Presentations

1. Introduction and general report (Orlando Rojas)
2. NFC from residual biomass (OR/Ana Ferrer)
3. Composites with bacterial cellulose (OR/Cristina Castro)
4. Click chemistries (Ilari Filpponen)
5. SP Aerogels (Julio Arboleda)
6. Effect of lignin on enzymatic hydrolysis of bicomponent cellulose/lignin films (Raquel Martin)
7. Biocomponent Films (Laura Taajamaa)
8. Bio-modification (Oriol Cusola)

Influential factors:
- Lignin content
- Cellulose crystallinity
- Degree of polymerization
- Available surface area
- Particle size
Inhibition of Enzymatic Hydrolysis by Residual Lignins from Softwood—Study of Enzyme Binding and Inactivation of Lignin-Rich Surface


Biotechnology and Bioengineering 2011, 108 (12): 2823-2834

Endoglucanase
Exoglucanases (CBH-II)
β-glucosidases
Crystalline cellulose
Amorphous cellulose
Crystalline cellulose

Trichoderma cellulase enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Conc (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBH I</td>
<td>50-60</td>
</tr>
<tr>
<td>CBH II</td>
<td>15-18</td>
</tr>
<tr>
<td>EG I</td>
<td>12-15</td>
</tr>
<tr>
<td>EG II</td>
<td>9-11</td>
</tr>
<tr>
<td>EG III</td>
<td>0-3</td>
</tr>
<tr>
<td>EG V</td>
<td>0-3</td>
</tr>
</tbody>
</table>

Tolun, 2002 Clean Techn Environ Policy 3, 339-345
Objective

Roll of lignin on the enzymatic hydrolysis of cellulose

Previous work: Hoeger’s work

Bicomponents films: Cellulose / Lignin

Enzymatic hydrolysis using QCM-D: enzymes mixture

News objectives:
- CTA/AcL instead of TMSC/AcL
  - One step to convert them into Cellulose/Lignin
- Monocomponents enzymes
  - Different interactions depending on enzyme type

Deacetylation of lignin
Desilylation of TMSC
Organosolv lignin from Aspen

1. Blend CTA / AcL (conc. of the major component fixed at 5 g/l)
   - Dissolved AcL 10 g/l

2. Spin coating on silicon wafers (at 4000 rpm for 1 min)
   - Dissolved CTA 10 g/l

3. Deacetylation (NH₄OH atmosphere at room T for 3 days followed by washing with water)

4. Cellulose / Lignin films (Ce/L)
   - Blend CTA / AcL
   - Deacetylation
   - After deacetylation

Contact Angle

Cellulose (Ce/L 1:0)
- 61° CTA
- 26° Cellulose
- Deacetylation 68h NH₄OH atm

Lignin (Ce/L 0:1)
- 71° Ac. Lignin
- 76° Lignin
- Deacetylation 68h NH₄OH atm
Enzymatic treatment in QCM

Enzymatic hydrolysis of Cellulose/lignin films:

- QCM-D: monitoring in situ and in real time
- Bicomponent films: 1:0, 5:1, 1:1 and 0:1
- Monocomponents enzymes: Cellulbiohydrolase CBH I Endoglucanase EG I

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</tr>
<tr>
<td>EG V</td>
<td>0-3</td>
</tr>
</tbody>
</table>

Trichoderma cellulase enzymes
Enzymatic treatment in QCM

**Cellobiohydrolases: CBH I**

- CBH-I are adsorbed on both lignin and cellulose, but more on cellulose.
- Lignin reduce the hydrolysis rate of cellulose (comparing Ce/L 1:0 to 5:1) until complete inhibition for 1:1.
- After a few minutes, the adsorption on lignin reach a maximum.

**Cellbiohydrolases: CBH I → Influence of enzyme concentration**

- If we reduce the concentration of the enzyme, similar results was obtained:
  - The cellulose hydrolysis depend mainly on the amount of CBH I adsorbed
Endoglucanase: EG I

- EG-I are adsorbed on both lignin and cellulose, but on cellulose the hydrolysis rate is higher than the adsorption rate, in the first minutes.
- Lignin reduce the hydrolysis rate of cellulose (comparing Ce/L 1:0 to 5:1 and 1:1) until almost complete inhibition.
- More EG-I can be adsorbed on lignin after 20 minutes.

Enzymatic treatment in QCM

Endoglucanase: EG I → Influence of enzyme concentration

- If we reduce the concentration of the enzyme, both the adsorption and the hydrolysis decrease.

Adsortion after 20 min

- Data for 0.5 mg/ml and 0.25 mg/ml.
Enzymatic treatment in QCM

**Endoglucanase: EG I → Overcrowding**

- After long adsorption stage → Inhibition of hydrolysis in cellulose films → Overcrowding

The dense binding of enzyme molecules hinder each other from accessing the cellulose with their catalytic domains (Suchy et al. Langmuir 2011, 27, 8879-8828)

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**AFM**

<table>
<thead>
<tr>
<th>Ce/L</th>
<th>Before hydrolysis</th>
<th>After CBH I (5 mg/ml)</th>
<th>After EG I (0.5 mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:0</td>
<td>Roughness 1.81 nm</td>
<td>Roughness 3.32 nm</td>
<td>Roughness 3.04 nm</td>
</tr>
<tr>
<td>5:1</td>
<td>Roughness 3.27 nm</td>
<td>Roughness 3.50 nm</td>
<td>Roughness 3.19 nm</td>
</tr>
<tr>
<td>1:1</td>
<td>Roughness 1.88 nm</td>
<td>Roughness 2.35 nm</td>
<td>Roughness 2.45 nm</td>
</tr>
<tr>
<td>0:1</td>
<td>Roughness 4.44 nm</td>
<td>Roughness 0.94 nm</td>
<td>Roughness 0.93 nm</td>
</tr>
</tbody>
</table>

Roughness increase:
- Cellulose hydrolysis
- Enzyme adsorption
Roughness $0.443 \text{ nm}$

Before hydrolysis

After CBH I (0.5 mg/ml)

After EG I (0.5 mg/ml)

Cellulases immobilized on PIII-treated polystyrene (PIII=Plasma immersion ion implantation)

Increase in roughness from 0.5 to 0.9 nm

Hirsh et al. Langmuir 2010, 26 (17), 14380-14388

Enzyme adsorption on lignin film

Individual enzyme molecules: size 10-15 nm
Conclusions

✓ We successfully and reproducibly prepared bicomponents films containing both cellulose and lignin (CTA and acetylated lignin)

✓ Enzyme activity: different behaviors observed for CBH I and EG I.

✓ CBH I: higher affinity for cellulose. EG I: higher affinity for lignin.

✓ Lignin: inhibits the enzymatic hydrolysis of cellulose (CBH I and EG I). EG I is more sensitive to lignin.

✓ CBH I inhibition: irreversible enzyme adsorption and physical blocking.

✓ EG I inhibition: overcrowding.

Future works

✓ Study the effect of the type of lignin on the interaction with enzymes:
  • Films from different lignin sources:
    • Spruce
    • Spruce after steam pre-treatment
    • Wheat straw
    • Wheat straw after steam pre-treatment
    • Interaction of enzymes with these films (QCM experiments)

✓ Study the effect of non-ionic surfactants on the enzymatic hydrolysis of bicomponent films:
  • If surfactants are adsorbed selectively onto lignin, this adsorption might prevent the adsorption of enzymes onto the lignin, and then the hydrolysis of cellulose domains could be not affected by the lignin presence.
Acknowledgements

- Orlando Rojas and Janne Laine
- Leena-Sisko Johansson and Joe Campbell – XPS measurements
- Lignocell Project

THANK YOU FOR YOUR ATTENTION

QUESTIONS ???

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Thin functional films from cellulose

Steering group meeting 11.5.2012

Laura Taajamaa

OUTLINE

- Ultrathin films
  - (Taajamaa, Rojas, Laine and Kontturi, Soft Matter 2011 (7), 10386)

- Porous fibres

- Current work
Background - THIN FILMS

MOTIVATION FOR THIN FILMS

Modeling aspect
- well-defined chemistry
- well-defined morphology

Materials aspect
- sensors
- transistors
- photonic devices
- receptors
- templated nanomaterials
- coatings
- etc.

Background - POLYMER BLENDS

**Polymer blend**: two or more macromolecular substances blended to create a new material with novel physical properties

**Immiscible polymer blends** phase separate during spin coating. The patterns formed are regular due to the constraints caused by the interfaces.

![Polymer blend diagram](image)
After either of the blend components has been converted to cellulose, the remaining one can be selectively dissolved.

Alternatively, also the second component can be converted to cellulose.

A matrix of materials with unique morphological and surface energy properties.

Background - CELLULOSE DERIVATIVES

**Trimethylsilyl cellulose (TMSC) DS=3**

**Cellulose triacetate (CTA) DS=3**

Gas phase acid conversion

‘Gas phase’ base conversion

Cellulose

Cellulose

Background - OBJECTIVE

**FILMS and FIBERS**

Unmodified

TMSC/CTA

After selective conversion

Cellulose*/CTA

TMSC/Cellulose*

After selective conversion or dissolution

Cellulose*/Cellulose*

Cellulose*/Cellulose

Cellulose*/Cellulose

Cellulose*/Cellulose

Cellulose*/Cellulose

Cellulose*/Cellulose

Cellulose*/Cellulose

Cellulose*/Cellulose

Cellulose*/Cellulose

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Cellulose*/Cellulose

Cellulose*/Cellulose
ULTRATHIN CELLULOSE DERIVATIVE FILMS

TMSC/CTA films
- TMSC conversion to cellulose

Cellulose/CTA films
- CTA dissolution

Cellulose films

Blend ratio (w/w)
- 10:1
- 5:1
- 2:1
- 1:2
- 1:5
- 1:10

FLMS

TUNABLE MORPHOLOGY

Humidity and blend ratio played an important role in the morphology formation.
Pore growth essentially linked to CTA rich phase, but presence of humid air vital.

Humidity increases

Aalto University
School of Chemical Technology
Hypothesis - MORPHOLOGY FORMATION

Blends with excess of one component

Transient bilayer theory (three top images):
- Entropic optimization
- vertical separation
- upper CTA layer dewets
- holes filled by the TMSC-rich layer from underneath (lateral separation)

Intermediate blend ratios

Additional dewetting

Layer inversion:
- Film strives to minimize its surface energy
- material from the lower layer forms a continuous layer at the topmost surface
To transfer the porous, high surface area 2D film structures into 3D fiber mats.

**VISION**

**FILMS and FIBERS**

**POROUS CELLULOSE DERIVATIVE FIBERS**
**FIBERS**

**WETTING CHARACTERISTICS**

![Graph showing wetting characteristics of fibers](image)

- **TUNABLE WETTING PROPERTIES**
- **ADHESIVE SURFACE**

**CHEMICAL MODIFICATIONS**

![Diagram showing chemical modifications of fibers](image)

- **TUNABLE CHEMISTRY**
- The network was destroyed only when CTA, as the majority component, was selectively dissolved:

  → CTA formed a continuous matrix

- Contact angle measurements:

  → TMSC formed a layer on top of the fibres. TMSC was also hypothesised to exist as interconnected domains embedded in the CTA matrix.

CURRENT WORK—starting points

COMPONENT 2

COMPONENT 1

BLEND FILMS

Modified cellulose surface

Mutual attraction

Cellulose surface

Modified cellulose in suspension

Double click: Ilari, Hannes, Eero, Janne

FUNCTIONAL SURFACES

Schematics not to scale
1. Polysaccharide activation

"Polysaccharide" has affinity to cellulose surface.

2. Activated polysaccharide is adsorbed into a solid cellulose substrate (e.g. NFC)

3. Attaching click counterpart to adsorbed activated polysaccharide in aqueous solution leads to modified cellulose surface.

J H Choi et al. Nanotechnology 2009

Surface with specific and non-specific adsorption sites for the modified cellulose

The amount of modification can be tailored
Double click for thin blend films

FUNCTIONAL SURFACES

Spin coating of a polymer blend with click activated polysaccharide

→ Cellulose derivative blend film with activated patches

→ Modified cellulose film

The amount of modification can be tailored

Surface with specific and non-specific adsorption sites for the click counterpart

J H Choi et al. Nanotechnology 2009

Films to be modified

FUNCTIONAL SURFACES

PS

Cellulose films

1:2
1:5
1:50

Tested:

PS

Cellulose, CMC/PEO, CMC/PEG

→ Did not form phase-separated patterns

PS

Cellulose

5*5 μm²

10*10 μm²

25*25 μm²

Schematics not to scale
**GOAL!**

- sensors
- transistors
- photonic devices
- receptors
- templated nanomaterials

**FUNCTIONAL SURFACES**

**PS/Cellulose films + Alkyne-modified CMC + Azide-modified silver nanoparticles**

**FUNCTIONAL SURFACES**

**Modified silver nanoparticles (AgNP)**

**AgNP**

- COOH activation
- Facilitating bond formation

**Azide-modified AgNP**

Mixing for 2h, washing by centrifugation

Zetapotential: -45 mV → -21 mV

**Schematics not to scale**

EDC = 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
NHS = N-Hydroxysuccinimide
Amine-N$_3$ = 11-azido-3,6,9-trioxaundecan-1-amine
Adsorption tests

**FUNCTIONAL SURFACES**

**Surface morphology – AFM**

**BEFORE**

**AFTER**

**Sensitive balance – QCM-D**

Increased viscoelasticity

Increased adsorbed mass/bound water

---

**FUNCTIONAL SURFACES**

**Surface morphology – AFM**

**BEFORE**

**AFTER**

**Sensitive balance – QCM-D**

---
**Adsorption tests**

**Surface morphology – AFM**

**BEFORE**

1 x 1 µm

**AFTER**

500 x 500 nm

**Sensitive balance – QCM-D**

- Time (min)
- Δf (Hz)
- ΔD (10^-6)

1. alkyne-CMC (in 50 mM NaCl)
2. Rinsing (50 mM NaCl)
3. Rinsing (UHQ-water)
4. azide-AgNP + CuSO4 + ascorbic acid
5. Rinsing (UHQ-water)

**Challenges**

- Too high ionic strength (e.g. catalyst Copper) destabilizes the AgNPs, but at low Copper amounts the click reaction not effective
  - Solution? protective ligand for copper, increase the stability of the AgNPs

- Non-selective adsorption of azide-AgNPs to PS due to adsorption of alkyne-modified CMC to polystyrene?
  - Solution? alkyne-modify the AgNPs

**FUNCTIONAL SURFACES**
**ACKNOWLEDGEMENTS**

- Janne, Eero Kontturi & Orlando
- Soledad Peresin – Electrospinning
- Leena-Sisko Johansson & Joe Campbell @ Aalto CHEM – XPS measurements
- Chuck Mooney @ NCSU – SEM measurements
- Kati Vilonen @ Aalto CHEM – N\textsubscript{2} adsorption measurements
- Karoliina Junka & Ilari @ Aalto CHEM – Click Chemistry & chemicals
- All LignoCell partners and especially Kalle & Heidi for a nice visit to Imatra!
Thank you!

Questions, comments?

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8. Enzymatic treatments of fibers: surface modification (Oriol Cusola)
Enzymatic treatments of paper surface using hydrophobic compounds and lignosulfonates

Objectives

To find a new way to functionalize cellulosic surfaces (paper) by means of enzymatic treatments

Obtaining added-value products

To confer hydrophobicity / slow down liquid absorption

Already reported for fiber suspensions.

Surface treatments

Usual places where enzymatic treatments are performed.

New locations?

Surface treatments

Treatments applied onto an already formed sheet:

- Advantages:
  - It doesn’t affect the formation process.
  - Lower product amounts.
  - Compatible with the high speed of the paper machines.
  - Application at different points.
  - Several application methods.

Size press

Metering bar

Spray
Enzymatic functionalization

- Addition of functional groups onto a surface to modify its properties.
- **Laccase-mediator system.**
- Use of the Laccase-mediator system to fix low molecular weight compounds.

**KUDANGA, Tukayi, et al.**

**ARACRI, Elisabetta, et al.**

**Surface functionalization**

**Surface treatments**

- Use of the Laccase-mediator system with **Lauryl Gallate (LG)** as reactant.
- Substrate to be treated: Comercial filter paper FILTERLAB®.
- Treatment conditions:
  - Laccase: 40U/g
  - Consistency: 3%
  - Reactant (LG): 4% with respect to paper
  - pH: 4 (sodium tartrate) 50mM
  - Temperature: 50°C
  - Time: 1h

- Result: Sheets become hydrophobic, but with a very **bad LG distribution** along the surface of the sheet.
Surface treatments

- Hydrophobic property not uniform along the surface.
- No solubility of LG in water!

Need to introduce a surfactant.
- Lignosulfonate and LG.
- Lignosulfonate dose: Same as the reactant (4%).
- Result: Hydrophobicity with better distribution!
Surface treatments

- Study of the reaction time.
- Times: 0'-5'-10'-15'-30'-45'-60'-75'

Laccase: 40U/g
Consistency: 3%
Reactant (LG): 4% with respect to paper
pH: 4 (adjusted with tartrate) 50mM
Temperature: 50ºC
Time: variable

Surface treatments

- Absorbance curves of the LG vs reaction time:
Surface treatments

Residual enzymatic activity vs reaction time:

- LG
- Lignosulfonate
- LG+ Lignosulfonate

Paper sheets on the reaction

Surface treatments

Internal sizing/hydrophobicity characterization: WDT (T835 om-08)
Surface treatments

Internal sizing/hydrophobicity characterization vs the reaction time.

<table>
<thead>
<tr>
<th>Time</th>
<th>0'</th>
<th>5'</th>
<th>10'</th>
<th>15'</th>
<th>30'</th>
<th>45'</th>
<th>60'</th>
<th>75'</th>
<th>90'</th>
<th>105'</th>
<th>120'</th>
<th>135'</th>
<th>150'</th>
<th>240'</th>
<th>390'</th>
<th>1359'</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV%</td>
<td>18.51</td>
<td>16.81</td>
<td>16.00</td>
<td>7.57</td>
<td>19.74</td>
<td>13.04</td>
<td>11.14</td>
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<td>18.09</td>
<td>18.66</td>
<td>13.53</td>
<td>18.87</td>
<td>20.66</td>
<td>62.92</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Hydrophobicity evolution with reaction time

Surface treatments

Hydrophobicity characterization

Surface contact angle

Contact angle

< 90°  → Hydrophobic

> 90°  → Hydrophilic

WITHOUT TREATMENT

TREATED SHEET

CA: 54°

CA: 130°
Surface treatments

**CONTACT ANGLE DECREASE**

- Non-treated paper
- Treated paper

Up to 1h to fall to 0%

**WATER DROP ABSORPTION - EVAPORATION**

- LG Treated paper
- Teflon (evaporation)

**Influence of the dosage and the temperature:**

- Laccase: 40U/g
- Consistency: 3%
- Reactant (LG): 4% with respect to paper
- pH: 4 (adjusted with tartrate) 50mM
- Temperature: 50ºC
- Time: 1h

- St.
- St. + LGx2
- St. + Heat treatment
- St. + LGx2 + Heat treatment
Conclusions

- It is possible to apply enzymatic treatments on the surface of an already formed paper sheet.

- Use of lignosulfonates:
  - Improves the product (LG) distribution
  - Helps preserving the enzyme activity.

- Sheets reached a high hydrophobic behavior with this method.

- Time, dosage and temperature are factors that affect the level of hydrophobicity reached.

THANK YOU FOR YOUR ATTENTION
Plans for Next Semester