Chemo-Enzymatic Modification of High-Lignin Content Fibers with Laccase

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“If it Ain’t Broke Don’t Fix it?”

- Kraft pulping
  - Long, strong fibers
  - Energy and chemical recovery
  - 45-50% pulp yield for bleached grades
What happens when we pulp to higher lignin contents to preserve yield?
The Effects of Lignin on Fiber-to-Fiber Bonds

The Effect of Lignin Content on Paper Strength Properties

At higher kraft pulp yields, both tensile and Z-direction strength of paper decrease.


High-Yield Kraft Fibers

Stop kraft cook earlier = High-Kappa Kraft Pulp
  • Poor bonding characteristics limit applications
    – High lignin content
  • Surface lignin content prevents bonding
  • Stiff fibers – conformability
  • Restriction of fiber swelling

Can the properties of high-yield kraft pulps be improved to broaden their range of applications?
What is currently being done to modify fibers to affect pulp quality?

- Tree choice
- Pulping
- Chipping
- Papermaking chemicals

Chemical formula: $\text{CH}_2\text{CH}_2\text{NH}_2$
Next Generation?

- Enzymes from wood-degrading fungi can be used for altering pulp properties

**Cellulase**
- Enzymes modify fibers rendering them more flexible and aid drainage on paper machines

**Hemicellulase**
- Enzymes modify fibers to aid in bleaching

**Ligninases**
- Manganese peroxidase, Lignin peroxidase, and **LACCASE**
  - Employed primarily for delignification studies
  - Laccase different than MnP and LiP
Laccase

- Japanese lacquer tree (Rhus vernicifera)
- Basidiomycete fungi and plants
- Oxidoreductase enzyme
- Biodelignification studies with kraft pulps
- Polymerizes monolignols in solution to form polymers with lignin-like properties
Laccase

• Oxidation of monomeric phenols has been shown to result in coupling to lignin macromolecule (Lund 2001)
Rationale

• There has been limited work in pulp and paper research exploiting laccase’s polymerizing ability to modify pulps.

• The high surface lignin content of high-kappa kraft pulps* makes them prime candidates for reaction with laccase.

*Laine, 1994
Hypothesis

Oxidizes a wide array of phenolic structures

$O_2 + 2H^+ \rightarrow H_2O_{ox}$

no rxn

Fiber Grafting?
Possibilities...

- Polymerization of phenols with themselves (self-condensation)
- Polymerization of phenols with fiber
- Polymerization of fiber lignin
Objectives

- Devise and evaluate the feasibility of a system utilizing laccase to co-polymerize (graft) water soluble compounds with high kappa kraft pulp
- Determine if lignin was the main target for modification of the laccase-facilitated grafting system
- Determine conditions where the laccase-facilitated grafting system was the most effective for modifying fibers
- Evaluate the effects of the laccase-facilitated grafting treatment on paper strength properties and surface chemical properties
Phase 1: Initial Experiment

4-Hydroxybenzoic acid

Syringic acid

Vanillic acid

- Kappa Number
- Carboxylic Acid Groups
- X-ray Photoelectron Spectroscopy
Experimental

Water
45°C

Kapak® bag

Water Bath

Pulp
- Kappa 90
- 20% csc
- pH 4.5

1. Phenolic
2. Laccase

Phenolic

Laccase
Pulp before and after treatment

Control and Brownstock
TB: 20.2

Laccase
TB: 18.3

4-Hydroxybenzoic acid
TB: 19.2

Laccase + 4-Hydroxybenzoic acid
TB: 15.3
Phase 1:
Kappa Number

- Kappa increased in presence of laccase and phenolic acids
- 4-hydroxybenzoic acid (4-HBA) treatment with laccase resulted in largest kappa increase

LSD: 3.75
Phase 1: Bulk Carboxylic Acid Groups

- Acid group results correlate with kappa number results
- Laccase + 4-hydroxy benzoic acid approx. doubled the bulk acid groups

LSD:
0.02 meq/g
Phase 1: XPS Results

- Lac case and 4-hydroxybenzoic acid increased the percentage of acid groups on the surface.

SD: 0.08%
Phase 1: Conclusions

- Laccase treatment with phenolic acids increases both kappa number and bulk carboxylic acids.
- Strong evidence for laccase facilitated coupling of phenolic acids to fiber surface.
- Laccase most effective in coupling of 4-hydroxybenzoic acid which may be due to absence of methoxyl groups on aromatic ring.
Phase 2: Treatment Conditions

- Used 4-hydroxybenzoic acid to determine effect of treatment conditions
- Varied presence of oxygen, dosage of 4-hba, pulp consistency, treatment time
Phase 2: Oxygen Pressure Vs. Ambient Conditions

- Laccase treatments for biodelignification most effective when pressurized with O₂
- Compare to treatments performed in bags
- Treatments performed with identical conditions

- No real difference between treatments performed in O₂ reactor or atmospheric
Phase 2: Factorial Design

- Three factors $3^3$ factorial with kappa number as the response
- Variables
  - 5, 10, & 20% pulp consistency
  - 0.1, 1, & 10 mmol dosage of 4-Hba
  - 2, 4, & 8 hours treatment time

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<tr>
<td>Consistency (CSC)</td>
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<td>Dosage*Time</td>
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<tr>
<td>CSC*Time</td>
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- Consistency and dosage
  Have the most significant effects on grafting reaction
- Consistency and dosage also have the strongest interaction
Phase 2: Periodic Addition of 4-Hba

- Alkaline extraction removes adsorbed un-grafted material
- 4x addition promoted polymerization to the pulp

Entire dose of 4-Hba added to the pulp at the beginning of the reaction vs. periodic addition of 4-Hba throughout the duration of the reaction
To decrease 4-Hba self-condensation and increase coupling to the pulp
Additions in two hours:
  - 1x – Entire dosage of 4-HBA
  - 2x – 1 addition per hour
  - 4x – 1 addition each 30 minutes
  - 8x – 1 addition each 15 minutes
Treatments followed by alkaline extraction to remove adsorbed 4-Hba
Phase 2: Conclusions

• It is not necessary to perform the laccase/phenol/pulp reaction in a reactor pressurized with $O_2$
• A high consistency and high dosage of phenol (4-Hba) is necessary for obtaining higher amounts of phenol coupled to the pulp
• Periodic addition was effective in increasing the amount of 4-hba coupled to the pulp
Phase 3: Effects of Laccase-Grafting Treatment on Lignin

Fully Bleached Pulp → Laccase/4-Hba Treatment

Isolated Lignin → "Lignin-Impregnated Fibers"

Laccase/4-hba Treatment

Lignin

Molecular Weight

Structural Changes
Phase 3: Application to Fully Bleached Pulps

- Applied 4-hba with laccase
- No significant increase in acid groups when compounds applied with laccase
- Indicates pulp lignin is necessary for coupling to fibers
Phase 3: Lignin Impregnated Fibers

- Blender
- Filter paper
- Acetone extract
- Kraft (kappa 92)
- Lignin extraction
  - 9:1 dioxane:1N HCL
  - 2 hours w/acid precipitation

- Lignin+filter paper fibers
  - 20% csc in dioxane
- Roto-evaporate
- Lignin impregnated fibers

React with laccase+4-Hba
Phase 3 Lignin Impregnated Fibers: $^{31}$P NMR

- $^{31}$P NMR for quantifying hydroxyl groups on lignin
- Coupling of phenolic acids to lignin should increase carboxylic acid groups on lignin
- Carboxylic acid groups increased indicating coupling of 4-hydroxybenzoic acid to lignin

![Bar chart showing mmol/g lignin for different treatments: BS, Control, Laccase, 4-HBA, Lac+4-HBA.](chart.png)
Both non-condensed and condensed phenolic groups decreased in the presence of laccase indicating the predominant reaction was the reaction of laccase with phenols.
Phase 3
Lignin Impregnated Fibers: Molecular Weight

LSD: 1545 g/mol
Phase 3: Conclusions

• NMR data suggests increase in acid groups that indicate the attachment of 4-hydroxybenzoic acid to lignin.

• NMR data also shows that laccase decreases both the non-condensed and C5 condensed phenols during grafting.

• Sensitivity of molecular weight analysis may be insufficient to illustrate changes imparted by grafting of 4-hba to lignin.
Phase 4: Pulp Surface Materials

High-kappa kraft pulp

Laccase - Grafting Treatment

Isolate Surface Material

Molecular Weight  Carboxylic Acid Groups
Phase 4: Isolation of Pulp Surface Material

35 g pulp Sample

Suspend at 10% Consistency

Filter 5x through Whatman no.41 filter paper retaining >20 um material and freeze dry

Disintegrate for 200,000 Revolutions

Yield of 40-50 mg Per sample

* Heijnessson et al 1995
Phase 4:
Pulp Surface Material
Molecular Weight

- Pulps treated with laccase and 4-hba followed by isolation of surface material
- Dissolved and acetylated with DMSO/DMF/Pyridine mixture
- Molecular weight measured by gel permeation chromatography

- Molecular weight increased with laccase+4-hba treatment
- Indicates laccase-facilitated coupling of hba to the fiber surface

LSD: 752 g/mol
Phase 4
Pulp Surface Material: Acid Group Titration

- Pulps pre-treated with laccase with tyrosine, 4-hydroxyphenylacetic acid, and guaiacol sulfonate
- Acid groups on surface material increased with laccase-grafting treatments

**Tyrosine**

**Guaiacol Sulfonate**

4-hydroxy phenylacetic acid

Tyrosine = tyrosine, Pa = Phenylacetic acid, Gs = Guaiacol Sulfonate
Phase 4: Conclusions

- Laccase-facilitated grafting treatments result in increases in the molecular weight and carboxylic acid groups on material isolated from the pulp fiber surface
Phase 5: Paper Physical Properties

High-kappa pulp → Lac case grafting treatment → Tensile, Tear, Burst

- Wet-tensile
- Zero-span
- Z-direction
- Tensile
Phase 5
Compounds Studied

Compounds applied to high-kappa kraft pulps:

- Syringic acid
- 4-Hydroxybenzoic acid
- Vanillic acid
- 4-hydroxy phenylacetic acid
- Tyrosine
- Guaiacol Sulfonate
- Celestine Blue
- Gallic Acid
Phase 5

Paper Physical Properties: Apparent Density

Kappa 91, Refined 2000 rev. PFI

Kappa 91, Refined 1000 rev. PFI
Phase 5

Paper Physical Properties: Apparent Density

Kappa 91, No Refining

Kappa 91, Refined 2000 rev. PFI
Phase 5

Paper Physical Properties: Burst

Kappa 91, Refined 2000 rev. PFI

Kappa 91, Refined 1000 rev. PFI
Phase 5

Paper Physical Properties: Burst

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Kappa 91, No Refining

Kappa 91, Refined 2000 rev. PFI
Phase 5
Paper Physical Properties: ZDT

COOH

HBA

OH

kPa

Con  Lac  Hba  Lac+Hba

100 120 140 160 180 200 220 240 260 280
Phase 5
Paper Physical Properties: Tear Resistance

Kappa 91, Refined 1000 rev. PFI
Kappa 91, No Refining
Phase 5

Paper Physical Properties: Zero Span

Kappa 91, Refined 2000 rev. PFI

Kappa 91, No Refining
Phase 5
Paper Physical Properties: Tensile Strength

Kappa 91, Refined 2000 rev. PFI

Kappa 91, Refined 1000 rev. PFI
Phase 5

Paper Physical Properties: Tensile Strength

Kappa 91, No Refining

Kappa 91, Refined 2000 rev. PFI
Phase 5

Paper Physical Properties: Wet-Tensile Strength

![Chemical structure of GA](image)

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Kappa 91, No Refining

Kappa 91, Refined 2000 rev. PFI
Phase 5: Conclusions

- Addition of phenolic acids to the pulp resulted in the best performing papers during paper strength testing.
- Laccase treatment with gallic acid provided the largest increases in wet and dry tensile strength of all the compounds tested.
- The increases in strength with laccase/gallic acid treatment were not accompanied by changes in sheet density.
Phase 6: Further Investigation

Laccase + Radical Scavenger

Contact Angle  Vertical Wicking
Phase 6
Radical Scavenger

• Observe if gallic acid coupling to kraft pulp is due to radical coupling reactions
• Radical scavenger
• Added in 10:1 molar ratio to gallic acid due to laccase
• Has been shown to scavenge radicals of gallic acid in grape juice (Tulyathan 1989)
• Reduces quinones formed by laccase oxidation back to phenols (Bocks 1967)
Phase 6
Radical Scavenger

- Lac+Gal ineffective in the presence of ascorbic acid (asc)
- Wet-tensile strength of original pulp preserved with ascorbic acid
Phase 6
Contact Angle

- Compatibility of sheet surface with water
- Measured with camera shooting 1 frame/0.067sec
- Slight decrease in contact angle observed with gallic acid treatment
- Higher dosages of gallic acid could not be measured since there was no drop holdout
Phase 6
Vertical Wicking

- Important for absorbent characteristics of non-woven structures
- Kinetic relationship can be used to relate structural and surface properties
Phase 6
Vertical Wicking

• Lucas-Washburn Wicking Equation:

\[ h = \left( \frac{r \sigma \cos \theta}{2 \tau^2 \mu} \right)^{1/2} \sqrt{t} = k \sqrt{t} \]

- \( h \)=distance traveled
- \( r \)=capillary radii
- \( \sigma \)=surface tension
- \( \tau \)=tortuosity factor
- \( \mu \)=viscosity

• Square-root time should be linear to vertical distance traveled (Hodgson and Berg 1988)
• Increasing slope of distance vs. square root time curve should indicate enhanced liquid absorbing capability of material
• Increased compatibility with \( \text{H}_2\text{O} \) may indicate propensity for hydrogen bonding during paper sheet formation
Phase 6
Vertical Wicking

Laccase+Gallic Acid
• Steepest slope
• Lowest contact angle

Test Duration = 5 min
Sample weight = 0.15g +/- 0.01g
Sample density = 0.27g/cm³ +/- 0.01 g/cm³
Average of 2 tests

Lac+gallic wicked 20% further and absorbed 30% more H₂O than control sample
Phase 6: Conclusions

• Ascorbic acid inhibits the laccase/gallic acid treatment strongly suggesting a free-radical mechanism

• Laccase/gallic acid treatment results in an increase in hydrophilicity of fibers
Thesis Conclusions

• It is possible to use laccase to couple phenolic compounds to high-kappa kraft pulps
• Coupling imparts significant changes to the pulp fiber surface
• Lignin is the main site of coupling for laccase generated phenoxy radicals
• Coupling of gallic acid to the fiber results in tremendous increases in wet/dry tensile strength coupled with an increase in hydrophilicity of fibers.
• Strength improvements may be a combination of improvement of surface characteristics and cross-linking of phenoxy radicals between fibers in the sheet
• Chandra, R.P., Lehtonen, L.K., and Ragauskas, A.J., Accepted in Biotechnology Progress. “Modification of High-Yield Kraft Pulps with Laccase to Improve Strength Properties: Laccase Treatment in the Presence Gallic Acid”
Publications and Presentations


- Chandra, R.P., Dyer, T.J. and Ragauskas, A.J., Publication to be submitted, “The Aptitude of Laccase to Attach Compounds to Bleached Chemical Pulps”
Future Lignocellulosics

Tip of the iceberg
• Many new avenues
  – New grafting agents and substrates
  – Various application methods
  – Chemo-enzymatic opens door for novel fiber modification possibilities
    • Energy efficient
    • Environmentally friendly
    • Untapped potential
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