Hydrolysis of biomass at high dry matter content: effect of different pretreatments and process strategies

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## ENEA’s role in the project

<table>
<thead>
<tr>
<th>PARTNER</th>
<th>WP1</th>
<th>WP2</th>
<th>WP3</th>
<th>WP4</th>
<th>WP5</th>
<th>WP6</th>
<th>WP7</th>
<th>WP8</th>
<th>WP9</th>
<th>WP10</th>
<th>WP11</th>
<th>WP12</th>
<th>WP13</th>
<th>WP14</th>
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<tbody>
<tr>
<td>ENEA</td>
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</tbody>
</table>

**WP1**: Selection, supply and pretreatment of feedstock

**WP2**: Selection enzymes cocktails

**WP3**: Fermentation technology

**WP4**: Basic design of high solid concentration enzymatic hydrolysis reactor

**WP5**: Basic design of SSF reactor

**WP6**: Preliminary design of complete industrial demo plant

**WP7**: Detail design of industrial demo plant

**WP8**: Purchase of equipment and material, plant construction and commissioning

**WP9**: Plant testing, monitoring, enzyme cocktail and microorganism supply

**WP10**: Product utilisation

**WP11**: Creation of functioning infrastructure

**WP12**: Integrated assessment of sustainability

**WP13**: Dissemination and exploitation

**WP14**: Coordination and IPR management
Outline on processes

• Biomass hydrolysis after two different pretreatments
• Biomass hydrolysis with several enzymatic mixtures at high biomass loading (*CTEC*, *CTEC2*, *NS22140*, *CTEC3*)
• Various process configurations, including biomass, and enzymes fed-batch
• Biomass hydrolysis during SSF
• Conclusions
Arundo donax contains significant percentages of C5 sugars. This implies the importance of selecting pretreatment conditions that minimizes the degradation of pentoses
Biomass pretreatments

ENEA’s pretreatment facilities

TARGET: High biomass destructuration+high C5 recovery

1. mild thermal conditions along with small amounts of acid catalysts (i.e. \( \text{SO}_2 \), \( \text{H}_2\text{SO}_4 \))
2. Chemtex process, two steps process: hemicellulose is separated before steam explosion
Composition of the slurry after the acid catalyzed steam explosion pretreatment

CONDITIONS: 200° 5’ 1.4% sulphuric acid

<table>
<thead>
<tr>
<th>Component</th>
<th>Value ± Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>glucose monomers%</td>
<td>0.29 ± 0.03</td>
</tr>
<tr>
<td>xylose monomers%</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>glucose soluble oligomers%</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>xylose soluble oligomers%</td>
<td>0.44 ± 0.03</td>
</tr>
<tr>
<td>insoluble glucose%</td>
<td>5.73 ± 0.38</td>
</tr>
<tr>
<td>insoluble xylose%</td>
<td>0.66 ± 0.05</td>
</tr>
<tr>
<td>acetic acid%</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>5HMF%</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>furfural%</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>lignin%</td>
<td>3.85 ± 0.2</td>
</tr>
<tr>
<td>H2O</td>
<td>~85 %</td>
</tr>
</tbody>
</table>

After the pretreatment the solids were separated from the liquid and then recombined up to the desired DM level.
# Chemtex products

## DOUBLE- STEPS CHEMTEX PRE-TREATMENTS CONDITIONS

<table>
<thead>
<tr>
<th>Code of product</th>
<th>Conditions during the hemicellulose separation</th>
<th>Steam explosion temperature [°C]</th>
<th>Steaming time [min]</th>
<th>logR0</th>
</tr>
</thead>
<tbody>
<tr>
<td>7610</td>
<td>-</td>
<td>198°C</td>
<td>4</td>
<td>3.49</td>
</tr>
<tr>
<td>5310</td>
<td>-</td>
<td>206°C</td>
<td>4</td>
<td>3.72</td>
</tr>
<tr>
<td>5610</td>
<td>+ water</td>
<td>198°C</td>
<td>4</td>
<td>3.49</td>
</tr>
<tr>
<td>5710</td>
<td>+ water</td>
<td>206°C</td>
<td>4</td>
<td>3.72</td>
</tr>
</tbody>
</table>
Hydrolizability of Arundo donax after different pretreatments

STEAM EXPLOSION IN BATCH DIGESTOR

S/L 2%; pH 4.8; T 40°C, CTEC 0.24g/g_{biomass}

ACID CATALYZED STEAM EXPLOSION IN BATCH DIGESTOR

HYDROLIZABILITY OF THE ARUNDO DONAX FIBER FROM THE TWO STEPS CHEMTEX PRETREATMENT
Summary on process assessments

- Biomass hydrolysis after two different pretreatments
- Biomass hydrolysis with several enzymatic mixtures at high biomass loading (*CTEC, CTEC2, NS22140, CTEC3*)
- Various process configurations, including biomass, and enzymes fed-batch
- Biomass hydrolysis during SSF
- Conclusions
Hydrolizability of *arundo donax* (washed fibers) from acid catalyzed steam pretreatment (enzymes mixture NS22140)

Composition of the substrate: Glucan: 53.91%, Xylan: 6.02%

Process conditions: T 50°C; pH 5; 0.17 g ENZYME /g GLUCAN

Process scale: 500 mL shaken flasks
Effect of the feeding strategy during hydrolysis at high DM content

Fed-batch processes avoid high viscosities→ poor mixing
Fed batch of biomass and enzymes ensure that the same specific activity is maintained

<table>
<thead>
<tr>
<th>Batch</th>
<th>Fed-batch fiber</th>
<th>Fed-batch fiber and enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber + ENZYME + hemicellulose</td>
<td>fiber ENZYME + hemicellulose</td>
<td>fiber hemicellulose ENZYME</td>
</tr>
</tbody>
</table>
Different feeding strategies produced different results depending on the substrate, and hydrolysis set-up (namely, enzymatic cocktail, pH, bioreactor geometry, etc).

Substrate: *Arundo* fiber CTX 5710 (206°C + water);
Process conditions: T 50°C; pH 5.5; 18% s/l; 0.27 g<sub>CTEC2</sub>/g<sub,GLUCAN</sub>

Substrate: *Arundo* donax slurry, from ENEA acid catalyzed SE pretreatment
Process conditions: T 50°C; pH 5; 30% s/l; 0.27 g<sub>ns22140</sub>/g<sub,GLUCAN</sub>
CTEC3 Performance

Hydrolysis of the cellulose rich fraction of *Arundo donax* pretreated by acid-catalyzed steam explosion and water-washed (ENEA batch plant).

Experimental setup
T: 50°; 20% s/L; pH 5
0.17g of enzyme/gram of biomass
Shaken flasks 500mL

ASSESSMENT OF THE MINIMUM CTEC3 DOSAGE
CTEC3 inhibition by soluble glucose

In a fed-batch process, hydrolysis of fresh biomass by enzymes can be affected by the amount of soluble products. The inhibition effect of soluble glucose was investigated during the hydrolysis.

Process conditions: T=50° pH5; CTEC3 0.11g/g glucan

Glucose was slightly inhibiting toward the hydrolysis of fresh biomass at 15% s/L and enzyme-to-glucose ratio of 0.15 g/g.

Higher glucose concentrations corresponding to 0.080 g/g enzyme-to-glucose ratio reduced the hydrolysis yield by 18% → product removal could reduce the enzymes inhibition.
At high DM content an effective mixing of the slurry could produce different process yields

Composition of the substrate: overall slurry from acid catalyzed steam explosion at 30% S/L  
Process conditions: T 50°C; pH 5; CTEC3 0.08 g$_{ENZYME}$/g$_{GLUCAN}$; fed-batch strategy

In vertical bioreactor, the addition of fresh biomass at 72 hours did not increase the glucose yield

Gravimetric shaking in rotating drum system (containing flasks) was much more effective. The final glucose yield was 80%

Bioreactor and impeller geometry are very important to improve the process yields
Hydrolysis by CTEC3 with product removal

TEST BY USING PRODUCT REMOVAL + GRAVIMETRIC SHAKING SYSTEM

Glucose yield at 88h: 80%

- Removal of part of the liquid hydrolyzate;
- Addition of hemicellulose fraction
- Addition of fresh biomass;
- No addition of further enzyme

Glucose concentration increased again: new Y 75%

Ctec3 maintained its activity in prolonged operations → recovery and reuse for several process batches.
Biomass liquefaction

**BIOLYFE PROJECT**

- Double-steps steam explosion pretreatment
- Enzymatic liquefaction at high dry matter level
- Simultaneous saccharification and cofermentation (SSF) with different microorganisms

*Arundo donax*
Hydrolizability of the product from CTXI viscosity reduction step

Conditions: 50° pH5
500mL shaken flasks
Enzyme: CTEC3

- The activity of the original enzymes can be reduced by various handling steps
- Need to adding fresh CTEC3 to enhance the cellulose conversion
Simultaneous saccharification and fermentation of pre-liquefied biomass

YEASTS FOR FERMENTATION TESTS

- Saccharomyces cerevisiae (SIGMA II)
- Saccharomyces cerevisiae M861 (isolated by ENEA and alcohol tolerant)
- Kluyveromyces marxianus 6271 (DBVPG collection)
- Kluyveromyces marxianus 6858 (DBVPG collection)
Two SSF set-ups were evaluated to *increase the cellulose hydrolysis*:
1. SSF at common process conditions
2. SSF with intermittent step-wise increase of temperature
Typical cells viability during SSF with modulation of temperature

Cells viability of SIGMA II as function of the process strategy

- SIGMA II batch
- SIGMA II fed-batch
Traditional SSF

Substrate: product from the CHEMTEX viscosity reduction step
Conditions: 32° pH5
500mL shaken flasks
Enzyme: CTEC3 0.23g/g of residual glucan
Yeast seeding: 4g/L
Traditional SSF compared to SSF with the temperature modulation

K. marxianus strains

S. cerevisiae strains

k. marxianus 6271

SIGMA II

k marx. 6858

M861
The maximum ethanol yields from glucose was 80% by using 0.22 g ENZYME/g glucan (46 g/L). The process yields at higher temperature and lower enzymes loading were lower (36 g/L).

Hydrolysis of residual cellulose in the hybrid SSF process is the bottle neck of the process. ~2.5 times more enzyme than in the hydrolysis tests at optimized conditions (T 50°C) is required to achieve comparable process yields around 80%.

<table>
<thead>
<tr>
<th>yeast</th>
<th>temperature</th>
<th>pH</th>
<th>g CTEC3/g residual glucan</th>
<th>ethanol yield%</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIGMA II</td>
<td>32°</td>
<td>5</td>
<td>0,22</td>
<td>80</td>
</tr>
<tr>
<td>SIGMA II</td>
<td>32°</td>
<td>5</td>
<td>0,09</td>
<td>55</td>
</tr>
<tr>
<td>SIGMA II</td>
<td>37°</td>
<td>5</td>
<td>0,09</td>
<td>61</td>
</tr>
</tbody>
</table>
Conclusions

1. ACSEP at 200°C and the Chemtex process in which the SE step was carried out at 206 and 4 min had similar hydrolizability.

2. CTEC3 is 1.5 more effective than CTEC2 and NS22140.

3. Hydrolysis at high DM content (~30%) by using 80 mgCTEC3/g GLUCAN produced 80% glucose yields (GLU+XYL=137 g/L).

4. Optimization of the process strategy includes fed-batch feeding of biomass and enzymes along with optimized mixing conditions.

5. Inhibition of enzymes by glucose was observed at 100 g/L glucose and enzymes-to-glucose ratio of 0.08 g/g.

6. Enzymes adsorption on fresh biomass and product removal enables the enzymes recovery and reuse.

7. ~2.5 times more enzyme than in the hydrolysis tests at optimized conditions (T 50°C) is required to achieve comparable SSF process yields of 80%.

8. Hydrolysis of residual cellulose in the hybrid SSF process is the bottle neck of the process.
Thanks for the attention

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