Seaweed biorefinery: production of fuels and chemicals from native North Sea seaweed species

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Outline

- The ‘Seaweed Biorefinery’ project: general description
- Composition of seaweed species for biorefinery
- Biorefinery of green seaweeds (local *Ulva lactuca*)
- Biorefinery of brown seaweed species:
  - *Saccharina latissima* as model feedstock
    - mannitol and alginate extraction
    - fermentation of mannitol/glucose to acetone, butanol and ethanol
Seaweed Biorefinery project

Aim: Development of biorefinery technologies for chemicals and fuels production

- Biochemical and chemical conversion of sugars
- Valorization remaining fractions: proteins, minerals, residues
- Design, economic evaluation, LCA

Project coordinator: ECN (Dr Jaap van Hal)
Other partners: PRI-WUR, ATO, Ocean Harvest, Process Groningen
http://seaweed.biorefinery.nl
Seaweed Biorefinery

North Sea seaweeds as feedstock for Biorefinery:

High density cultivation techniques, near- and offshore, under development

Chemical composition: sugars (for fermentation, chemistry, digestion), uronic acids (for chemistry), protein (food, feed), minerals (fertilizer, P). No lignin.

Laminaria digitata\(^2\)  Saccharina latissima\(^1\)  Palmaria palmata (dulse)\(^1\)  Ulva lactuca (Sea lettuce)

\(^1\) Source photo: www.seaweed.ie © M.D. Guiry
\(^2\) Source photo: www.seaweeds.uib.no
## Composition of seaweed species

<table>
<thead>
<tr>
<th></th>
<th>Laminaria digitata</th>
<th>Saccharina latissima</th>
<th>Palmaria palmata</th>
<th>Ulva lactuca</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Harvest month</strong></td>
<td>June</td>
<td>July</td>
<td>March</td>
<td>February</td>
</tr>
<tr>
<td><strong>Sugars</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total sugars, % d.m.</td>
<td>14.5</td>
<td>17.6</td>
<td>40.5</td>
<td>11.3</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.9</td>
<td>6.6</td>
<td>3.8</td>
<td>5.4</td>
</tr>
<tr>
<td>Xylose</td>
<td>0.4</td>
<td>0.2</td>
<td>31.1</td>
<td>1.3</td>
</tr>
<tr>
<td>Fucose</td>
<td>1.9</td>
<td>1.6</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Mannose</td>
<td>1.9</td>
<td>0.3</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Arabinose</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.7</td>
<td>0.8</td>
<td>5.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>0.1</td>
<td>0.1</td>
<td>0.0</td>
<td>4.1</td>
</tr>
<tr>
<td>Mannitol</td>
<td>3.6</td>
<td>8.1</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Total water extrac. % d.m.</strong></td>
<td>25.2 (no mono-)</td>
<td>47.9 (mannitol)</td>
<td>32.2 (no mono-)</td>
<td>38.3 (no mono-)</td>
</tr>
<tr>
<td>Solvent extract. % d.m.</td>
<td>4.7</td>
<td>9.6</td>
<td>8.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Uronic acids, % d.m.</td>
<td>7.3</td>
<td>7.6</td>
<td>--</td>
<td>4.9</td>
</tr>
<tr>
<td>Ash (550°C) % d.m.</td>
<td>27</td>
<td>36.3</td>
<td>19</td>
<td>19.4</td>
</tr>
<tr>
<td>Protein, % d.m. (Kjeldahl)</td>
<td>10.8</td>
<td>12.4</td>
<td>17.8</td>
<td>23.5</td>
</tr>
</tbody>
</table>
**Pre-treatment and saccharification of *Ulva lactuca***

*Ulva lactuca* was harvested in Zeeland (NL), freeze-dried, and milled (2mm)

- **Pre-treatments at small scale:**
  - 150°C, pH 2 (set with H$_2$SO$_4$), 10 min
  - 150°C, water, 10 min
  - 85°C, 6% NaOH (g/g DM *Ulva*) 4 hours
  - 85°C, water, 4 hours

- **Enzymatic saccharification:** GC220*, 96 h, 50°C

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*GC220 = cellulase enzyme by Genencore*
Pre-treatment and saccharification of *Ulva lactuca*

![Graph showing the solubility of different sugars (rhamnose, galactose, glucose, xylose) by pretreatment and enzymes from remaining pellet at 85°C and 150°C.]

- **85°C**
  - Sodium hydroxide (NaOH)
  - 100%

- **150°C**
  - Sulfuric acid (H2SO4)
  - 100%
Fermentation of *Ulva lactuca* hydrolysate to acetone, butanol and ethanol (ABE)

**Preparation of hydrolysate:**

- 150°C, water, 10 min
- Enzymatic saccharification: GC220, 50°C, 24h
- Starting material, 15% d.m. slurry, sugar yield 75%

<table>
<thead>
<tr>
<th>Sugar</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>8.4</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>8.1</td>
</tr>
<tr>
<td>Xylose</td>
<td>5.2</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Total sugars in hydrolysate.
Fermentation of *Ulva lactuca* hydrolysate to ABE by *Clostridium beijerinckii*

1,2-propanediol found in low (0.2 g/L) concentration
Fermentation of *Ulva lactuca* hydrolysate

### Fermentation of hydrolysate (H) and control cultures by *C. beijerinckii*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Control G/R</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sugars at t=0h (g/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>54.8</td>
<td>19.2</td>
<td>7.8</td>
</tr>
<tr>
<td>Xylose</td>
<td>1.9</td>
<td>0.8</td>
<td>2.4</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>36.7</td>
<td></td>
<td>5.5</td>
</tr>
<tr>
<td>Total</td>
<td>56.6</td>
<td>56.6</td>
<td>15.7</td>
</tr>
<tr>
<td><strong>Sugars at t= 140 h (g/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>17.1</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Xylose</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>10.2</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17.1</td>
<td>10.2</td>
<td>2.8</td>
</tr>
<tr>
<td><strong>Products at t=140h (g/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.4</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>0.0</td>
<td>0.4</td>
<td>1.3</td>
</tr>
<tr>
<td>1,2-propanediol</td>
<td>nd</td>
<td>9.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Acetone</td>
<td>3.7</td>
<td>4.1</td>
<td>1.3</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.5</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Butanol</td>
<td>10.7</td>
<td>6.9</td>
<td>3.0</td>
</tr>
<tr>
<td>Total ABE</td>
<td>14.8</td>
<td>11.3</td>
<td>4.5</td>
</tr>
<tr>
<td>Yield (g ABE/g sugar)</td>
<td>0.37</td>
<td>0.24</td>
<td>0.35</td>
</tr>
</tbody>
</table>

1,2-PD route from deoxy-sugars:

- **L-Rhamnose**
  - Rhamnose isomerase
  - **L-Rhamnulose**
    - Rhamnulokinase
    - **Rha-1-P**
      - Aldolase
      - Lactaldehyde
      - DHAP
      - Oxidoreductase
      - 1,2-Propanediol

Ref: Van der Werken *et al* (2008), Bennett & San (2001)
North Sea seaweed species have been biochemically characterised. Wide diversity in composition is observed.

Sugars in the green seaweed *Ulva lactuca* were solubilised at high yields (> 90%, small scale) using mild pretreatment (with no addition of chemicals) and enzymatic hydrolysis.

Sugars in hydrolysate were fermentable by *C. beijerinckii*, resulting in ABE and 1,2-PD formation.

*C. beijerinckii* utilized rhamnose, and in control cultures with high concentration of this sugar, 1,2-propanediol (9.7 g/L) was produced.
Biorefinery of brown seaweeds

Saccharina latissima (June), fresh

Press liquid

Brown seaweed

Chopping & pressing

Press cake

Extraction

Mannitol

Fermentation

Butanol

Dehydration

Isomannide

Hydrolysis Oxidation Dehydration

2,5-FDCA

Hydrolysis

Extraction residue

Sugars

Fermentation

ABE
Biorefinery of brown seaweeds

Chopping & Pressing: *Saccharina latissima, freshly harvested*

- Cutting of fresh seaweed using a guillotine chopper
- Pressing of cut seaweed using an expeller (oil press).

Press cake:
- 70 % of initial weight
- 17% d.m

Press liquid:
- 22 % of initial weight
- 12 % d.m
- 16 g/L mannitol
Extraction and purification of mannitol

- **Feedstock**: press juice of *Saccharina latissima*: 16 g / L of mannitol, 1 g/L glucose

- **Procedure**:
  - drying, followed by extensive Soxhlet extraction with methanol
  - mannitol slowly precipitates in the extract
  - yield of pure white mannitol: 70% (based on 16 g / L in press juice)
Biorefinery of brown seaweeds

- $^{13}$C NMR spectrum (DMSO-d6):

pure mannitol from 
*S. latissima*
Biorefinery of brown seaweeds

Extraction of alginate from *Saccharina latissima* press cake

- Acidified press cake (160 g) suspended in 4% Na$_2$CO$_3$ (800 mL) for alginate extraction → increasing viscosity. Centrifugation, supernatant stored overnight at 4°C
- Addition of H$_2$SO$_4$ to alginate solution → gel
- Gel was filtered through cheese cloth → wet alginic acid (light brown)
- Alginic acid dried in oven overnight → 5.5 g of brown solid (residual acid?)
- Product needs to be identified as alginic acid

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1 Vauchel et al. *Food Bioprocess Technol* 1, 297–300 (2008)
Biorefinery of brown seaweeds

- Conversion of alginate to bio-based chemical building blocks

- Challenge: alginites are notoriously difficult to fully hydrolyse to monomeric uronic acids
Fermentation of *Saccharina latissima* fractions

Very viscous
- Mannitol 16 g/L
- Glucose 1 g/L

Approx. 70% of original seaweed (17% d.m.)
Hydrolysis of sugar polymers
Fermentation of *Saccharina latissima* fractions

Mannitol and glucose/mannitol mixes fermentation by *C. beijerinckii* to acetone, butanol and ethanol (ABE)

![Graph showing consumption of glucose/mannitol by C. beijerinckii](image)

![Graph showing production of ABE from glucose/mannitol by C. beijerinckii](image)
Fermentation of *Saccharina latissima* fractions

Fermentation of press cake (PC) to ABE by *C. beijerinckii*

<table>
<thead>
<tr>
<th>Products (g/L)</th>
<th>PC hydrolysate</th>
<th>PC Hydrolysate 2x dil.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABE</td>
<td>0 (no growth)</td>
<td>3.8</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>0</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Toxicity of the hydrolysate may be due to:

- High salt concentration?
  - in hydrolysate, conductivity approx. 20x higher than in control medium.
- Other?
Because of diversity, a species-dependent approach to biorefinery needs to be defined for seaweeds.

A biorefinery approach for brown seaweeds has been defined. Steps in which conversion of different fractions into valuable chemicals and energy carriers have been studied:

- Mannitol in press liquid has been purified
- Alginic acid from press cake has been isolated
- Fermentation of mannitol and mannitol/glucose mixtures to ABE has been screened
- Fermentation of sugar fractions to ABE has been performed
Agknowledgements

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Thank you!