Outline presentation

- Wheat straw biorefinery
- Alkaline pretreatment
- Analytical tools
- Highlights results
- Available samples for Round Robin COST PF0901
Wheat straw biorefinery concept

Clark et al. 2006
Pretreatment

- **Goal**
  - Fractionation of lignocellulosic to cellulose, hemicellulose and lignin
  - Make cellulose and hemicellulose fractions more accessible for enzymatic hydrolysis
  - Make lignin available for higher-valued products

- **Numerous processes**
  - Mechanical, chemical, enzymatic
  - Acids, alkaline, organic solvents
  - Combinations thereof
Alkaline pretreatment (high pH)

- Alkali induces swelling of biomass, leading to increase of internal surface area
- Disruption of lignin structure (lignin removal)
- Reduction of cellulose crystallinity

- Results in improved accessibility of cellulose and hemicellulose towards enzymatic hydrolysis
Alkaline pretreatment

- Common alkaline pretreatment route
  - Base as catalyst (NaOH, Ca(OH)$_2$, ammonia); T<120 °C
  - Carried out under (close to) atmospheric conditions
  - Long reaction times (hours)
  - Need for chemical recycling

- Improve alkaline pretreatment
  - Apply milder process conditions
    - Less formation of degradation products (e.g. inhibitors)
    - Lower operational costs
  - Improve accessibility of sugars to enzymes
  - Obtain lignin of high purity
From wheat straw to fermentable sugars

Wheat straw → Pretreatment → Enzymatic hydrolysis → Fermentation

Pretreatment efficiency:
- Properties of solid fraction (cellulose-rich)
  - Biochemical composition
- Properties of liquid fraction
  - Sugars, organic acids and lignins in solution
  - Recovery of lignin

Enzymatic hydrolysis:
- Conversion of solid fraction
  - Glucan to glucose
  - Xylan to xylose
- Standard procedure
  - 50 °C, 120h
  - Excess of enzyme (GC220)

Fermentation:
- Ethanol or ABE
Experimental setup

**Base case**: 4h alkaline pretreatment in stirred reactor

- **Preliminary experiments** (2 L, 70 °C)
  - Base case
  - Effect NaOH-concentration

- **Main experiments** (200 L, 90 °C)
  - Base case
  - Extrusion
  - Refining
Analytical tools (selection)

- Biochemical composition untreated & pretreated straw (solids)
  - Extraction (ASE)
  - Carbohydrates (HPAEC-PAD)
  - Lignin (AIL by weight, ASL by UV)
  - Ash (TGA, ignition)
  - Proteins (N by Kjehldahl or Dumas, AA by UPLC)
  - Morphology (SEM)
  - Cellulose crystallinity (WAXS)

- Liquids
  - Carbohydrates (monomers, oligomers)
  - Lignin
  - Fermentation inhibitors (phenolics, organic acids by LC)

- Isolated lignin
  - FT-IR
  - Impurities (carbohydrates, proteins, ash)
  - MWD (alkaline SEC)\(^1\)
  - Functional groups (\(^{31}\)P NMR)\(^1\)

\(^1\)Gosselink, R.J.A. et al. Holzforschung 2010
Pretreatment efficiency

Solid fraction

![Bar chart showing biochemical composition of various NaOH concentrations](image)

- **Wheat straw**
- **0% NaOH**
- **6% NaOH**
- **9% NaOH**
- **12% NaOH**

**Biochemical Composition (wt%):**
- Other
- Ash (TGA)
- Lignin
- Hemicelluloses
- Cellulose

**NaOH-conc as wt% dry biomass**
Pretreatment efficiency

**Liquid fraction**

**IR-spectra of lignins isolated from liquid fraction**

- Wheat straw lignin reference
- 0% NaOH
- 6% NaOH
- 9% NaOH
- 12% NaOH

Wavenumber (cm⁻¹)

Absorbance

**CO stretching carbohydrates**

**Aromatic skeleton lignin**
Enzymatic hydrolysis

Solid fraction

- Enhanced enzymatic degradability due to pretreatment
  - Structural changes of wheat straw (e.g. delignification)
  - Minimal formation of degradation products due to mild conditions
Summary

Pretreatment
- Main action is delignification (max 50%)
- Minimum degree of hemicellulose and cellulose hydrolysis
- Solid fraction enriched with sugars due to pretreatment (from 60% to 75%)
- Optimal NaOH concentration 9 wt%

Lignin
- Low purity

Enzymatic hydrolysis
- Conversion of glucan and xylan improved from < 20% to > 80%

Input for further experiments
- Increase T from 70 to 90 °C
- NaOH concentration 6-9 wt% NaOH

Base case (2L)
Experimental setup

**Base case:** 4h alkaline pretreatment in stirred reactor

- Preliminary experiments (2 L, 70 °C)
  - Base case
- Main experiments (200 L, 90 °C)
  - Base case
  - Extrusion
  - Refining
Base case

- Chopped wheat straw (1-2 cm)
- 9 wt% NaOH based on dry straw
- 4h at 90 °C
Extrusion

1\textsuperscript{st} Delignification
Whole wheat straw
6 wt\% NaOH, 2h, 90°C

2\textsuperscript{nd} Delignification
6 wt\% NaOH, 2h, 90°C

- Transport of biomass to RSE (reversed screw element)
  - Accumulation and compression of material
  - High compression and shear forces
  - Material is dry and absorbs added chemicals very well (e.g. NaOH)

- Shortening of fibres and fibrillation
Refining

1\textsuperscript{st} Delignification
Chopped wheat straw
6 wt% NaOH, 2h, 90 °C

2\textsuperscript{nd} Delignification
6 wt% NaOH, 2h, 90°C

- Shear and compression forces
  - Increase of surface area
  - Shortening of fibres and fibrillation
  - Homogeneous, clean fibres
Scanning Electron Microscopy

Wheat straw
Base case
Extrusion
Refining
Pretreatment efficiency

- Direct analysis of sugar monomers

- After acid hydrolysis, 3h, 100°C
  - Substantial increase of sugars

- Liquid fraction
  - Sugars mainly present as oligomers (total 6%)

**Liquid fraction**

![Graph showing sugar concentration over time](image)

- Total sugar release:
  - Sugars (% dry ash)
  - Time (h)

**Alkaline: Monomers**

- Concentration (g/l)
  - Rhamnose
  - Arabinose
  - Galactose
  - Glucose
  - Xylose
  - Mannose

**Alkaline: Oligomers and monomers**

- Concentration (g/l)
  - Rhamnose
  - Arabinose
  - Galactose
  - Glucose
  - Xylose
  - Mannose

**Amounts 100x lower**
Pretreatment efficiency  Liquid fraction

- Liquid fraction
  - Acetic acid primary fermentation inhibitor
  - Lactic acid present
  - Phenolic compounds present at very low levels
Pretreatment efficiency  

Liquid fraction

IR-spectra of lignins isolated from liquid fraction

- Wheat straw lignin
- Base case
- Extrusion
- Refining

Aromatic skeleton lignin
CO stretching carbohydrates

Absorbance

Wavenumber (cm⁻¹)
Lignin: Chemical composition

![Biochemical composition chart](chart.png)

- **Lignin base case (conical):**
  - Lignin: 75%
  - Hemicelluloses: 10%
  - Other: 10%

- **Extrusion (B):**
  - Lignin: 75%
  - Hemicelluloses: 13%
  - Other: 18%

- **Lignin refining (atm P):**
  - Lignin: 73%
  - Hemicelluloses: 13%
  - Other: 13%

- **Lignin refining (elev P):**
  - Lignin: 68%
  - Hemicelluloses: 13%
  - Other: 18%

- **Biochemical composition (wt%):**
  - Other
  - Ash (TGA)
  - Lignin
  - Hemicelluloses
  - Cellulose
### Lignin: MWD by alkaline SEC

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference lignin(^1)</th>
<th>Base case</th>
<th>Extrusion</th>
<th>Refining (atmP)</th>
<th>Refining (elevP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn (Da)</td>
<td>700</td>
<td>690</td>
<td>1000</td>
<td>900</td>
<td>890</td>
</tr>
<tr>
<td>Mw (Da)</td>
<td>8100</td>
<td>5300</td>
<td>18000</td>
<td>17000</td>
<td>28000</td>
</tr>
<tr>
<td>PD</td>
<td>10</td>
<td>8</td>
<td>18</td>
<td>19</td>
<td>32</td>
</tr>
</tbody>
</table>

- Depending on pretreatment method different lignins obtained
- Biomass more accessible due to mechanical pretreatment: less degradation of lignins?  

\(^1\)Soda wheat straw lignin supplied by Granit SA
Lignin: Functional groups by $^{31}$P NMR

Amounts of different hydroxyl group species (mmol/g) in lignin

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Aliphatic OH</th>
<th>Aromatic OH</th>
<th>S+C</th>
<th>G</th>
<th>H</th>
<th>Carboxylic acid</th>
<th>Total OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base case</td>
<td>3.72</td>
<td>1.14</td>
<td>0.30</td>
<td>0.62</td>
<td>0.22</td>
<td>0.89</td>
<td>5.75</td>
</tr>
<tr>
<td>Refining (atmP)</td>
<td>4.00</td>
<td>0.49</td>
<td>0.03</td>
<td>0.37</td>
<td>0.09</td>
<td>0.57</td>
<td>5.05</td>
</tr>
<tr>
<td>Refining (elevP)</td>
<td>3.97</td>
<td>0.85</td>
<td>0.24</td>
<td>0.48</td>
<td>0.14</td>
<td>0.68</td>
<td>5.50</td>
</tr>
</tbody>
</table>

S + C = Syringyl + Condensed phenolic units, G = Guaiacyl phenolic units and H = $p$-Hydroxyphenyl phenolic units

$^{31}$P NMR analysis performed by VTT

IS = Endo-N-Hydroxy-5-norbornene-2,3-dicarboximide
## Pretreatment efficiency

<table>
<thead>
<tr>
<th></th>
<th>Base case</th>
<th>Extrusion</th>
<th>Refining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield solid fraction</td>
<td>70-75%</td>
<td>60-65%</td>
<td>60-65%</td>
</tr>
<tr>
<td>Delignification</td>
<td>40-50%</td>
<td>70-80%</td>
<td>70-80%</td>
</tr>
<tr>
<td>Sugars in liquid fraction</td>
<td>&lt;10%</td>
<td>20-30%</td>
<td>20-30%</td>
</tr>
</tbody>
</table>

- Extrusion and refining comparable results
- Higher degree of delignification accompanied by loss of sugars by hydrolysis
Enzymatic hydrolysis  Solid fraction

Polysaccharide conversion (wt%)

- Wheat straw
- Base case
- Extrusion
- Refining

- Glucose
- Xylose
Conclusions

- Alkaline pretreatment excellent for
  - Delignification (40-50%)
  - Improvement of enzymatic degradability (from 20 to 70-80%)
- Added value extrusion or refining
  - More delignification (70-80%)
  - Further improvement of enzymatic degradability (from 20 to 100%)
- Different lignins produced
  - Moderate purity (80%)
  - Molar mass relatively high
- Economic feasibility of combined mechanical/alkaline pretreatment depends on value of lignin produced
- Analytical tools suitable for process development
Available samples for Round Robin

- Wheat straw (kg)
- Base case: Alkaline pretreated wheat straw (kg)
  - Neutralised + washed + stored @ -20°C
- Black liquor stored @ -20°C (kg)
- Isolated soda wheat straw lignin (100-200g, expected early September)
Acknowledgements

- Biosynergy  www.biosynergy.eu
- Biobutanol  www.biobutanol.nl
Thank you for your attention
Any Questions?

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