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

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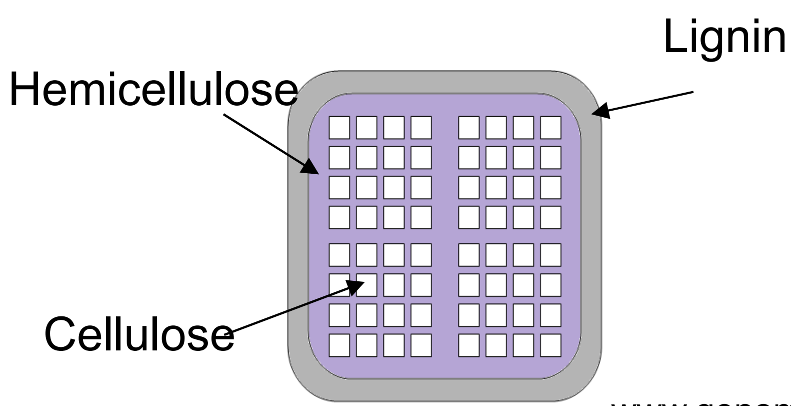
Background: Lignin and cellulose in biomass

- 2^{nd} 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



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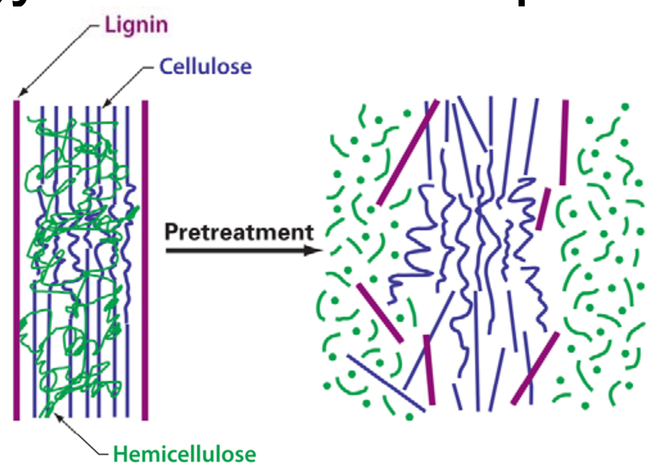
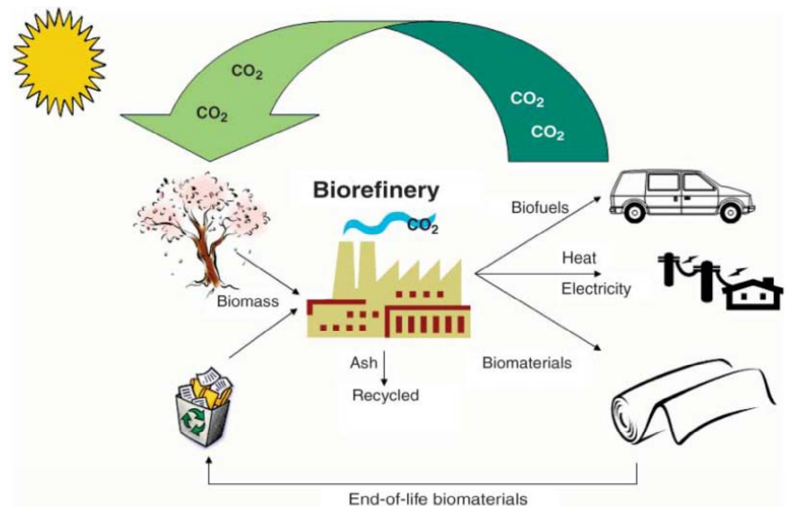


Organosolv pretreatment

- 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

Loblolly Pine biomass

- 65 % ethanol/water
- 1.1 % H_2SO_4

Heat at 170 ° C, 60 min

Volatile effluents (HMF + Furfural)

Unwashed pulp

Warm ethanol washing

Liquor with lignin

Washed pulp

Dilution with water

Filtration

Precipitate

Filtrate

Warm water washing

Ethanol organosolv lignin

Water-soluble fraction
- **hemicellulose**
- **depolymerized lignin**
- **HMF and furfural**

Organosolv treated biomass
- **Cellulose**
- **Residual lignin**

Enzymatic hydrolysis

Glucose

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

Pretreatment results

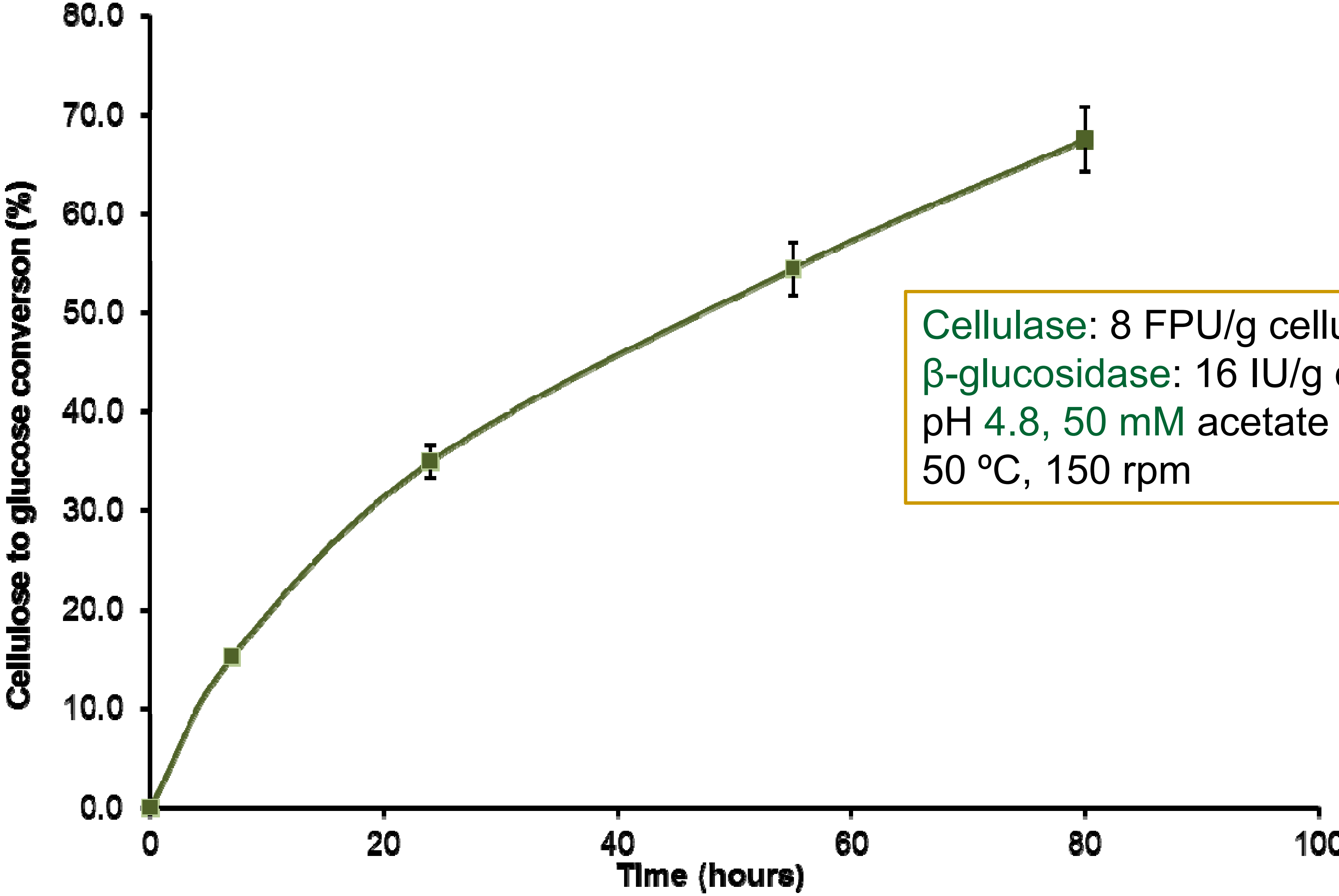
Untreated (100g)
- Lignin: 30g
- Cellulose: 42g
- Hemicellulose: 21g

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

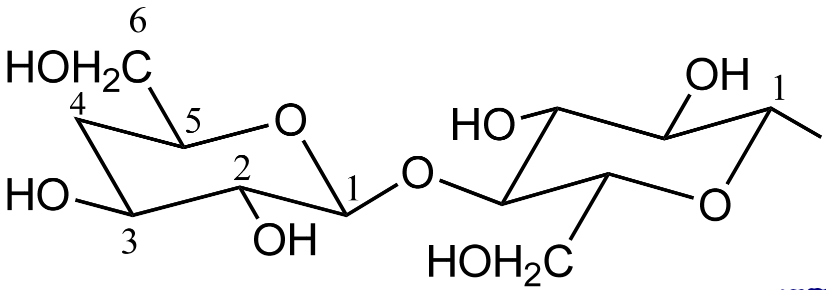


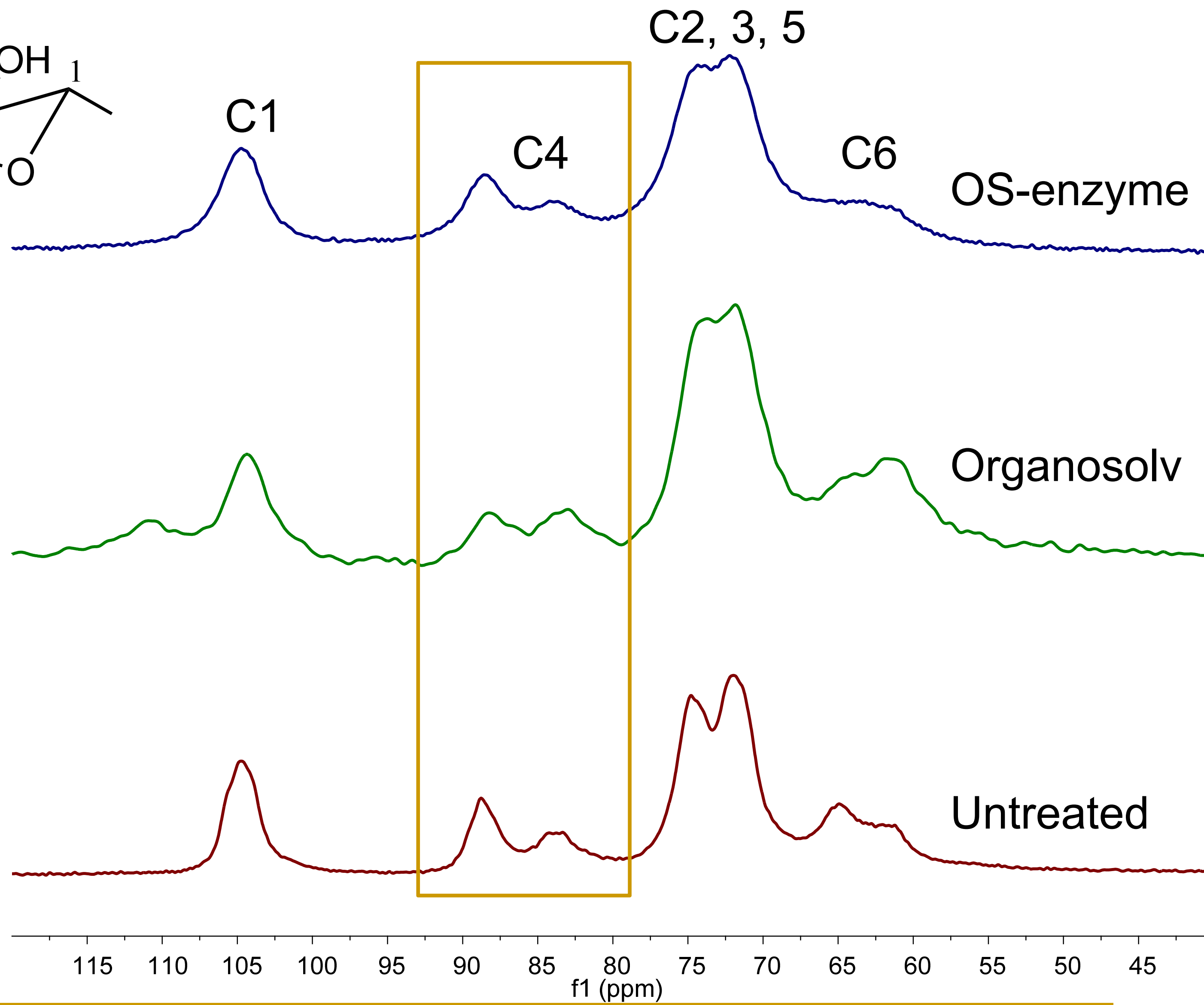
Cellulase: 8 FPU/g cellulose
β-glucosidase: 16 IU/g cellulose
pH 4.8, 50 mM acetate buffer
50 ºC, 150 rpm

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

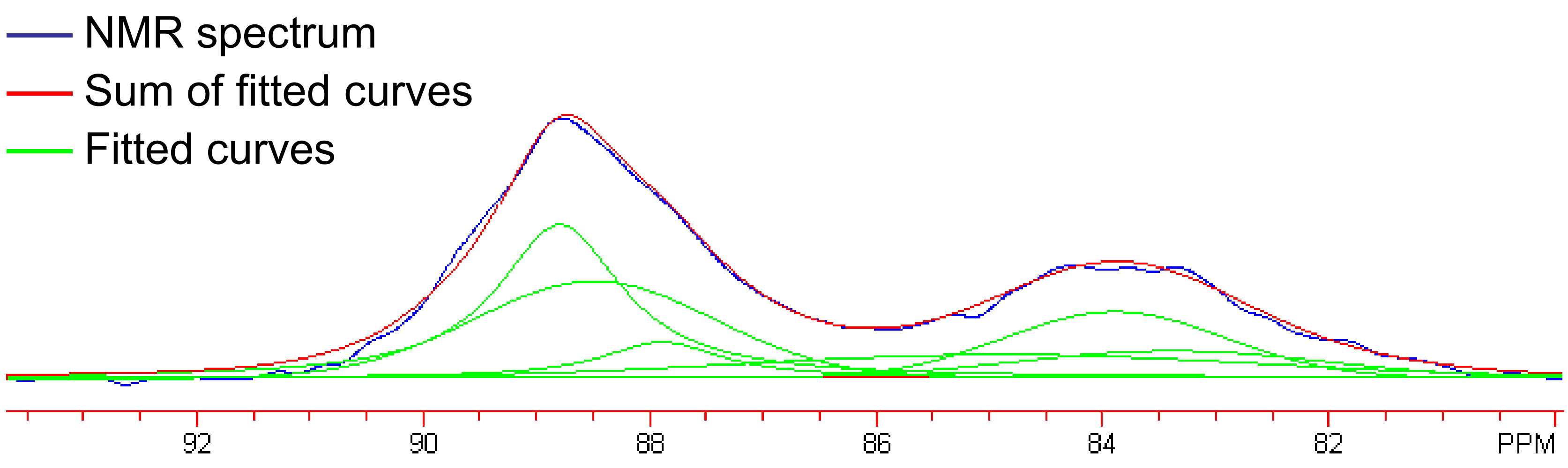
Cellulose NMR





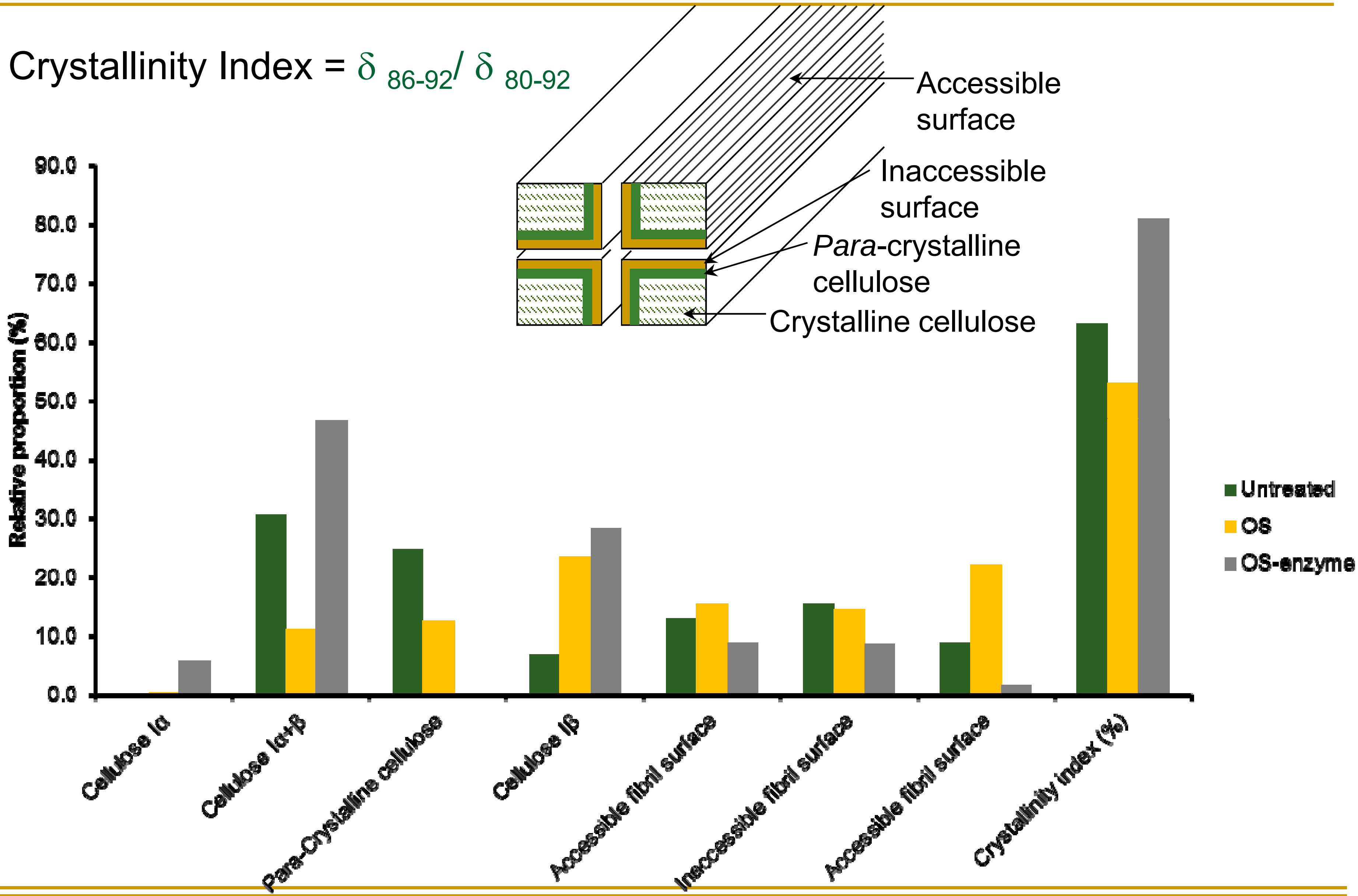
Cellulose NMR

- Line shape analysis of the C4 region to determine cellulose ultra structure
- Crystallinity Index = $\delta_{86\text{-}92} / \delta_{80\text{-}92}$



Cellulose structure and crystallinity

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



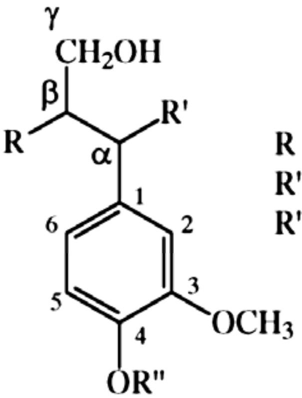
Changes in cellulose structure

- 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 $I_{\alpha+\beta}$

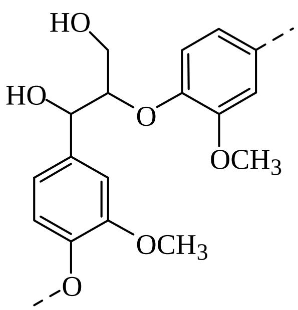
Lignin

- 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 phosphitylation
  - Gel permeation chromatography-- Molecular weight distribution

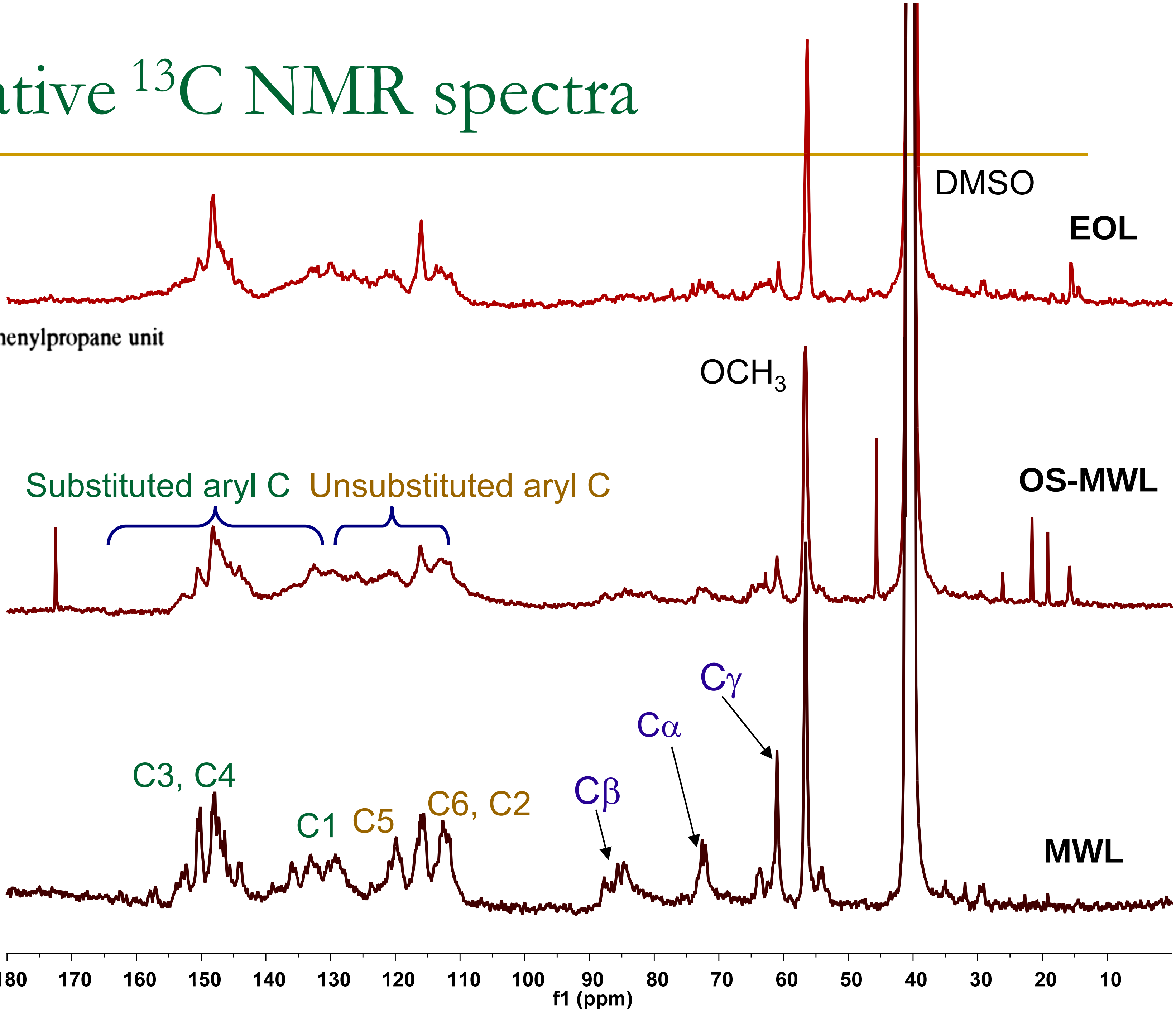
Quantitative ^{13}C NMR spectra



R = another phenylpropane unit
R' = OH or R
R" = H or R

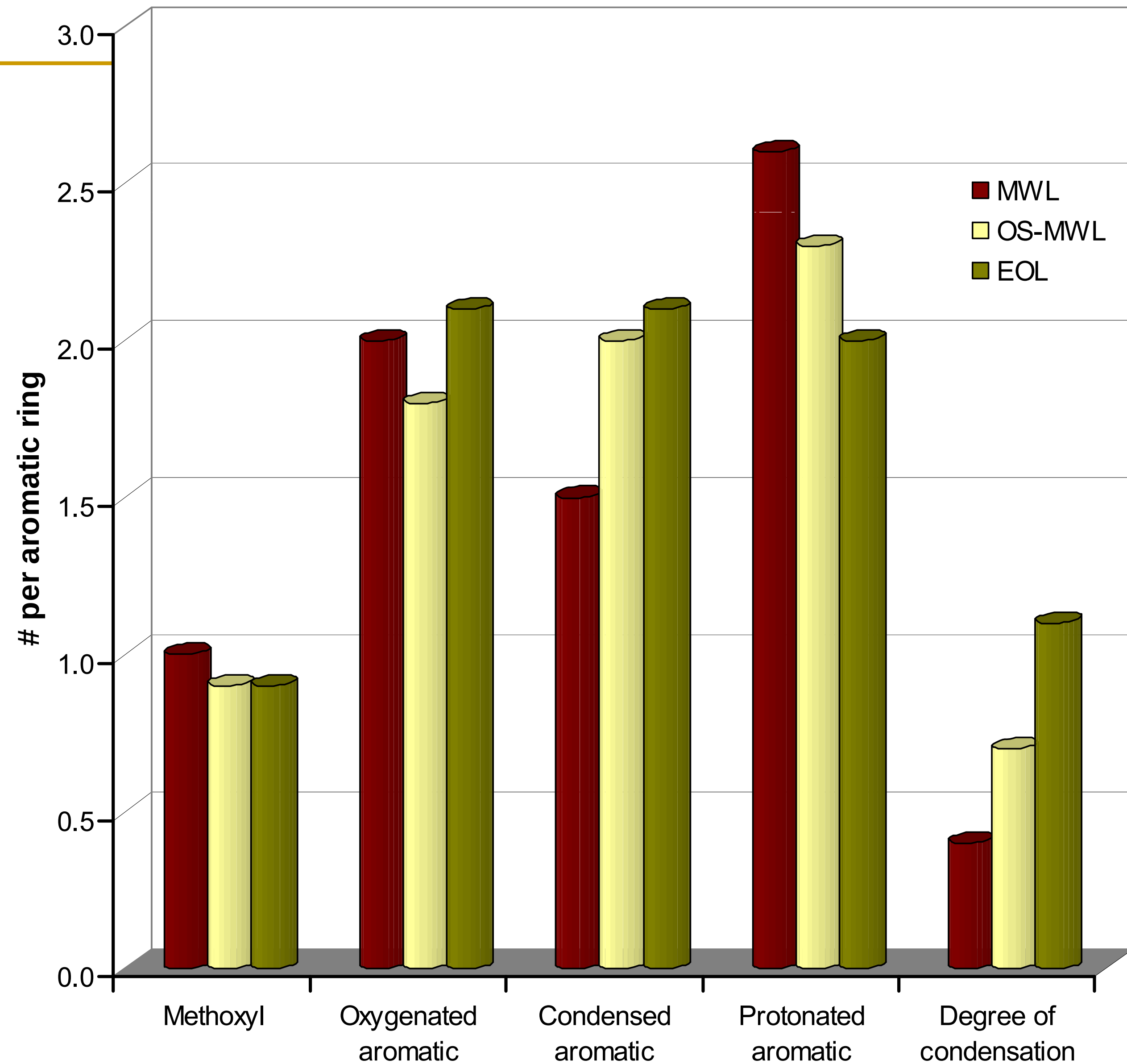


β-O-4

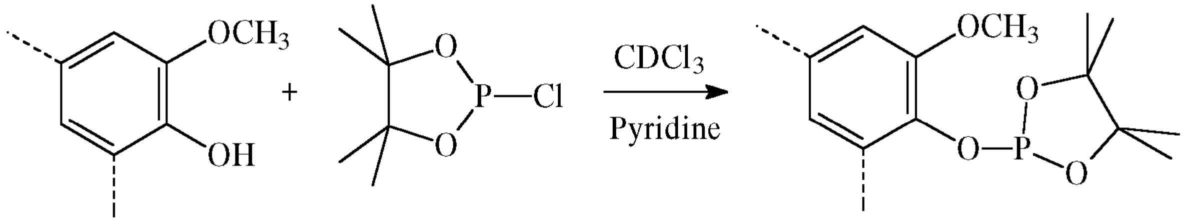


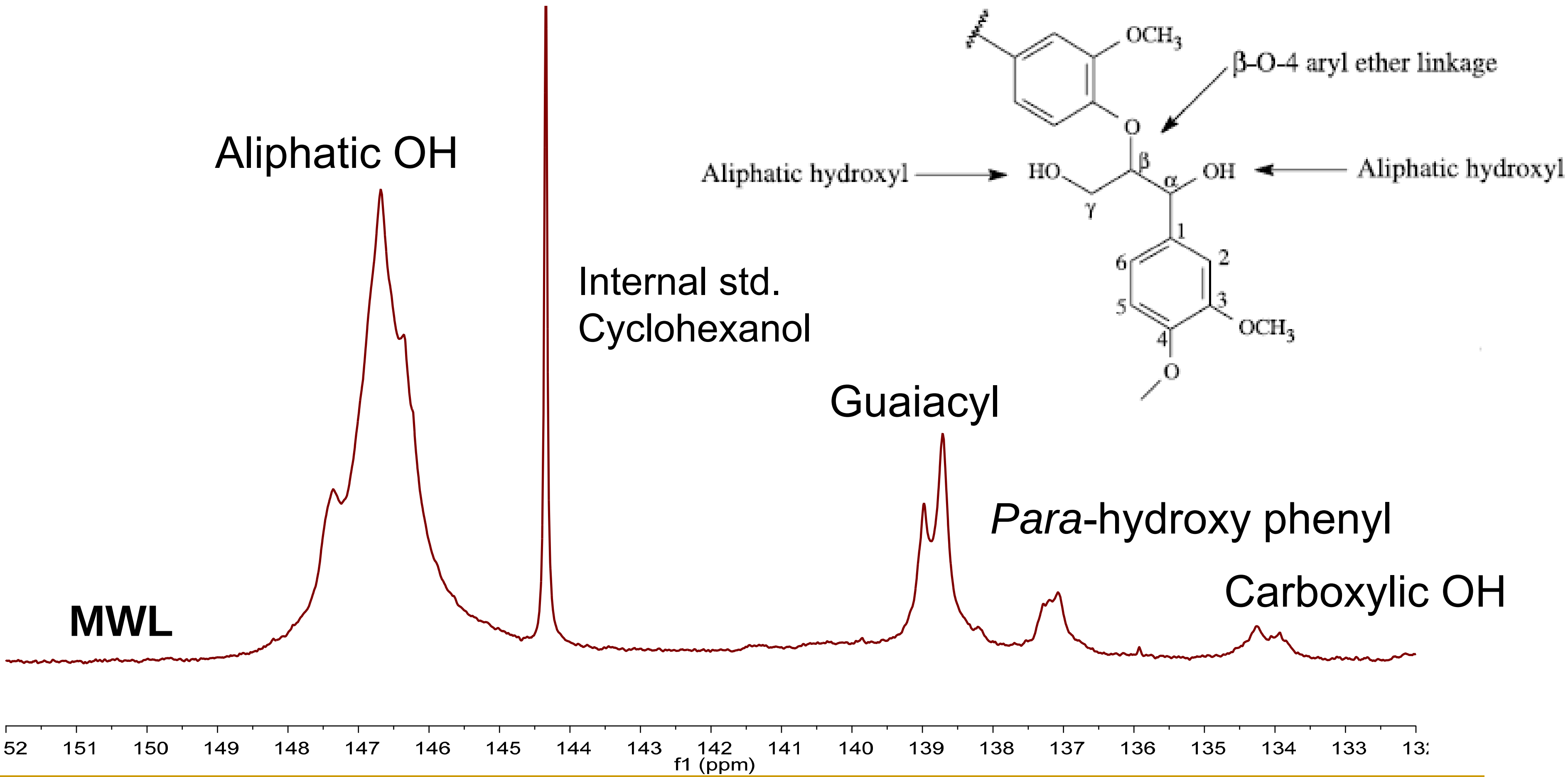
^{13}C NMR results

- 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



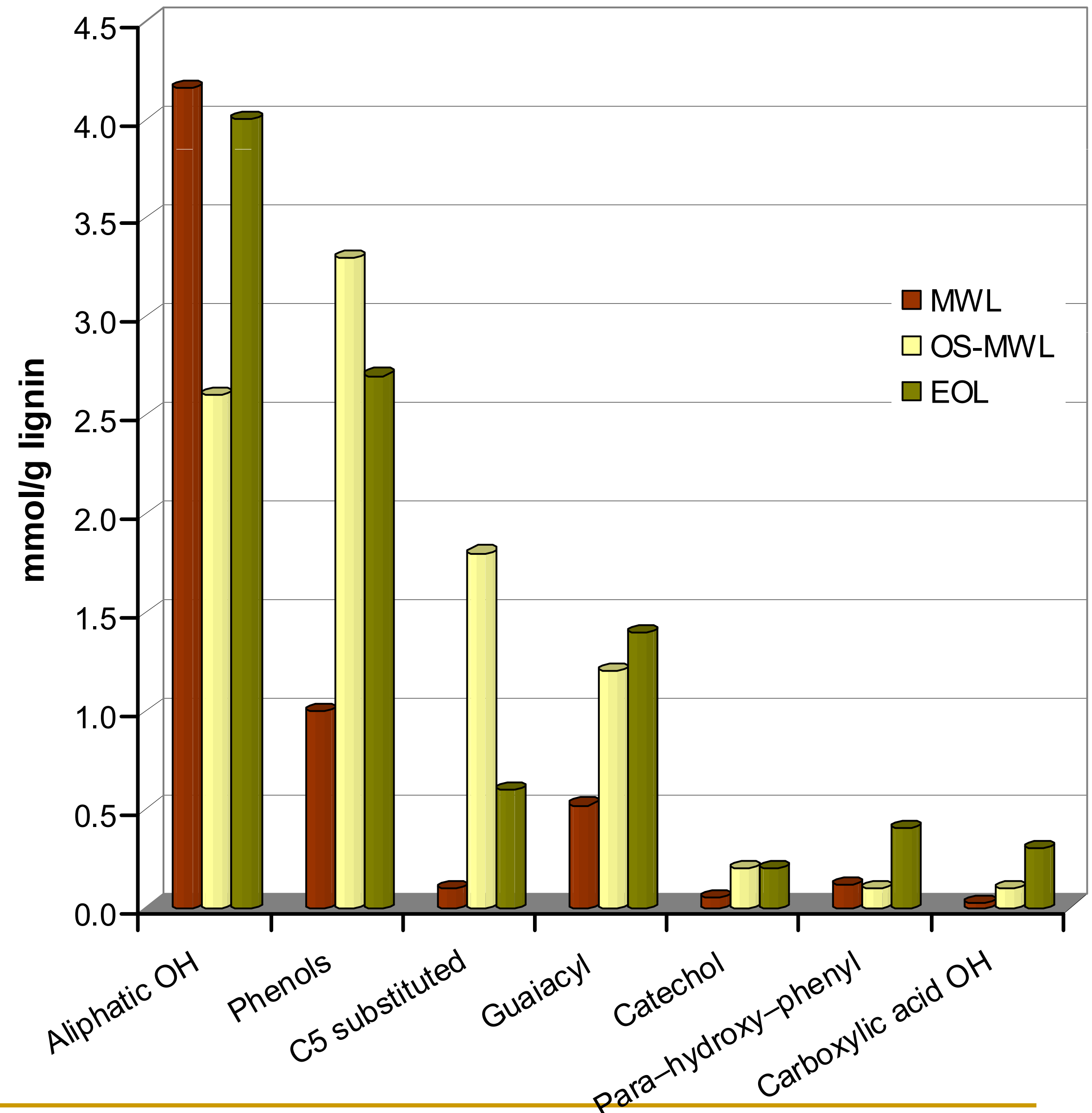
Lignin OH groups by ^{31}P NMR





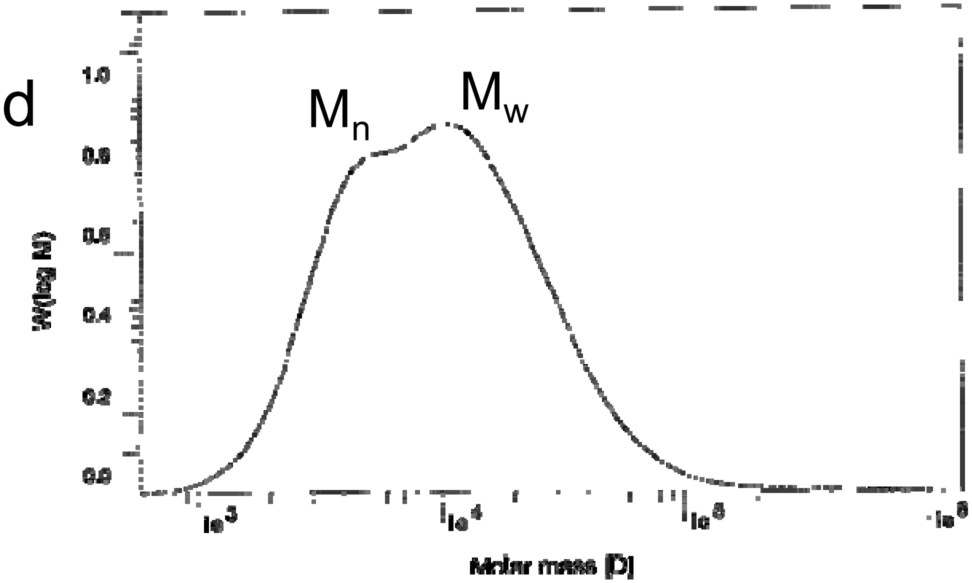
^{31}P NMR results

- 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

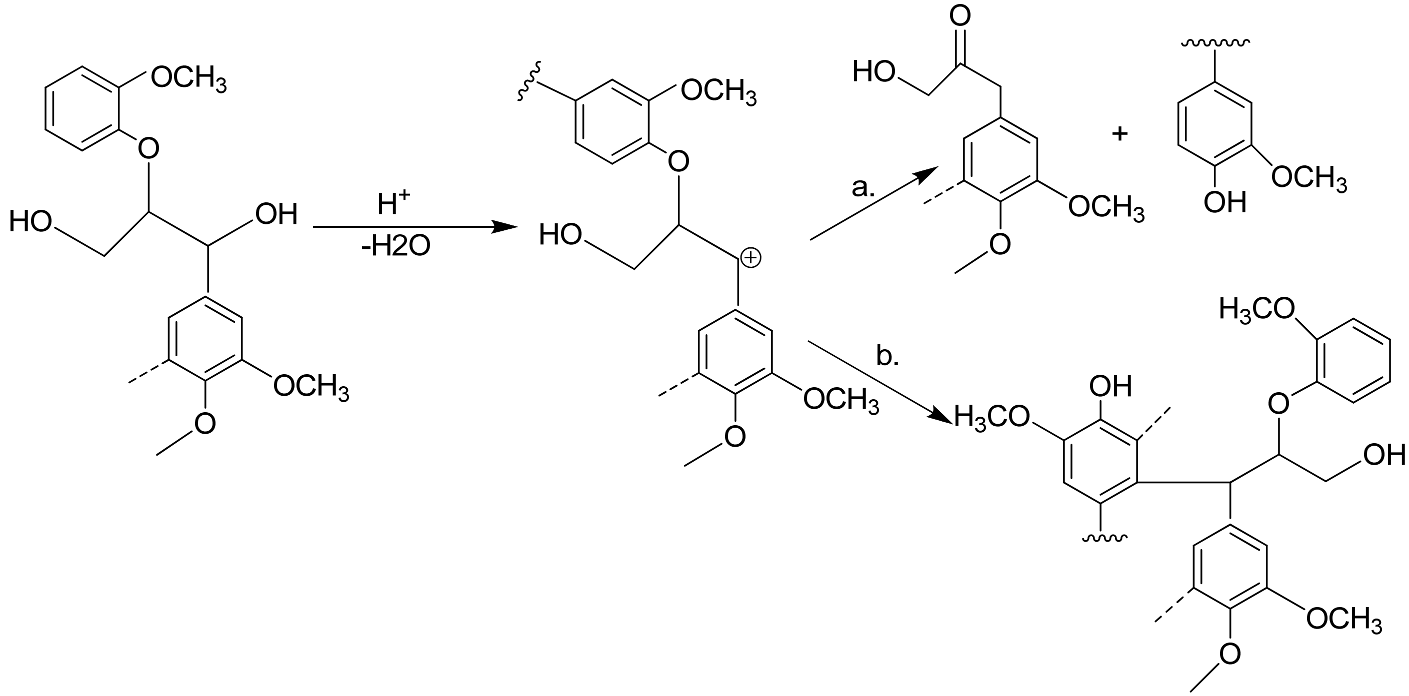
- Lignin samples were acetylated
- Analyzed by Gel Permeation Chromatography
  - THF as solvent



	$\overline{M_n}$ (g/mol)	$\overline{M_w}$ (g/mol)	$\overline{M_w} / \overline{M_n}$
MWL	7.6 x10^3	1.4 x10^4	1.8
OS-MWL	6.5 x10^3	1.7 x10^4	2.6
EOL	3.1 x10^3	5.4 x10^3	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)



a. Depolymerization

b. Repolymerization

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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