Fermentation Technology for Lignocellulose

Experiences from the BIOLYFE project

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Chemical Engineering, Lund University

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Brussels, 4 December 2013
Biomass *is not* sugar – but it contains sugar

Sugar \( \approx 200 \text{ g/L} \)

Milled pine wood, moisture content 50%, Glucan 35%, Mannan 12%

"Sugar" \( \approx 200 \text{ g/L} \)
The fermentation process

Sugar(s) + solids + inhibitors → Fermentation → Ethanol solids (lignin) by-products conversion products from inhibitors
The fermentation process!

Sugar(s) + cellulose + hemicellulose + solids (?) + inhibitors?

Hydrolysis

Fermentation

Ethanol solids (lignin) by-products conversion products

Maybe like this?
BIOLYFE: Demonstrating large-scale bioethanol production from lignocellulosic feedstocks

Hydrolysis

Cellulose + hemicellulose + solids (?) + inhibitors?

Fermentation

Sugar(s)

Ethanol solids (lignin) by-products conversion products

Or this?
BIOLYFE: Demonstrating large-scale bioethanol production from lignocellulosic feedstocks

Cellulose + hemicellulose + solids (?) + inhibitors?

Hydrolysis

Fermentation

Sugar(s)

Ethanol solids (lignin) by-products conversion products

Or this?
Sugar(s) + cellulose + hemicellulose + solids (?) + inhibitors?

Hydrolysis and Fermentation

Ethanol solids (lignin) by-products conversion products

THERE ARE OBVIOUSLY MANY OPTIONS IN THE PROCESS DESIGN!
<table>
<thead>
<tr>
<th>Type</th>
<th>Plant</th>
<th>Glucan</th>
<th>Xylan</th>
<th>Arabinan</th>
<th>Mannan</th>
<th>Galactan</th>
<th>Acetyl</th>
<th>Lignin&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Extrac-tives&lt;sup&gt;b&lt;/sup&gt;</th>
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<td>Godin et al., 2011</td>
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<td>0</td>
<td></td>
<td></td>
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<td>Foyle et al., 2007</td>
</tr>
</tbody>
</table>

Different distribution of carbohydrates

Here is some acid..

And here are lots of stuff..
Fermentation – a part in the integrated process

Feedstock

Pretreatment

Enzymatic hydrolysis

Fermentation

Down-stream processes
Fermentation – a part in the integrated process

The challenges in the fermentation are connected to all upstream steps:

- The *feedstock defines the sugars* to be converted, and also contains some components which may be inhibitory.
- The *pretreatment (may) produce (or liberate) inhibitory* compounds to the fermentation and enzymatic hydrolysis.
- The hydrolysis - if simultaneous to the fermentation – affects desired process temperature.
- The *process design (feeding strategies) defines the relative ratios between sugars and also the level of inhibitors* in case these are converted in the process (in-situ detoxification).
Feedstock

<table>
<thead>
<tr>
<th>Component</th>
<th>Content (% on oven-dry matter)</th>
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<tbody>
<tr>
<td>Cellulose</td>
<td>33.8%</td>
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<tr>
<td>Hemicellulose</td>
<td>25.6%</td>
</tr>
<tr>
<td>Lignin</td>
<td>24.0%</td>
</tr>
<tr>
<td>Extractives</td>
<td>12.2%</td>
</tr>
<tr>
<td>Ash</td>
<td>5.0%</td>
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</table>

Arundo Donax

Pretreatment

- The pretreatment defines the structure and chemical composition going into enzymatic hydrolysis.
- It also defines the relative ratios of oligosaccharides to monosaccharides in the liquid fraction
- and – to a large extent – the amount of inhibitors to be handled
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Pretreatment

Hemicellulose
- xylose, mannose, glucose, etc.
- Fermentation

Enzymatic hydrolysis

Cellulose
- glucose
- Fermentation

Temp 1

Temp 2
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**Pretreatment**

- Hemicellulose
  - xylose, mannose, glucose, ..
  - Enzymatic hydrolysis
  - More hemicellulose left

- Cellulose
  - glucose
  - Fermentation

Milder pretreatment
Less acid conditions also leaves more oligosaccharides in the liquid phase.
Fibre composition

<p>| | |</p>
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<tbody>
<tr>
<td>Glucan</td>
<td>48.2%</td>
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<tr>
<td>Xylan</td>
<td>3.8%</td>
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<tr>
<td>Lignin</td>
<td>41.7%</td>
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<td>Furfural</td>
<td>0.2 g/L</td>
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Soluble components

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<tr>
<th></th>
<th>Monomers</th>
<th>Total sugars</th>
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</thead>
<tbody>
<tr>
<td>Sugars</td>
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<tr>
<td>Glucose</td>
<td>2.5 g/L</td>
<td>14.2 g/L</td>
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<tr>
<td>Xylose</td>
<td>4.0 g/L</td>
<td>18.4 g/L</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>5.6 g/L</td>
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</tr>
<tr>
<td>HMF</td>
<td>n.d</td>
<td></td>
</tr>
<tr>
<td>Furfural</td>
<td>0.2 g/L</td>
<td>A lot of oligos</td>
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</table>
The eternal challenges..

- Rate
  - Investment costs "CAPEX"

- Yield
  - Raw material costs "OPEX"

- Product concentration
  - Operating costs (downstream) "OPEX"
<table>
<thead>
<tr>
<th>Rate</th>
<th>Yield</th>
<th>Product concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>High solids handling in enzymatic hydrolysis</td>
<td>Inhibition in fermentation</td>
<td></td>
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</tbody>
</table>
Increased final ethanol titer ➔ Higher fiber contents to be handled

Inhibitor problems

- Effects yeast metabolism
- Effects on enzymatic hydrolysis

Mixing issues

- Temperature control
- Distribution & blending
- Effects on process performance
- Viscosity
Viscosity is a significant factor in the processing of lignocellulosic feedstocks. The graphs illustrate the torque over time for different WIS (Weight in Solution) concentrations for Spruce and Arundo Donax. For Arundo Donax, there is a very rapid loss of viscosity, indicating low mixing energy requirements in hydrolysis.
What is the impact of pretreatment on SSF?

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Pretreated Arundo Donax, T = 34 C, 10% WIS
Yeast: Ethanol Red (industrial)

SO$_2$ catalyzed

Pretreated Arundo Donax, T = 34 C, 10% WIS
Yeast: Ethanol Red (industrial)

Pretreatment without acid catalysts leaves a material which is more difficult to enzymatically hydrolyse

Recalcitrance in enzymatic hydrolysis

Conversion of xylose/by-products in fermentation
#### Recalcitrance in enzymatic hydrolysis – Improved enzymes

<table>
<thead>
<tr>
<th>Reference enzyme mixture</th>
<th>Improved enzyme mixture</th>
<th>Change in ethanol yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellic CTec (+ HTec)</td>
<td>Cellic CTec2</td>
<td>No significant increase</td>
</tr>
<tr>
<td>Cellic CTec2</td>
<td>Intermediate enzyme blend</td>
<td>~ 15 % increase</td>
</tr>
<tr>
<td>Intermediate enzyme blend</td>
<td>Cellic CTec 3</td>
<td>~ 8 % increase</td>
</tr>
<tr>
<td></td>
<td><strong>Overall increase</strong></td>
<td>~ 24 %</td>
</tr>
</tbody>
</table>

*Batch SSF experiments at a WIS loading of 10 %. Yeast used: TMB3400 (Taurus Energy). T = 34 °C.*
Olofsson et al., Biotechnology for biofuels, 1:7, 2008

Xylose fermentation in Saccharomyces cerevisiae is improved by co-fermentation with a small amount of glucose

Olofsson et al., Biotechnology for biofuels, 1:7, 2008

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Conversion of xylose

SSCF

Enzymatic hydrolysis

34°C, pH 5.0, WIS content 22%
Xylose fermenting yeast TMB3400
CTec3 (0.075 g enzyme solution/g glucan)

34°C, pH 5.0, WIS content 22%
CTec3 (0.075 g enzyme solution/g glucan)
The basic process layouts

**Pre-treatment**
- Temperature 180-210°C

**Enzymatic hydrolysis**
- Temperature 45-50°C
- Temperature 30-32°C

**Fermentation**
- Temprature 180-210°C

**SHF**
- Enzymatic hydrolysis
- Fermentation

**SSCF**
- Enzymatic hydrolysis and fermentation

**Xylose fermentation is favored in SSF (co-utilization)**

**But the enzymatic hydrolysis is favored in SHF (high T)**
So maybe a hybrid in between these is a good idea.
Increased time for EH increases overall ethanol yield.

Xylose conversion relatively low (about 40%).

Palmqvist and Liden, submitted.
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Recalcitrance in enzymatic hydrolysis

Inhibition in fermentation

<table>
<thead>
<tr>
<th>Soluble components</th>
<th>Rate</th>
<th>Yield</th>
<th>Product concentration</th>
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<tr>
<td>Sugars</td>
<td>Monomers</td>
<td>Total sugars</td>
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<tr>
<td>Glucose</td>
<td>2.5 g/L</td>
<td>14.2 g/L</td>
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<tr>
<td>Xylose</td>
<td>4.0 g/L</td>
<td>18.4 g/L</td>
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<td>Acetic Acid</td>
<td>5.6 g/L</td>
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<td>HMF</td>
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<tr>
<td>Furfural</td>
<td>0.2 g/L</td>
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</tr>
</tbody>
</table>

Acetic acid inhibition is pH-dependent.
Xylose fermentation is particularly inhibited by acetic acid

\[ \text{HAc} \leftrightarrow \text{H}^+ + \text{Ac}^- \]
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- Improved xylose conversion
- Higher EtOH
- Lower EtOH concentration!
• The reason behind these, at first surprising results, lies in the fact that a higher pH favors growth and glycerol production.

• However, the effect is different under the carbon starved conditions in the SSF in comparison to the hybrid process.
Conclusion

- In the fermentation everything matters! – feedstock, pretreatment, process design and process conditions
- With a judicious choice of process design and conditions, significant improvements – in both hydrolysis yields and fermentation yields - can be made on top of the improvements in enzyme performance or strain performance
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Partners WP3

- Lund University
- Biochemtex
- ENEA
- Novozymes
- TAURUS Energy

Provider of yeast
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 Arianna Giovannini
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**Xylose/Xylitol**

- Improved xylose conversion

**Cell growth**

- Improved growth

Synthetic model medium with 8 g/L HAc