Integrated bioethanol and protein production from brown seaweed
*Laminaria digitata*

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Content

- Process Overview
- Ethanol production
- Concentrated Protein
- Highlights of WP3
- Future improvement
Laminaria digitata
(wild growing, harvested at Hamborg strand, north of Hanstholm at the Danish North Sea coast, in August 2012)

<table>
<thead>
<tr>
<th>Glucose (% DM)</th>
<th>Protein (% DM)</th>
<th>Lipid (%DM)</th>
<th>Mannitol (% DM)</th>
<th>Ash (% DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>56.7</td>
<td>3.8</td>
<td>0.5</td>
<td>6.5</td>
<td>8.3</td>
</tr>
</tbody>
</table>

Photo from: Annette Bruhn, AU
Process: Integrated Bioethanol and Protein Production

- **Wet seaweed biomass**
  - Conditioning
  - Pretreatment
  - Enzymatic hydrolysis
  - C6-Fermentation
  - Separation of fermented broth

**Inputs:**
- 100 kg *Laminaria digitata* (harvested on Aug 2012)
- 16.4 kg Glucose
- 1.1 kg Protein

**Outputs:**
- 8.3 kg Ethanol (theoretical maximum)
- Protein rich solid
- Liquid for distillation of Ethanol

**Quantities:**
- 100 kg *Laminaria digitata*
- 16.4 kg Glucose
- 1.1 kg Protein
- 8.3 kg Ethanol
Ethanol production

**Strategy I: SHF**
Optimal conditions for E & F

- **Wet Seaweed**
- **Drying**
- **Milling**
- **Re-adjusting DM**

- Dried in a Lytzen Oven at 50°C for 4 nights
- Grinded in a Fitzpatrick DAS06 comminatory knife mill

**Enzymatic Hydrolysis**

**Fermentation**

**Strategy II: SSF**
Compromise condition for E & F

**Enzymatic Hydrolysis & Fermentation**
Figure 1 Time course of glucose release during enzymatic hydrolysis of L. digitata, by CelluClast 1.5L and/or Alginate lyase at different concentrations.
## Ethanol production, SHF vs SSF

<table>
<thead>
<tr>
<th>Fermentation strategy</th>
<th>SHF</th>
<th>SSF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Substrate DM (% w/v)</strong></td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td><strong>Final ethanol concentration (g/L)</strong></td>
<td>11.3 ± 0.2</td>
<td>20.7 ± 0.5</td>
</tr>
<tr>
<td><strong>Ethanol yield (% theoretical)</strong></td>
<td>77.7 ± 0.9</td>
<td>70.6 ± 1.8</td>
</tr>
<tr>
<td><strong>Left glucose in the fermented substrate (g/L)</strong></td>
<td>0.7 ± 0.2</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td><strong>Hydrolysis efficiency (%)</strong></td>
<td>84.1 ± 4.6</td>
<td>72.7 ± 1.4</td>
</tr>
</tbody>
</table>

**SHF:** Hydrolysis at 50°C, 250 rpm, Fermentation at 30°C, 120 rpm

**SSF:** Hydrolysis and Fermentation at 32°C, 150 rpm
# Ethanol Production, SHF

<table>
<thead>
<tr>
<th>Substrate DM (% w/v)</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final ethanol concentration (g/L)</td>
<td>11.3 ± 0.2</td>
<td>20.7 ± 0.5</td>
<td>27.6 ± 0.1</td>
<td>30.3 ± 0.6</td>
</tr>
<tr>
<td>Ethanol yield (% theoretical)</td>
<td>77.7 ± 0.9</td>
<td>70.6 ± 1.8</td>
<td>63.4 ± 0.1</td>
<td>52.0 ± 0.1</td>
</tr>
<tr>
<td>Left glucose in the fermented substrate (g/L)</td>
<td>0.7 ± 0.2</td>
<td>2.6 ± 0.2</td>
<td>5.9 ± 0.2</td>
<td>10.6 ± 1.0</td>
</tr>
<tr>
<td>Hydrolysis efficiency (%)</td>
<td>84.1 ± 4.6</td>
<td>72.7 ± 1.4</td>
<td>72.3 ± 2.1</td>
<td>70.5 ± 3.6</td>
</tr>
</tbody>
</table>
Figure 2 Time course of SHF fermentation of 5% DM L. digitata, in 5L reactor with 3 L working volume. S. cerevisiae were inoculated at 48 hours. Horizontal dashed line indicated the ethanol concentration at 100% theoretical yield.

78% glucose recovery

100% theoretical ethanol yield

70% theoretical ethanol yield
CONCENTRATED PROTEIN

Total crude protein: 3.8 g/100g

Figure 3 Amino acid profile in Laminaria digitata harvested in Aug 2012

Solid residue recovery: 26%
Protein recovery: 97%

Total crude protein: 14.2 g/100g

Figure 4 Amino acid profile in the solid residue after fermentation

Methionine: 2% in this L. digitata
âˆ¼1% in normal soybean
Highlights

- Glucose recovery (on dried milled material: 84.1%)
- Overall ethanol yield: 77.7%
- Crude algal protein content was concentrated by 2.7 fold
- This algal protein has relatively high content of methionine
100 kg Laminaria digitata (harvested on Aug 2012)

29 kg dry weight

16.4 kg

13.8 kg Glucose

1.1 kg Protein

6.5 kg Ethanol

29 kg dry weight

Protein rich solid

Liquid for distillation of Ethanol

8.3 kg Ethanol (theoretical)
Future improvement

- High DM fermentation (e.g. Fed-batch; Strain improvement)

- More refinery paths (e.g. fucodian extraction before fermentation)

- Adapt the process to other products (e.g. butanol and other value-added products)
Thanks to Danish Council for Strategic Research for financing the project

Thank you all for your attention!