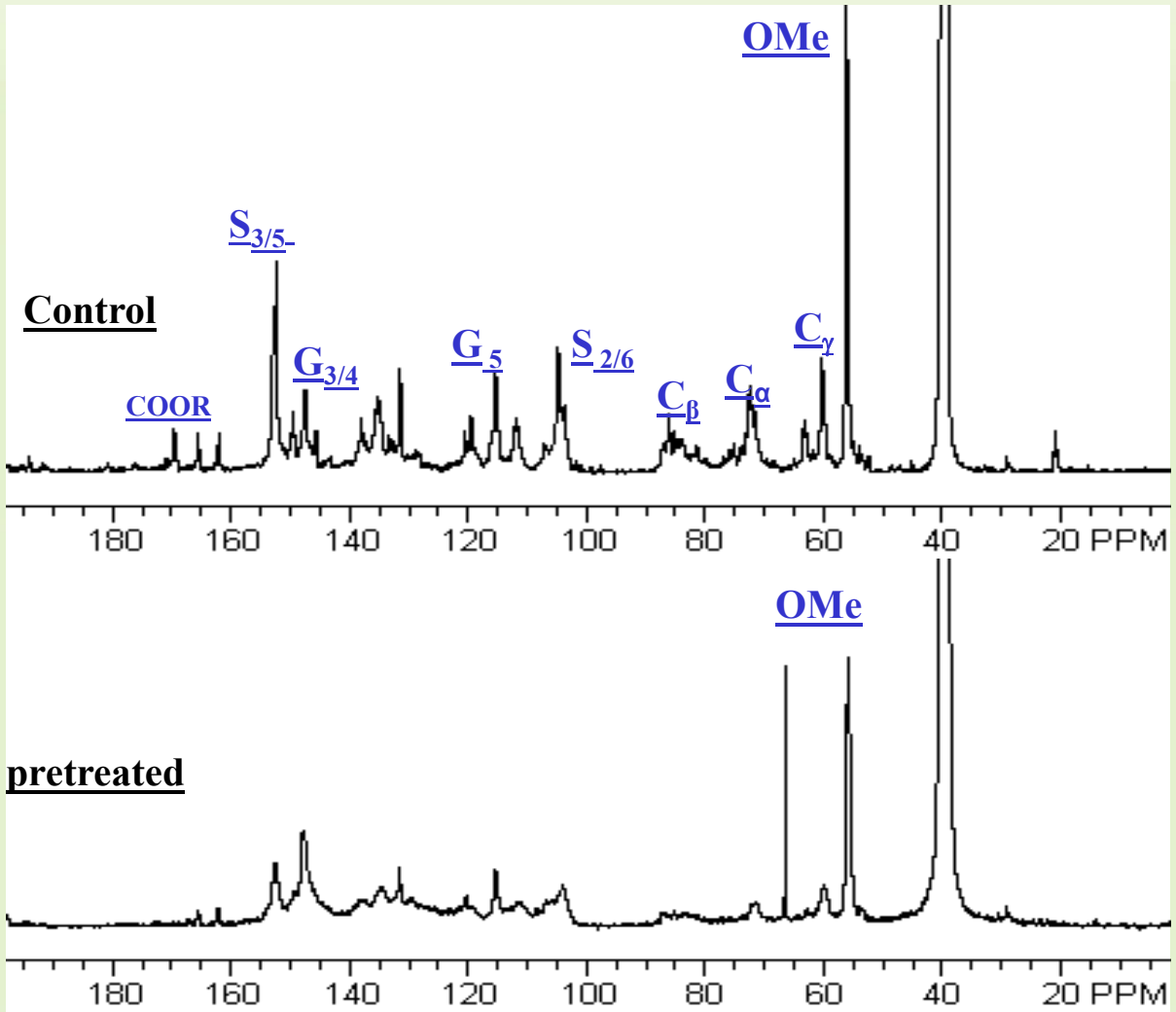


SEC analysis results of cellulose from dilute acid pretreated poplar.

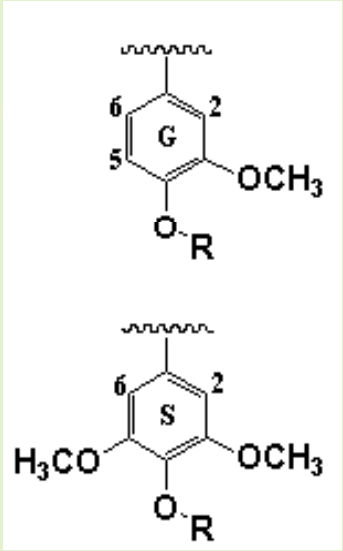


- Degree of polymerization decrease during dilute acid pretreatment;
- DP decreased rapidly at first 8 min pretreatment with ~70% decrease;
- Additional 17% decrease when pretreatment time extends to 27 min

Lignin: Quantitative ^{13}C NMR characterization



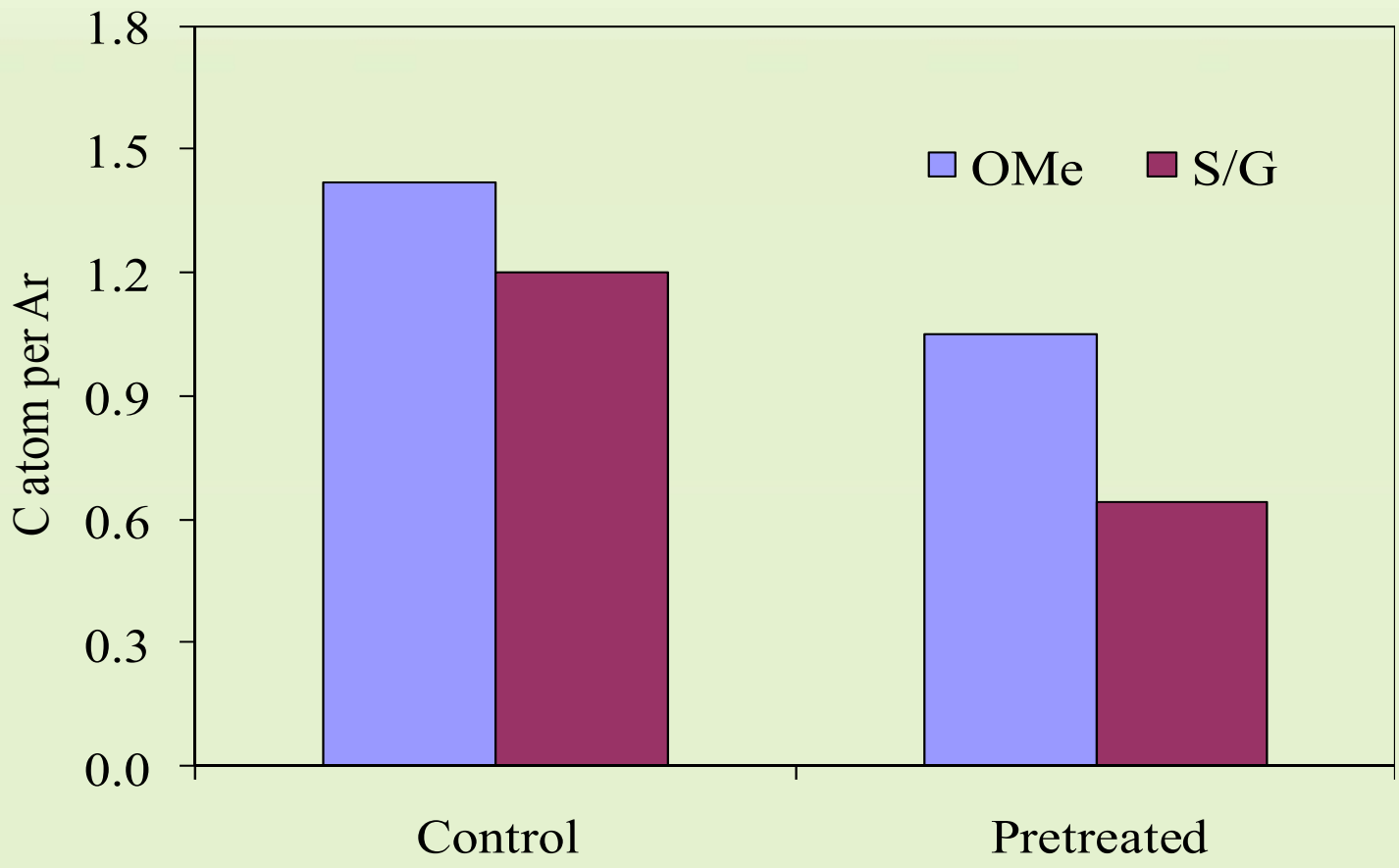
- DMSO as solvent
- Inverse gated pulse, 12 s delay, 50 °C
- 12288 scans



^{13}C quantitative NMR spectra of isolated poplar lignin

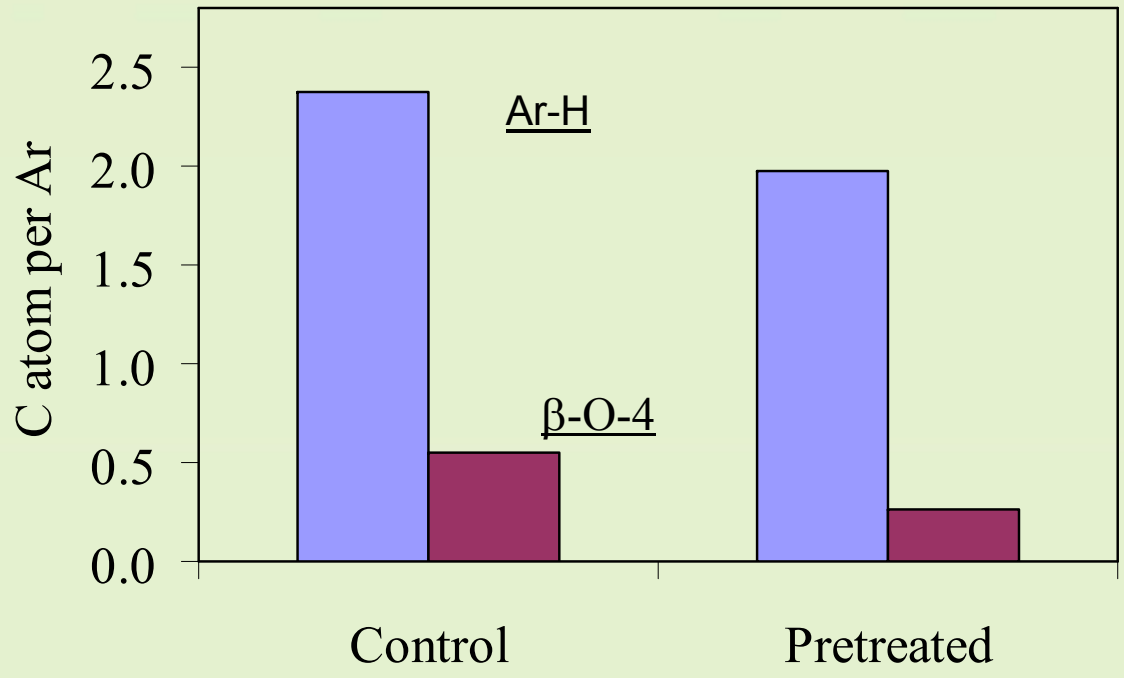
Lignin: Quantitative ^{13}C NMR Characterization

Methoxyl content and S/G ratio of isolated poplar lignin



- Methoxyl group content decrease by ~26%/demethoxylation
- S/G ratio decrease ~47%

Lignin: Quantitative ^{13}C NMR Characterization



- β-O-4 linkage decrease by ~52%;
- Ar-H content decrease, indicating condensation of lignin increase

Surface vs. Bulk Analysis Pretreatment

Fresh Poplar

↓

50um cross section at -9C

↓

Soxhlet extraction by CH_2Cl_2

↓

Diluted acid pretreatment * (DAP)

↓

Soxhlet extraction by CH_2Cl_2

→

Surface Analysis

ToF-SIMS

&

Bulk Analysis

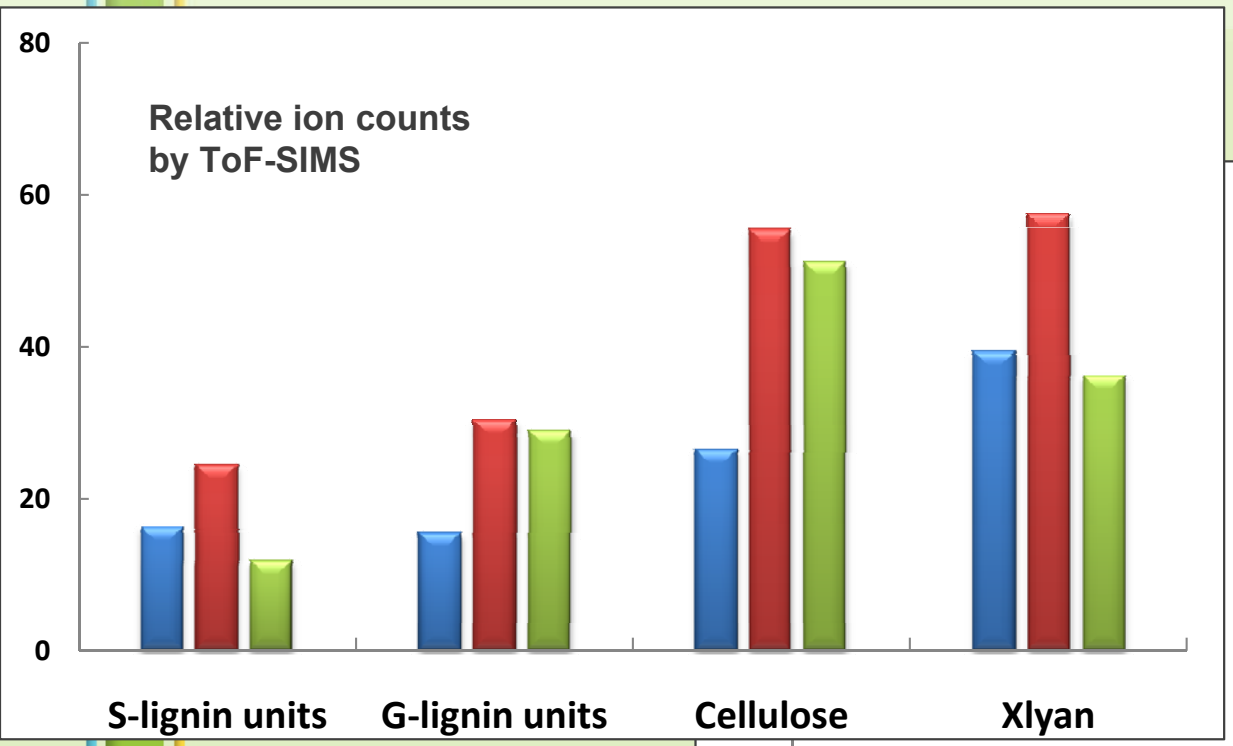
- Dilute acid pretreatment (DAP) - **1% H_2SO_4 @160°C 10 min or 2% H_2SO_4 @175°C 10 min**

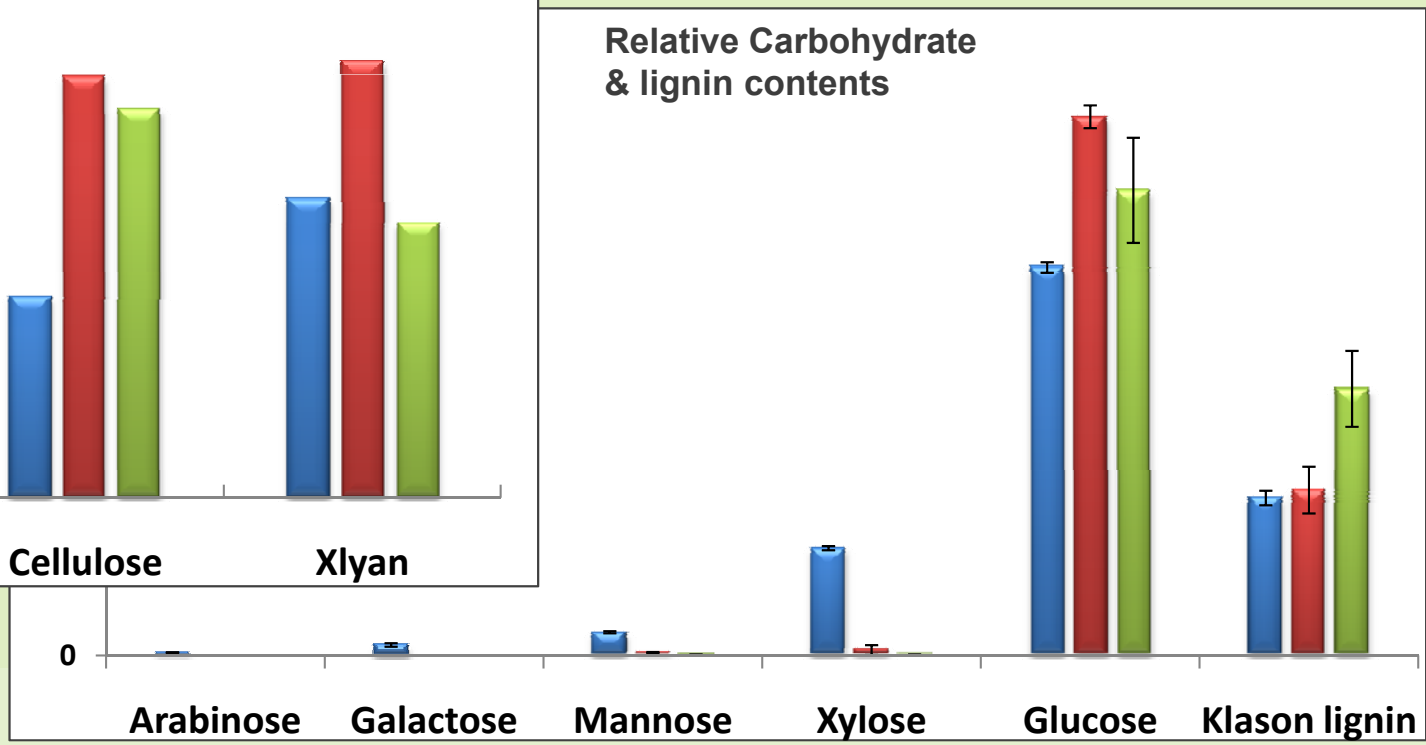
Surface vs. Bulk Analysis Pretreatment

- **Relative intensity by ToF-SIMS vs Bulk carbohydrate & Klason lignin analysis**

Dilute acid pretreatment (DAP)

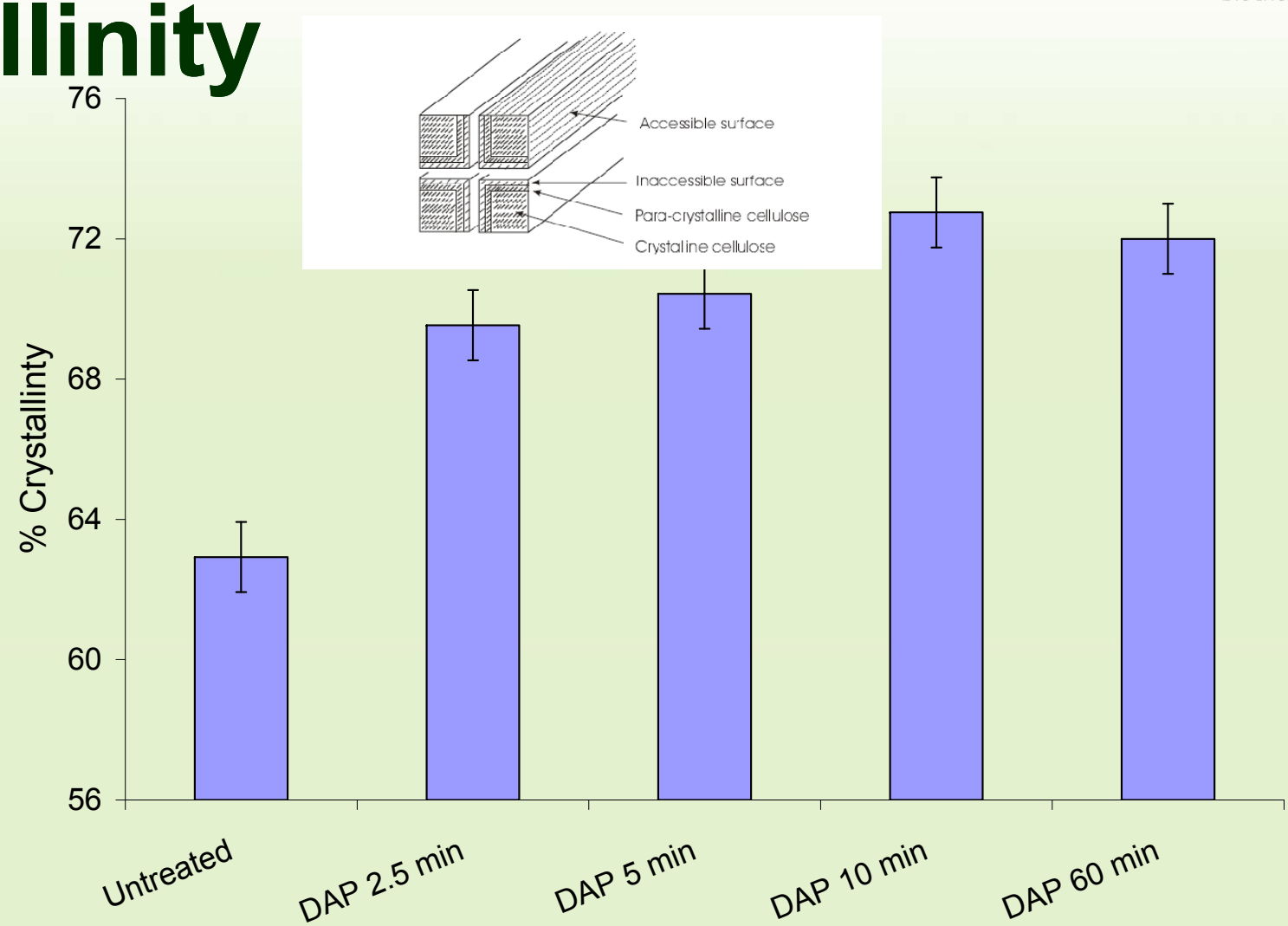
- **- 1% H_2SO_4 @160°C 10 min or 2% H_2SO_4 @175°C 10 min**





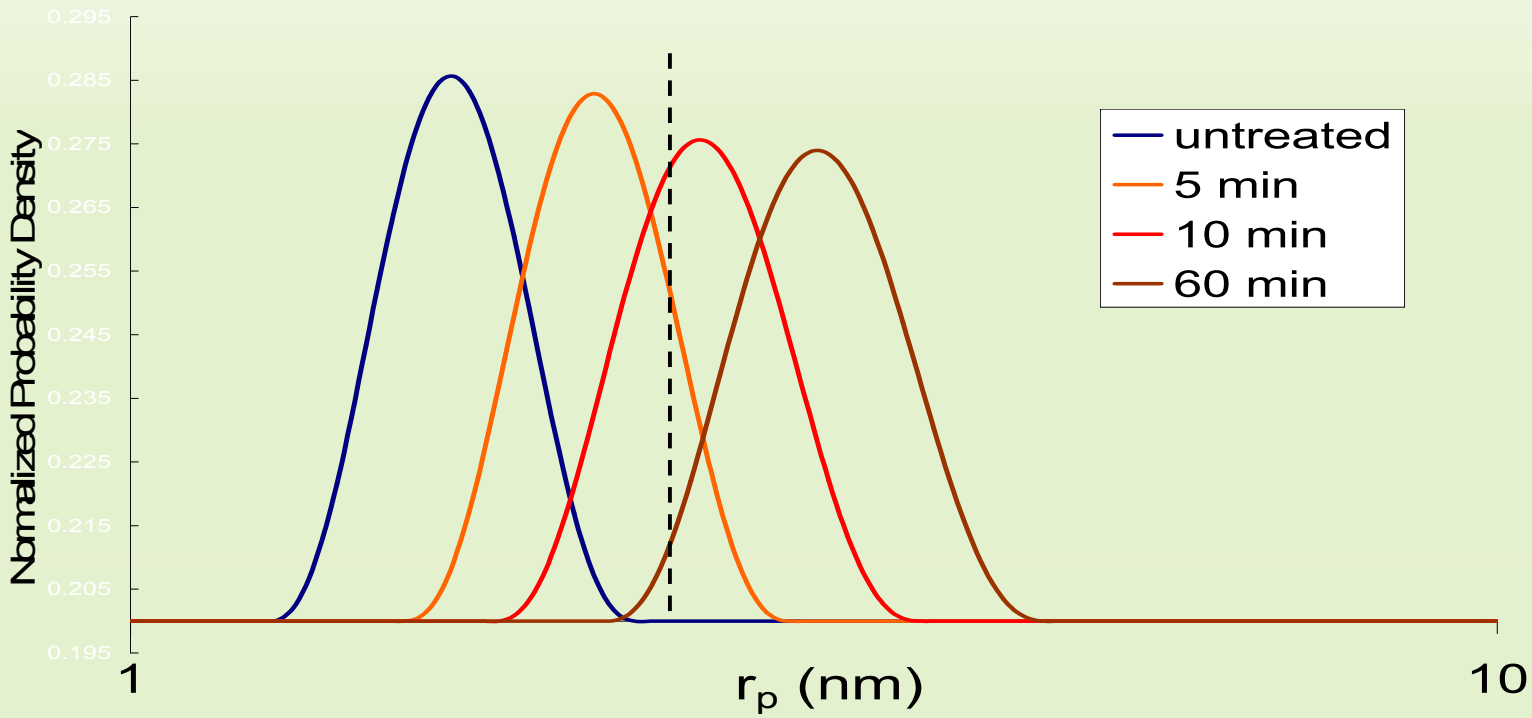
- **Bulk increase in lignin has been attributed to loss of cellulose or acid condensation polysaccharides**
- **Surface and Bulk have differences in Chemistry**

Poplar % Cellulose Crystallinity



- % Para-crystalline increase
- Total crystalline allomorph increase and then remain constant
- Observed cellulose I_α convert to cellulose I_β

Poplar Pore Size



Pore size distribution based on D_2O adsorption and ^2H T_1 relaxation for untreated and pretreated poplar biomass (pretreated in 0.10 M H_2SO_4 at 160 °C for various residences times) at a moisture content of 24 ± 5 %.

Conclusions

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- Changes in lignin structure –content impact recalcitrance for transgenics
- Dilute acid pretreatment
  - Removes most of xylan
  - *Role of LCC needs to be determined*
  - Cellulose CrI changes minimally or increases
    - Allomorphs I_α, I_β, paracryst. influenced, *role?*
    - *Need to identify viable approach to alter?*
    - Increase in pore size and accessibility
    - *Relationship?*
- Cellulose molecular weight decreases after dilute acid pretreatment, reducing sugars increase correlated with reduced recalcitrance
- Lignin structure was significantly changed after pretreatment
- Role of pseudo-lignin needs further examination
- Surface of pretreated material needs additional study

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Thank you!

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