
Structural modifications of cellulose and lignin in Loblolly pine arising from the ethanol organosolv pretreatment

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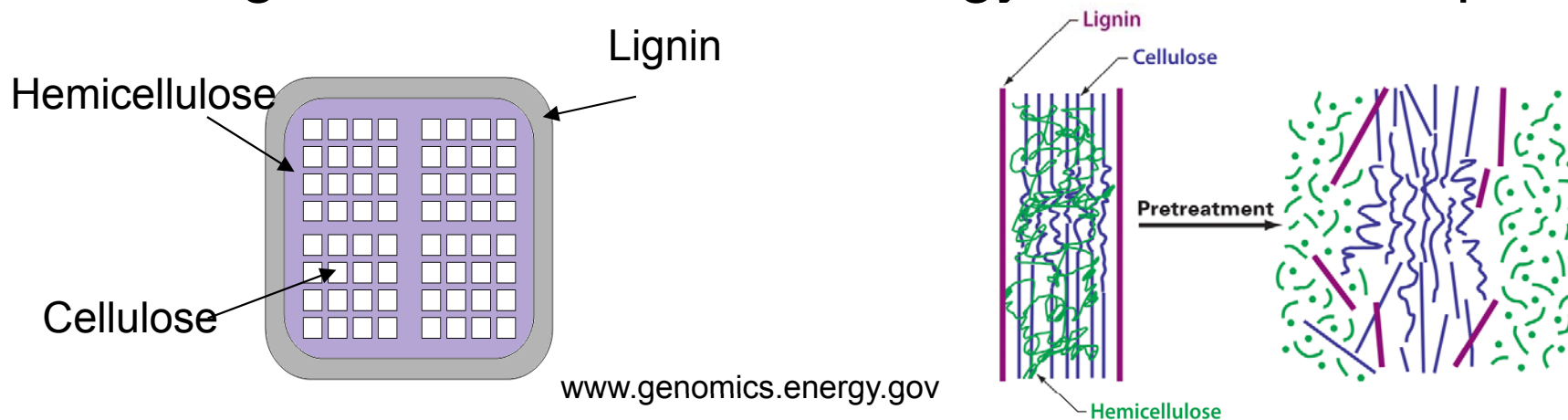
Background: Lignin and cellulose in biomass

- 2nd generation biofuels are cellulose-based
 - Lignin is generated as a by-product
- Cellulose structure varies with species
 - Can be modified during biofuels processing
- Lignin is the second most abundant biopolymer
 - Annual biosynthesis rate of 20×10^9 metric tons
- Structure and chemistry of lignin is species and process dependant
 - Can be manipulated by chemical and enzymatic pathways



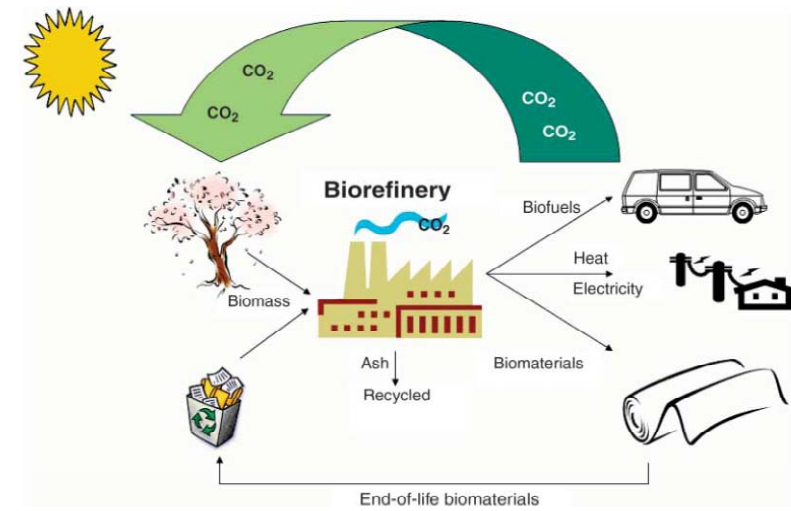
Background: Biomass pretreatments

- ❖ Pretreatments necessary to overcome inherent recalcitrance of lignocellulosic biomass towards enzymatic deconstruction
 - ❑ Steam explosion, dilute acid, AFEX, organosolv
 - ❑ Most current pretreatments produce co-products of low economic value
 - ❑ Lignin is used as heat/energy source for the process



Organosolv pretreatment

- Developed in Canada as the Alcell[®] process
- Biomass treated with water and organic solvent
 - Acid or base added as catalysts
- Has been optimized for softwood, hardwood and agricultural waste
- Acts like a biorefinery



Ragauskas et al. *Science* 311, 484-489

Lignocellulosic biomass

Organosolv pretreatment

Cellulose-rich solid

High-quality lignin

Hemicellulose-rich liquid



Objectives

- Investigate structural changes in Loblolly pine cellulose after organosolv pretreatment and enzymatic hydrolysis
- Determine lignin composition in untreated Loblolly pine, **residual lignin after organosolv pretreatment** and organosolv lignin
- Understand the mechanisms of changes in lignin structure during organosolv pretreatment

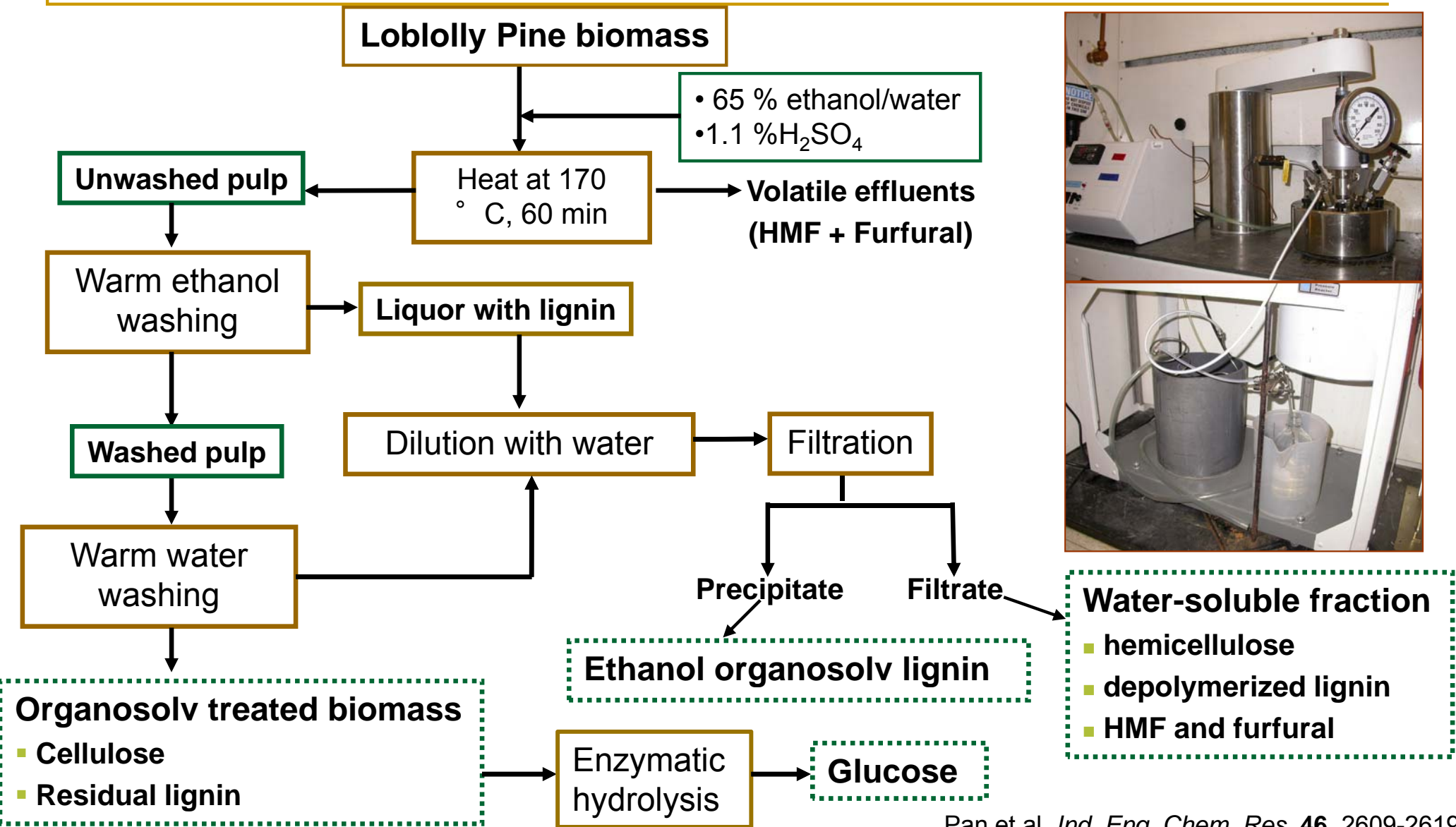


Biomass Feedstock: Loblolly pine

- Mature (15 yr. old) tree from Baldwin Co., GA
- Sections debarked and chipped, stored at -5°C
- Composite sample used for study
- Lignin: **30%**; Cellulose: **42%**; Hemicellulose: **21%**



Ethanol Organosolv pretreatment



Pan et al. *Ind. Eng. Chem. Res.* **46**, 2609-2619



Pretreatment results



Untreated (100g)

- Lignin: 30g
- Cellulose: 42g
- Hemicellulose: 21g



Organosolv treated

- Lignin: 12g
- Cellulose: 33g
- Hemicellulose: 1g



Organosolv lignin

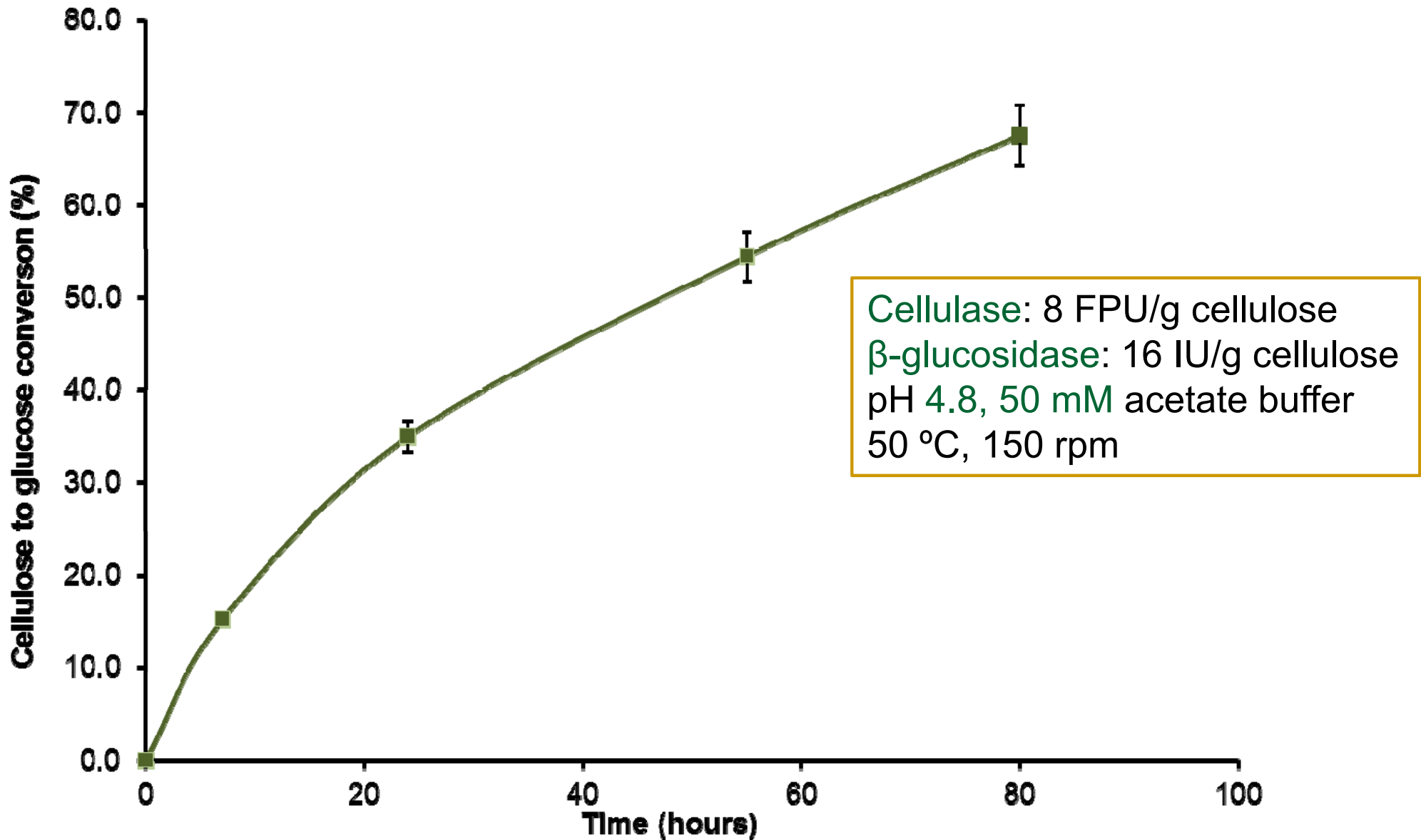
- Lignin: 6g

Liquid fraction

- Cellulose: 3g
- Hemicellulose: 15g
- Lignin: 6g



Enzymatic hydrolysis of organosolv treated Loblolly pine

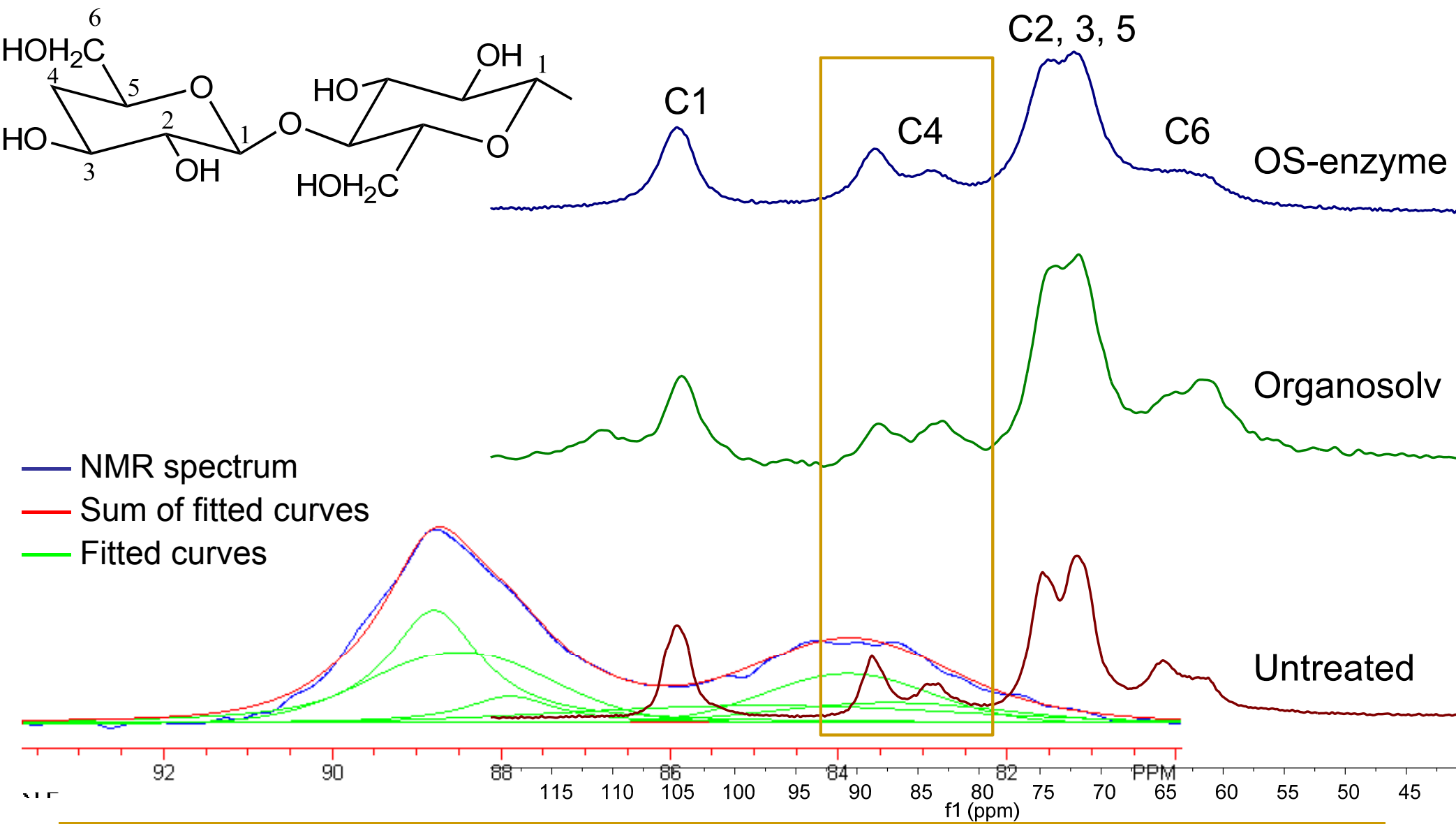


Cellulose

- Biomass samples delignified by holocellulose pulping (acetic acid and sodium chlorite)
- Cellulose isolated by hydrolyzing holocellulose with 2.5 M HCl
- Analyzed with solid-state ^{13}C CP/MAS NMR
- Structure determined by line fitting analysis of C4 region

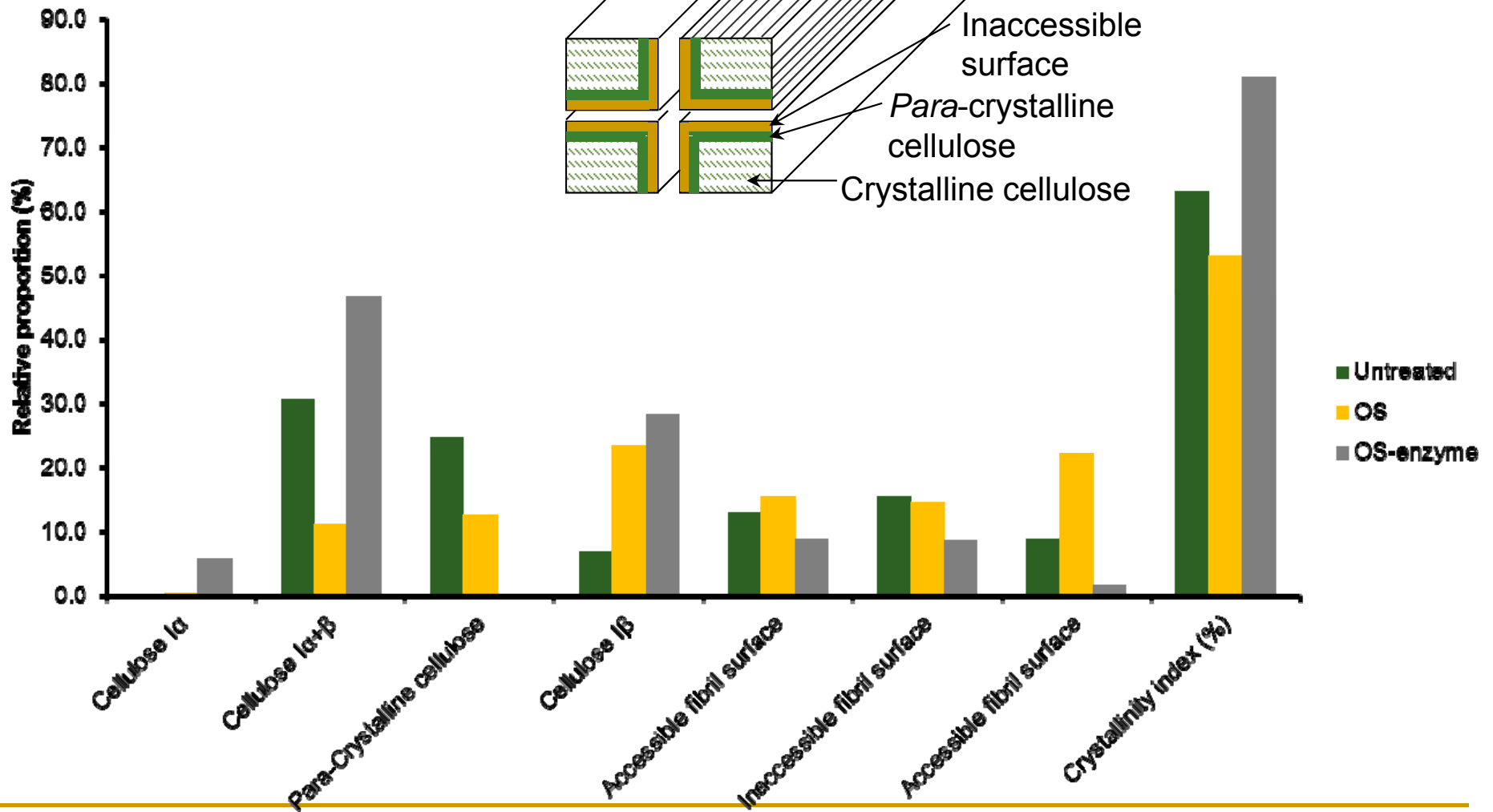


Cellulose NMR



Cellulose structure and crystallinity

$$\text{Crystallinity Index} = \delta_{86-92} / \delta_{80-92}$$



Changes in cellulose structure

- Cellulose crystallinity
 - Decreases after organosolv pretreatment
 - Pretreatment capable of decreasing ordering of cellulose
 - Together with delignification, produces substrate amenable to enzymatic hydrolysis
 - Increases after enzymatic hydrolysis
 - Enzymes selectively degrading less ordered forms of cellulose
- Cellulose structure after pretreatment
 - Increase in cellulose I_{β} , accessible fibril surfaces
 - Decrease in cellulose $I_{\alpha+\beta}$

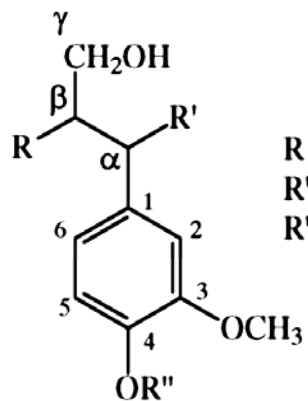


Lignin

- Milled wood lignin (MWL)
 - Isolated from very finely milled, extractives free biomass
 - Extracted with dioxane/water and purified
- MWL before and after organosolv pretreatment and Ethanol Organosolv Lignin studied with:
 - Quantitative ^{13}C NMR -- quantified as functional groups/aromatic ring
 - ^{31}P NMR-- Lignin OH groups quantified after phosphorylation
 - Gel permeation chromatography-- Molecular weight distribution



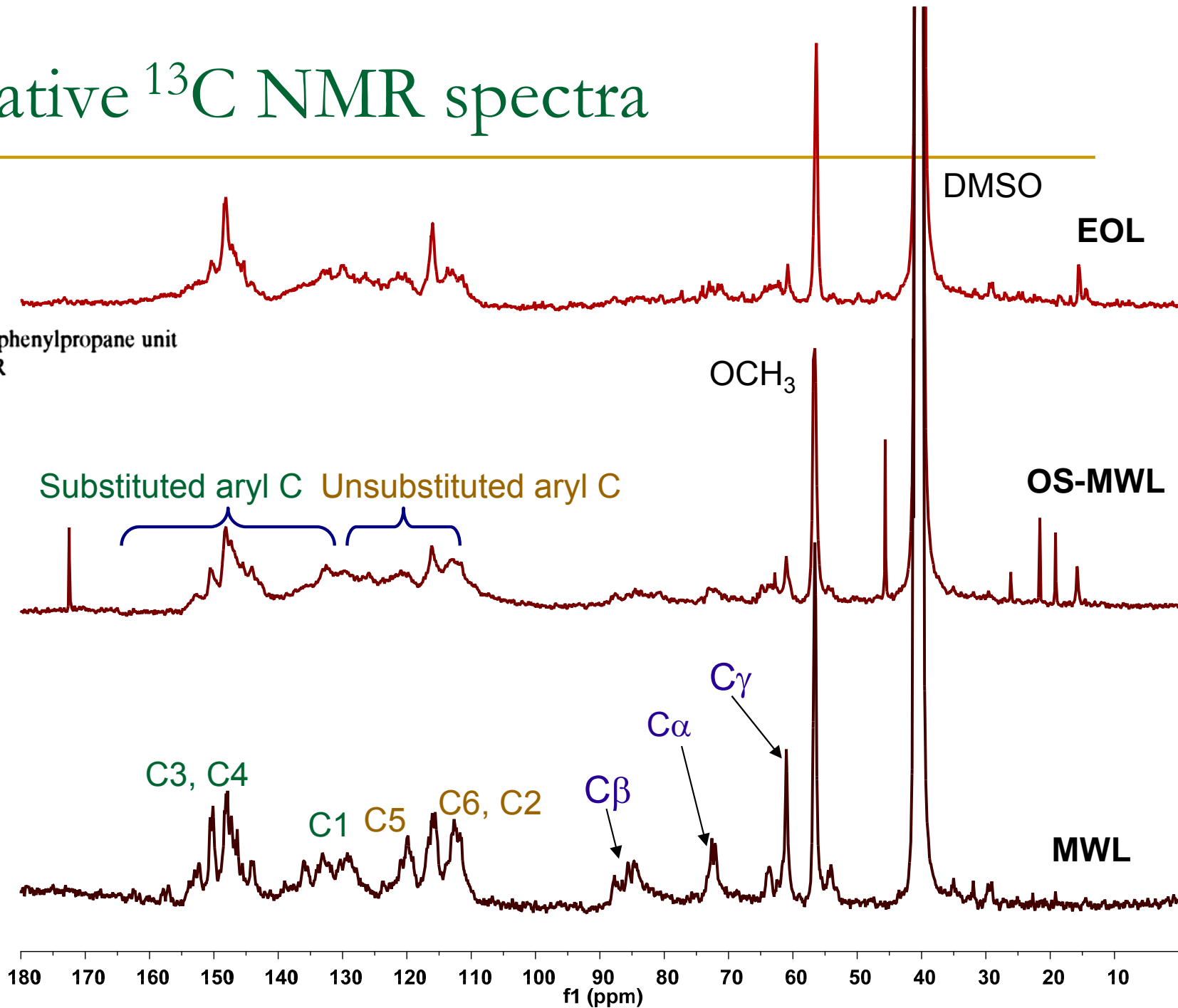
Quantitative ^{13}C NMR spectra



R = another phenylpropane unit

R' = OH or R

R'' = H or R

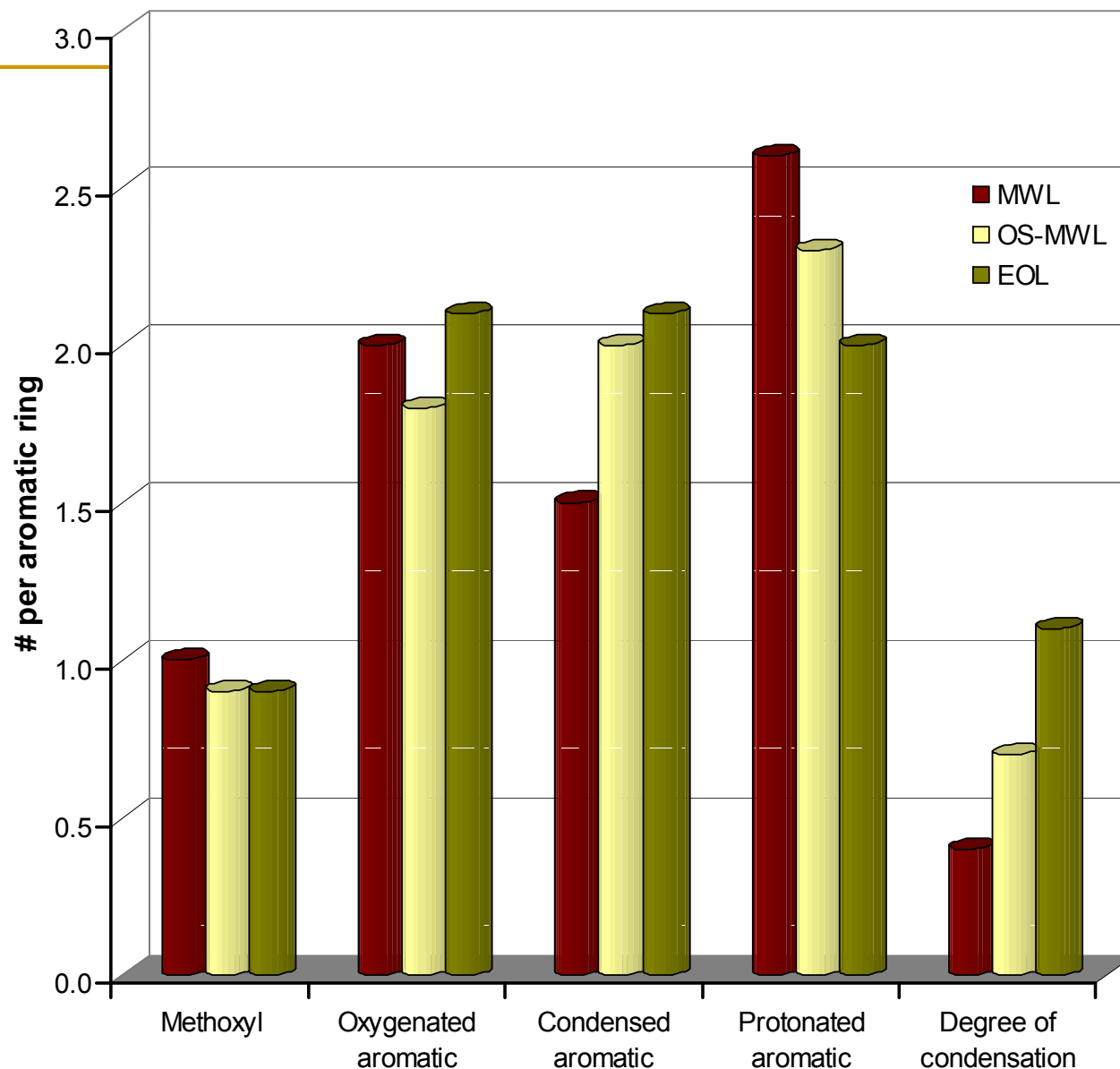


Ethanol Organosolv pretreatment of
Loblolly pine

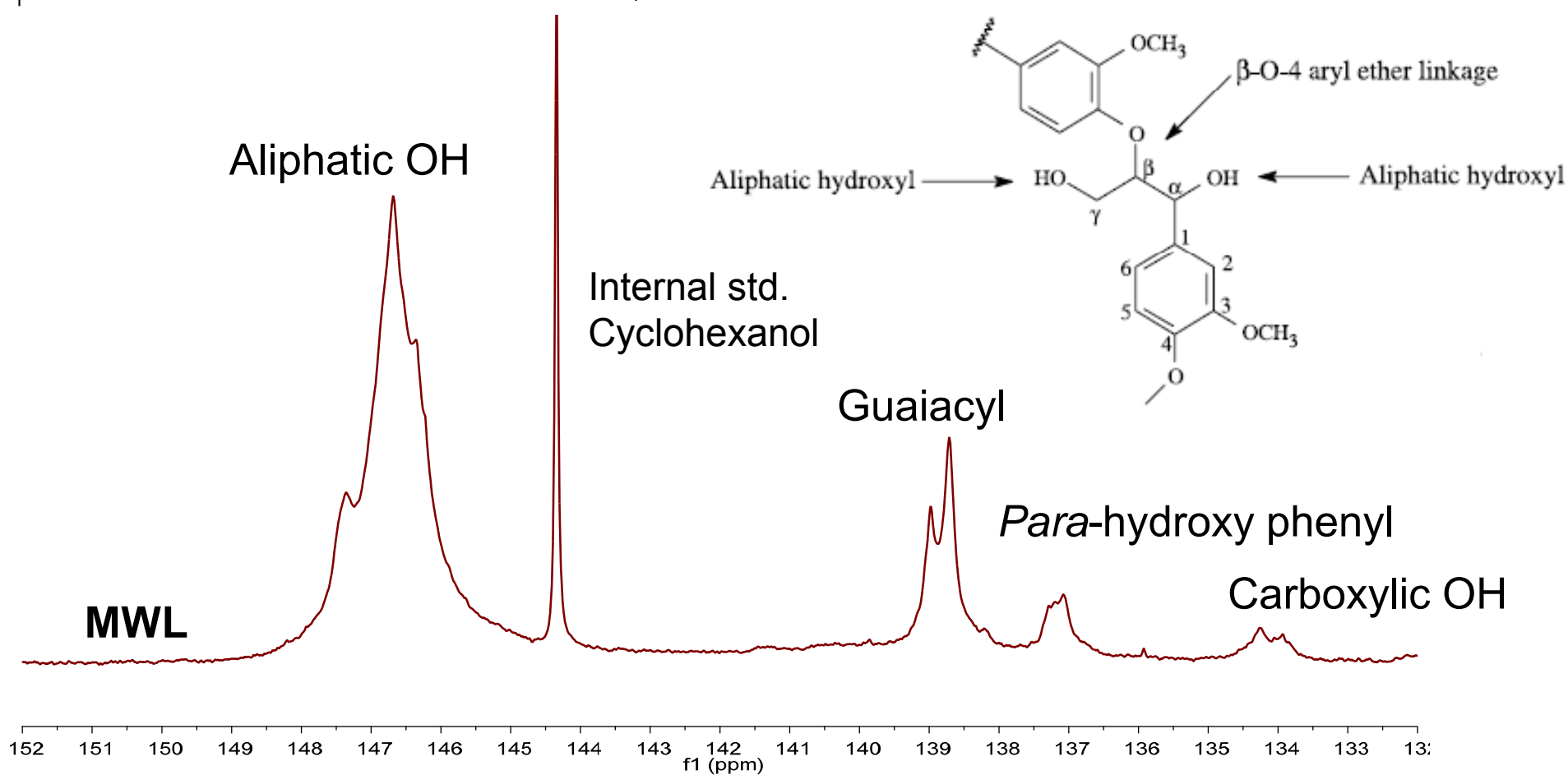
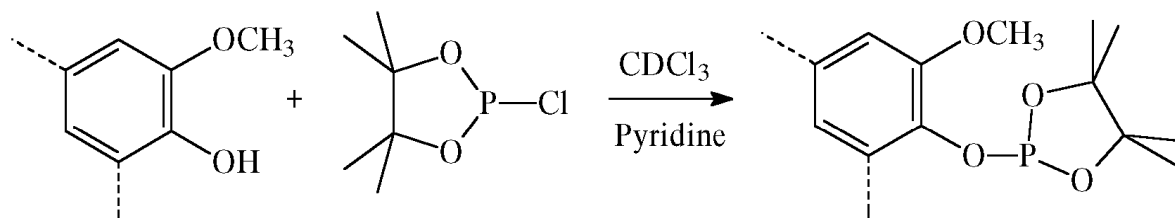


^{13}C NMR results

- β -O-4 linkages most abundant
- OS-MWL and EOL
 - Decrease in β -O-4
 - Acid catalyzed scission
 - Decrease in protonated aromatic C
 - Increase in condensed aromatic C
 - Increased condensation
 - Increase in carboxylic acids
 - Ester hydrolysis

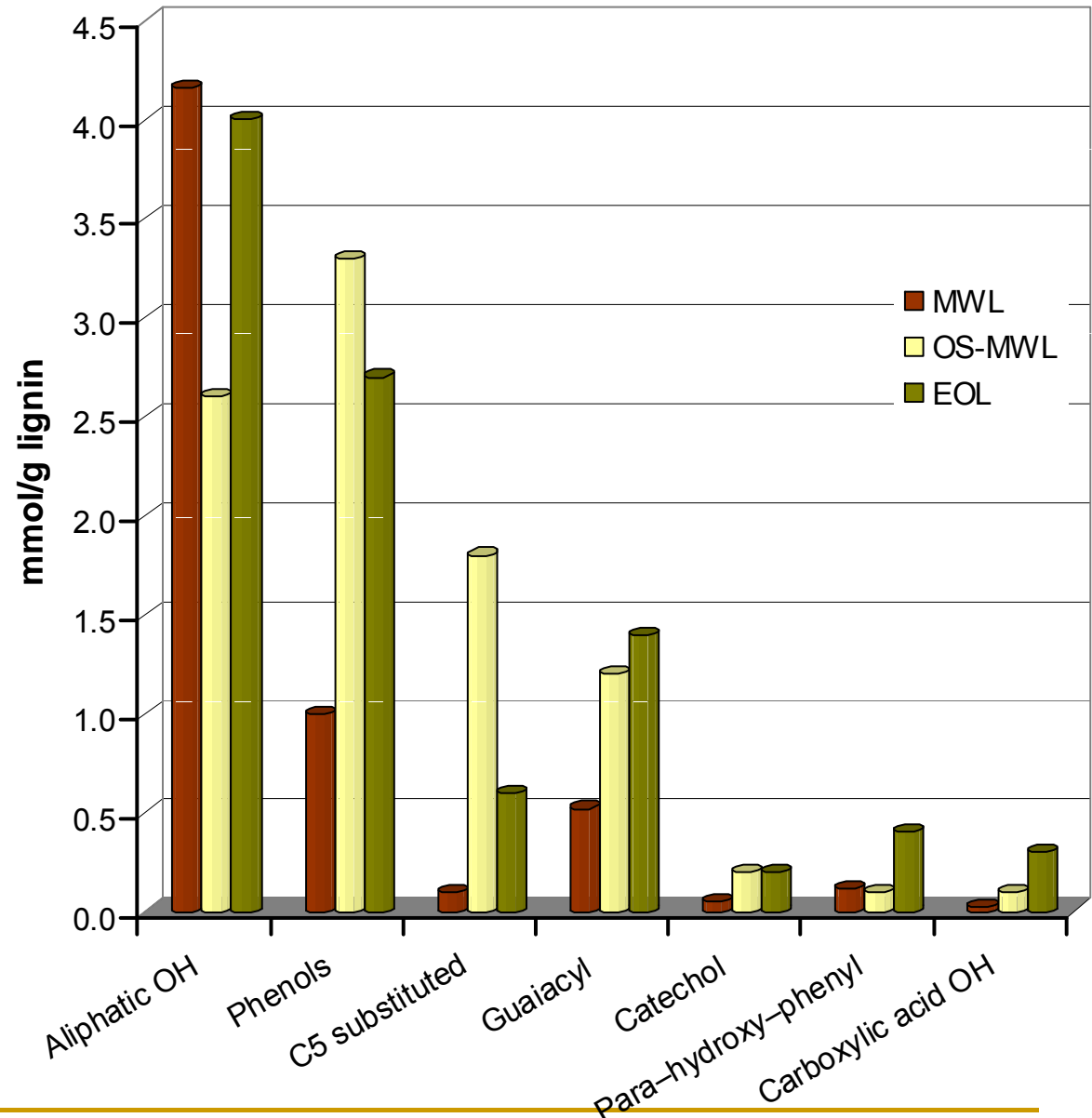


Lignin OH groups by ^{31}P NMR



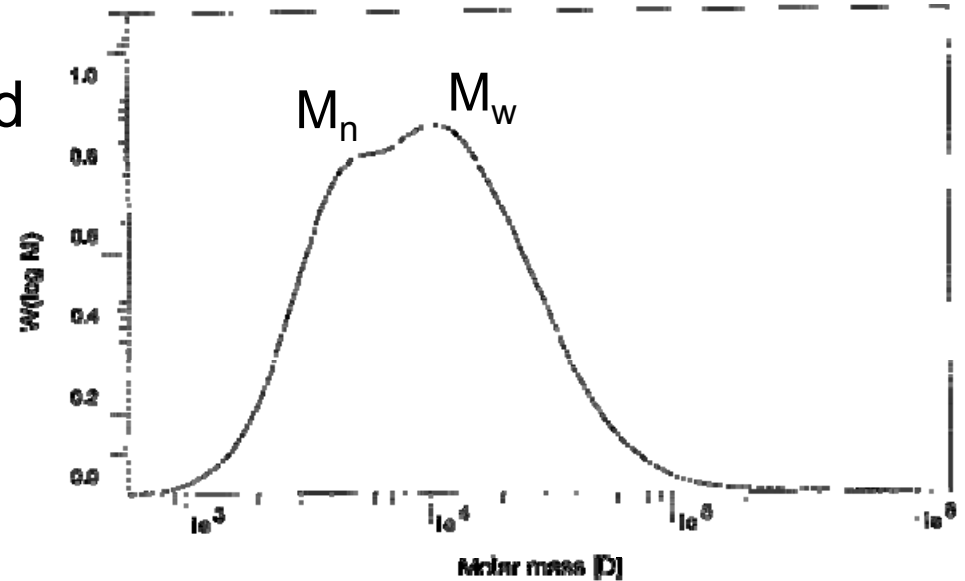
^{31}P NMR results

- Aliphatic OH dominant
- OS-MWL and EOL:
 - Lower aliphatic OH
 - β -O-4 cleavage
 - Higher phenolic OH
 - Aids in organosolv delignification
 - Higher condensed phenolic
 - Higher carboxylic OH



Lignin molecular weight distribution

- Lignin samples were acetylated
- Analyzed by Gel Permeation Chromatography
 - THF as solvent

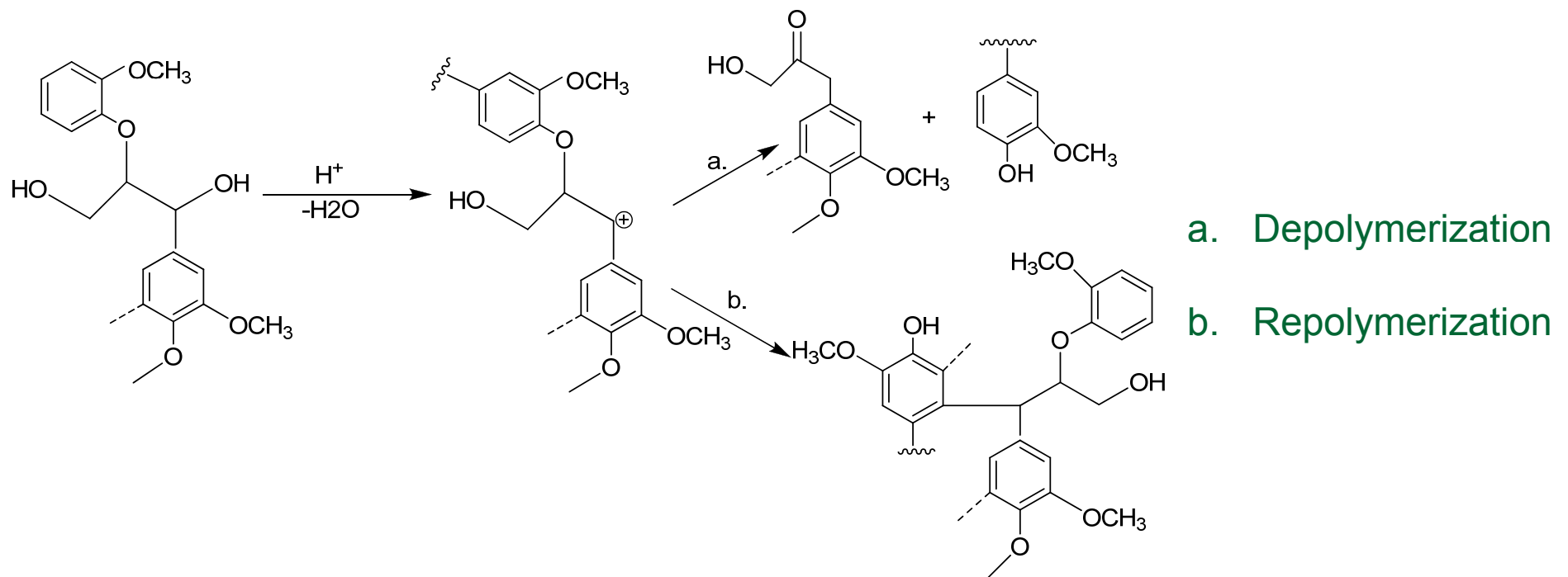


	\overline{M}_n (g/mol)	\overline{M}_w (g/mol)	$\overline{M}_w / \overline{M}_n$
MWL	7.6×10^3	1.4×10^4	1.8
OS-MWL	6.5×10^3	1.7×10^4	2.6
EOL	3.1×10^3	5.4×10^3	1.8



Structural changes with pretreatment

- Decrease in aliphatic groups
- Decrease in β -O-4 linkages
- Increase in phenolic groups, condensation
- Higher polydispersity in OS-MWL (lower M_n , higher M_w)



Potential uses of organosolv lignin

- Low molecular weight of EOL
 - ❑ Suitable for various industrial applications due to increased solubility
 - ❑ Also has low S content
 - ❑ Substitute for polymeric materials; precursor for chemicals; dispersant
- Coupled with the higher phenolic content
 - ❑ Higher radical scavenging potential
 - ❑ Imparts anti-oxidant activity by inhibiting oxidation of low density proteins
 - ❑ Anti-inflammatory, anti-carcinogenic

Pan et al. *Biotech. Bioeng.* **94**, 851-861



Summary and conclusions

- Organosolv pretreatment produces a substrate with reduced cellulose crystallinity and lignin content
 - Highly amenable to enzymatic deconstruction
- Cellulose crystallinity increases after enzyme hydrolysis
 - Preferential hydrolysis of less ordered cellulose
 - There is a need to develop enzymes capable of hydrolyzing crystalline cellulose



Summary and Conclusions

- Acid catalyzed cleavage of β -O-4 and ester are major mechanisms of lignin breakdown
- Residual lignin after organosolv pretreatment is more condensed
 - Also shows evidence of repolymerization
- Ethanol organosolv lignin
 - Low molecular weight
 - Higher phenol and carboxylic acid content
 - May be suitable as anti-oxidants or other value-added uses



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