Ensiling of seaweed and conservation of sugars for biofuel production

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Outline

• The MacroFuels project

• Reasons and aims for ensiling of seaweed

• Experiments with ensiling of *Saccharina latissima*
  • Biological ensiling
  • Chemical ensiling
The MacroFuels project

- Horizon project
- 6.3 million €
- January 2016 – December 2019
- 11 partners, from 6 countries

Objective: MacroFuels aims to develop technologies to produce advanced liquid transportation biofuels from seaweed, including ethanol, butanol, furanics and biogas.
The MacroFuels project

Various objectives along the value chain, including methods for conditioning, pre-treatment and storage of harvested seaweed.
Reasons and aims for ensiling seaweed

Ensiling allows conservation of wet biomass by decreasing pH to around 4.0. This can be achieved by:

• ‘Biological ensiling’: Production of organic acids by bacteria
  (e.g. Herrmann et al., 2015)
• ‘Chemical ensiling’: Addition of organic of inorganic acids
  (e.g. Sandbakken et al., 2018)

Energetically, ensiling may be advantageous compared to drying

Herrmann et al. 2015, Bioresource Technology, 196, 301-313
Biological ensiling / ‘ensilability’ of a biomass is generally improved by (Pahlow et al. 2002):

- Higher dry matter content (DM)
- Higher content of water soluble carbohydrates (WSC)
- Lower buffering capacity (BC)

Seaweeds are often low in DM, low in WSC and high in BC! And may be lacking lactic acid bacteria (Herrmann et al., 2015)

Therefore: Successful ensiling of seaweed may be a challenge!
Reasons and aims for ensiling seaweed

Various silage fermentation pathways – with different substrates, products and losses

How much sugar is conserved for e.g. ethanol fermentation?

<table>
<thead>
<tr>
<th>Organism</th>
<th>Substrate</th>
<th>Products</th>
<th>Loss (% substrate)</th>
<th>DM</th>
<th>Gross energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAB</td>
<td>Glucose</td>
<td>2 lactate</td>
<td>0</td>
<td>0</td>
<td>0.7</td>
</tr>
<tr>
<td>LAB</td>
<td>Glucose</td>
<td>1 lactate, 1 ethanol, 1 CO₂</td>
<td>24</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>LAB</td>
<td>3 Fructose</td>
<td>1 lactate, 1 acetate, 2 mannitol, 1 CO₂</td>
<td>4.8</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>LAB</td>
<td>2 Citrate</td>
<td>1 lactate, 3 acetate, 3 CO₂</td>
<td>29.7</td>
<td>-1.5</td>
<td></td>
</tr>
<tr>
<td>LAB</td>
<td>Malate</td>
<td>1 lactate, 1 CO₂</td>
<td>32.8</td>
<td>-1.8</td>
<td></td>
</tr>
<tr>
<td>Enterobacteria</td>
<td>2 Glucose</td>
<td>2 lactate, 1 acetate, 1 ethanol, 2 CO₂</td>
<td>17</td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td>Clostridia</td>
<td>2 Lactate</td>
<td>1 butyrate, 2 CO₂, 2 H₂</td>
<td>51.1</td>
<td>18.4</td>
<td></td>
</tr>
<tr>
<td>Yeasts</td>
<td>Glucose</td>
<td>2 ethanol, 2 CO₂</td>
<td>48.9</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

# General procedure for lab-scale ensiling

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Frozen <em>Saccharina latissima</em></td>
</tr>
<tr>
<td>2.</td>
<td>Drain collected after thawing</td>
</tr>
<tr>
<td>3.</td>
<td>The solid fraction chopped</td>
</tr>
<tr>
<td>4.</td>
<td>Solid and liquid fractions mixed to achieve composition as in fresh material</td>
</tr>
</tbody>
</table>
General procedure for lab-scale ensiling

5. Additives added into bags with 50 g of biomass
6. Additives mixed with biomass
7. Initial pH measured in the bag
General procedure for lab-scale ensiling

8. Vacuum packing in at least two layers of vacuum bags

9. Storage at 20°C and freezing after pre-planned ensiling time for later analysis

Duplicates or triplicates
Destructive sampling
Biological ensiling of *Saccharina latissima*
Biological ensiling

Sugar additive:
• Sugar beet molasses (43% sucrose)
• General dose: 24 g/kg FM = 10.3 g sucrose per kg FM
  ($\approx$ 100 g sucrose per kg DM at a DM content of 10 %)

Inoculum additive:
• Lactic acid bacteria culture SiloSolve® MC (3 LAB species)
Biological ensiling

Poor ensiling without additives
Lack of inoculum and especially WSC appears to limit ensiling

Letters indicate LSD groups (P<0.05)

S. latissima from ORF, July 2016
Biological ensiling

Final pH in silage decreases with the dose of molasses – up to a certain ‘saturation’ point

≈97 g sucrose per kg DM

*S. latissima* from ORF, July 2016
Biological ensiling

Significant differences in ensilability between different sources of *S. latissima*

Harvest dates: ORF 13th July 2016, SAMS 12th June 2018, AU 26th June 2018

Letters indicate LSD groups (P<0.05)

Initial pH 5.9-6.7

<table>
<thead>
<tr>
<th>Biomass source and DM content</th>
<th>10.7 % DM</th>
<th>12.1 % DM</th>
<th>20.9 % DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORF</td>
<td>a</td>
<td>d</td>
<td>e</td>
</tr>
<tr>
<td>SAMS</td>
<td>b</td>
<td>c</td>
<td>e</td>
</tr>
<tr>
<td>AU</td>
<td></td>
<td></td>
<td>e</td>
</tr>
</tbody>
</table>

Initial pH: 5.9-6.7

3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0

10.7 % DM 12.1 % DM 20.9 % DM

Harvest dates: ORF 13th July 2016, SAMS 12th June 2018, AU 26th June 2018

Letters indicate LSD groups (P<0.05)
Biological ensiling

pH may be related to initial concentration of laminarin/glucose
But may also be related to differences in buffering capacity etc.

Laminarin - storage glucan linked by β-1,6 and β-1,3 bonds

Harvest dates: SAMS 12th June 2018, AU 26th June 2018

Error bars indicate standard deviation of two replicates
Biological ensiling

Significant consumption of glucose during ensiling
Addition of molasses compensates for this (85 g sucrose/kg DM)

Harvest dates: SAMS 12th June 2018, AU 26th June 2018

Letters indicate LSD groups (P<0.05)

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Glucose concentration (% in DM)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Before ensiling</th>
<th>After 28 days ensiling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated SAMS</td>
<td>234 g/kg DM</td>
<td>70 g/kg</td>
</tr>
<tr>
<td>Molasses + inoculum SAMS</td>
<td>234 g/kg DM</td>
<td>70 g/kg</td>
</tr>
<tr>
<td>Untreated AU</td>
<td></td>
<td>234 g/kg DM</td>
</tr>
</tbody>
</table>

Harvest dates: SAMS 12th June 2018, AU 26th June 2018

Letters indicate LSD groups (P<0.05)

www.macrofuels.eu
Biological ensiling

No consumption of mannitol during ensiling

Harvest dates: SAMS 12\textsuperscript{th} June 2018, AU 26\textsuperscript{th} June 2018

Letters indicate LSD groups (P<0.05)

<table>
<thead>
<tr>
<th>Biomass and additive treatment</th>
<th>Before ensiling</th>
<th>After 28 days ensiling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated SAMS</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Molasses + inoculum SAMS</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>Untreated AU</td>
<td>c</td>
<td>c</td>
</tr>
</tbody>
</table>
Chemical ensiling of *Saccharina latissima*
Chemical ensiling

Lactic acid added to an initial pH of 3.5, 4.0 and 4.5 (10 minutes)
Initial measurements of pH may not represent later pH levels

Letters indicate LSD groups within ensiling times (P<0.05)

*S. latissima* from ORF, July 2016
Chemical ensiling

Clear relationship between lactic acid dose and pH after 28 days
But the relationship may differ between biomasses (BC, fouling)

\[ y = -0.319x + 6.2 \]
\[ P_{slope} < 0.001 \]
Long-term ensiling of *Saccharina latissima*
Long-term effects of ensiling

Does pH gradually increase when using lactic acid?
Will be continued up to one year of ensiling

![Graph showing pH changes over ensiling time for different treatments.](https://www.macrofuels.eu)

*S. latissima* from ORF, July 2016
Long-term effects of ensiling

Some mass loss - but DM content and composition also relevant
Should be verified at larger scale

S. latissima from ORF, July 2016
Ensiling of fresh versus frozen seaweed

Freezing may reduce microbial activity (Sandbakken et al. 2018)
Confirmed – but inoculum may counteract the effect

Initial pH 6.1-6.4

Additive treatment

Untreated    Molasses    Inoculum    Molasses+Inoculum

Additive treatment

Letters indicate LSD groups (P<0.05)

S. latissima from ORF, 15th May 2018
Verification at larger scale needed

Ensiling experiment in barrels started June 2018 at SAMS, Scotland

Chopping of seaweed

Addition of sucrose and inoculum to chopped seaweed in 60 L barrels

Photos by SAMS
Conclusions

- Ensiling is interesting for conservation of wet seaweed
  - But: Seaweed biomasses differ in ensilability

- Successful ensiling may require additives
  - Fermentable sugars, inoculum and/or acids

- Desirable with a ‘predictor’ for the need for additives

- Ensiling consumes glucose/laminarin

- Economy and downstream processing may determine the optimal additives for ensiling
Acknowledgements

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