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

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Seagriculture, 19th September 2012

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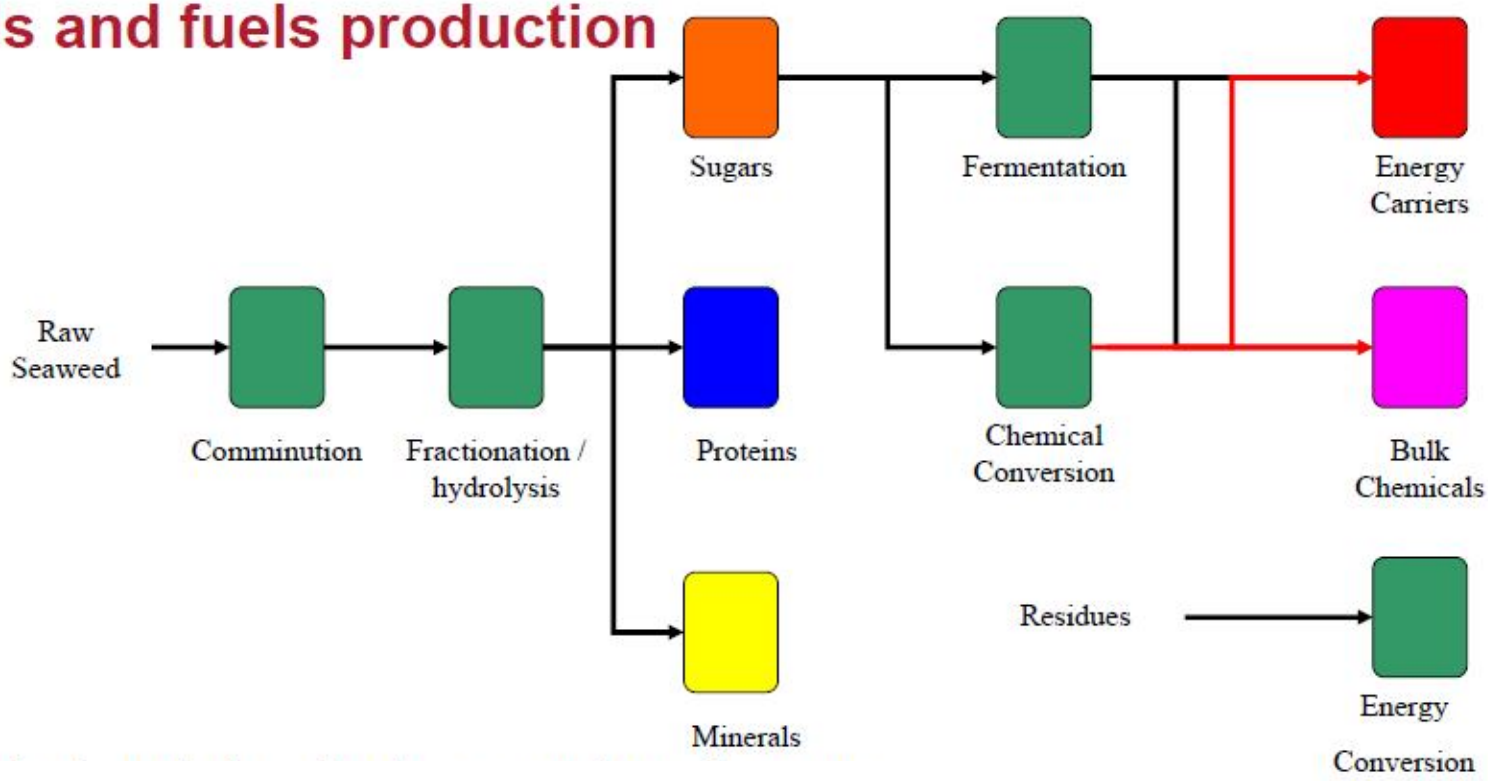
Outline

- 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

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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)**

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[1] Source photo: www.seaweed.ie © M.D. Guiry
[2] Source photo: www.seaweeds.uib.no

Composition of seaweed species

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

Pre-treatment and saccharification of *Ulva lactuca*

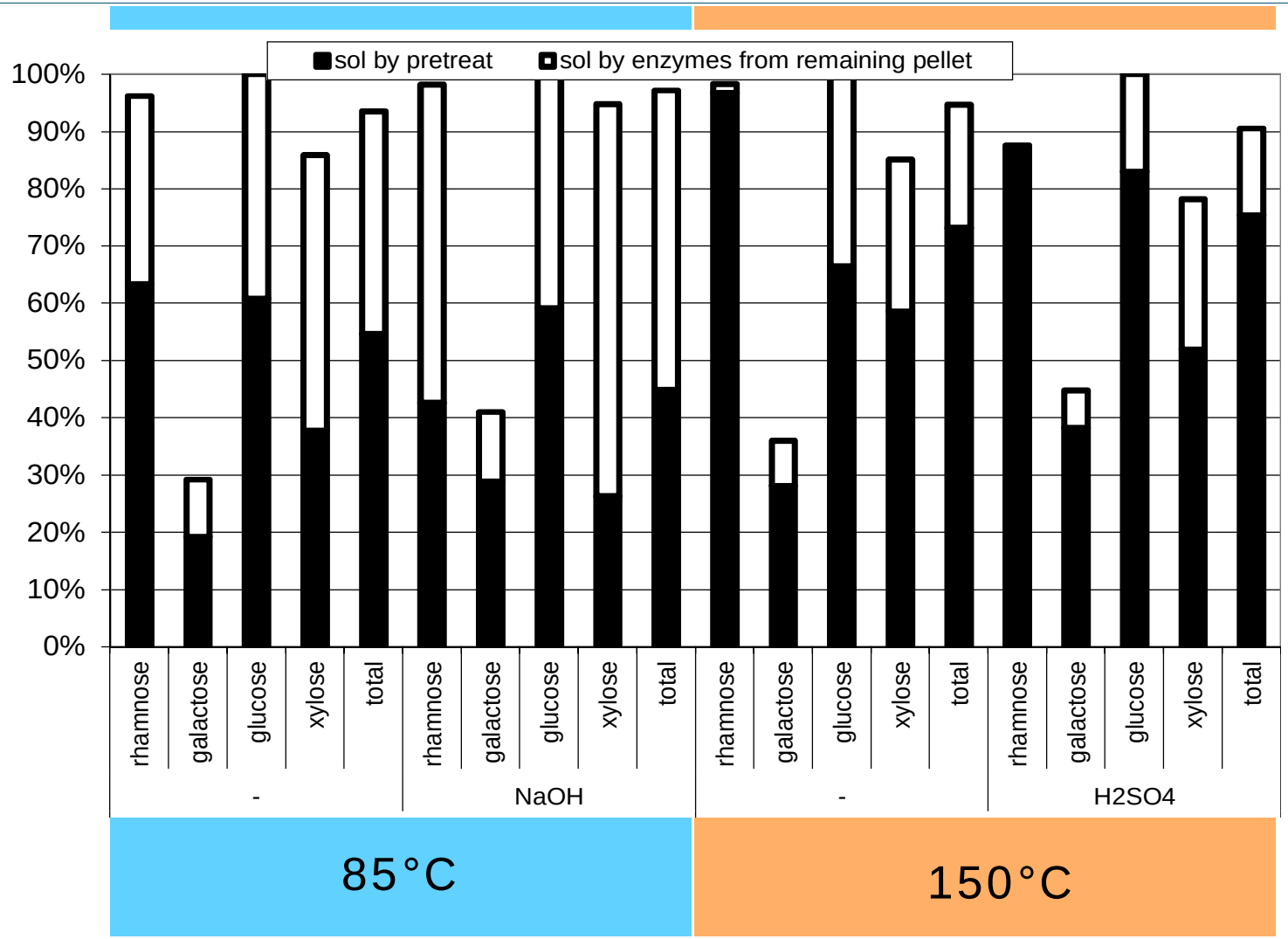
Ulva lactuca was harvested in Zeeland (NL), freeze-dried, and milled (2mm)

- Pre-treatments at small scale:
  - 150°C, pH 2 (set with H_2SO_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

**GC220 = cellulase enzyme by Genencore*

Pre-treatment and saccharification of *Ulva lactuca*



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Fermentation of *Ulva lactuca* hydrolysate to acetone, butanol and ethanol (ABE)

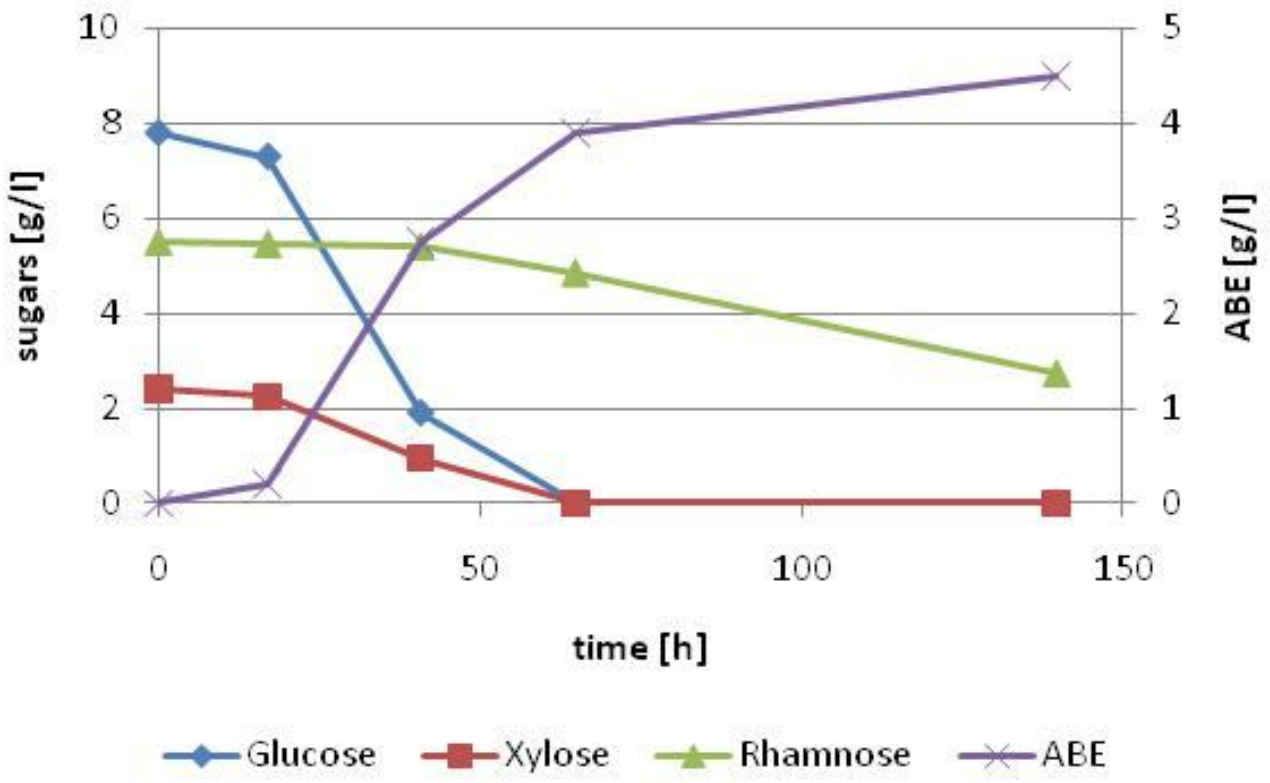
Preparation of hydrolysate:

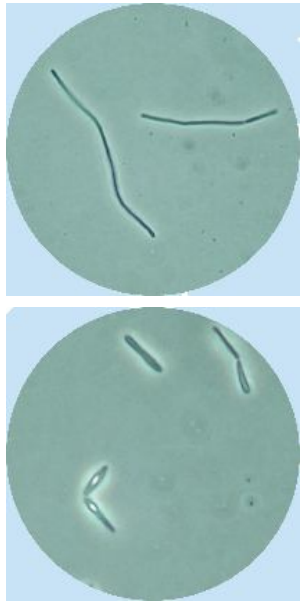
- 150°C, water, 10 min
- Enzymatic saccharification: GC220, 50°C, 24h
- Starting material, 15% d.m. slurry, sugar yield 75%

Total sugars in hydrolysate.

Sugar	g/L
Glucose	8.4
Rhamnose	8.1
Xylose	5.2
Galactose	0.7

Fermentation of *Ulva lactuca* hydrolysate to ABE by *Clostridium beijerinckii*





1,2-propanediol found in low (0.2 g/L) concentration

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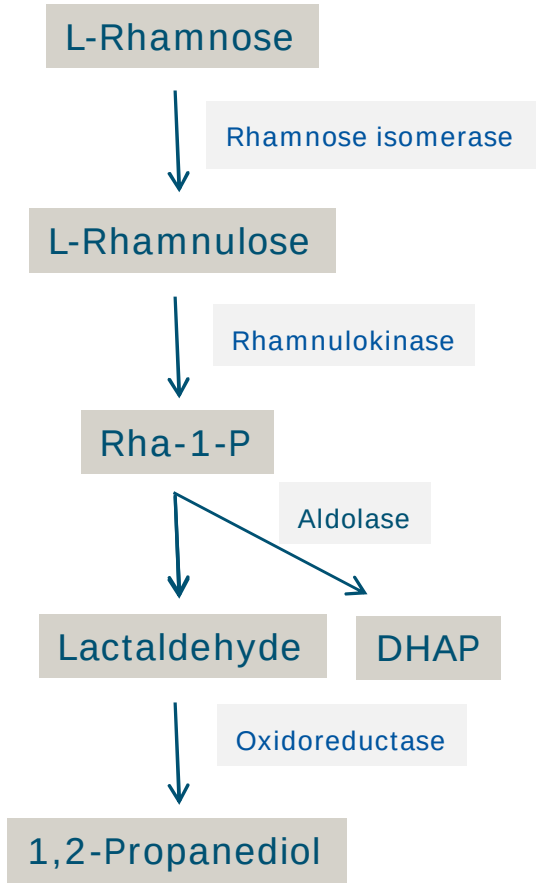
Fermentation of *Ulva lactuca* hydrolysate

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

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

1,2-PD route from deoxy-sugars



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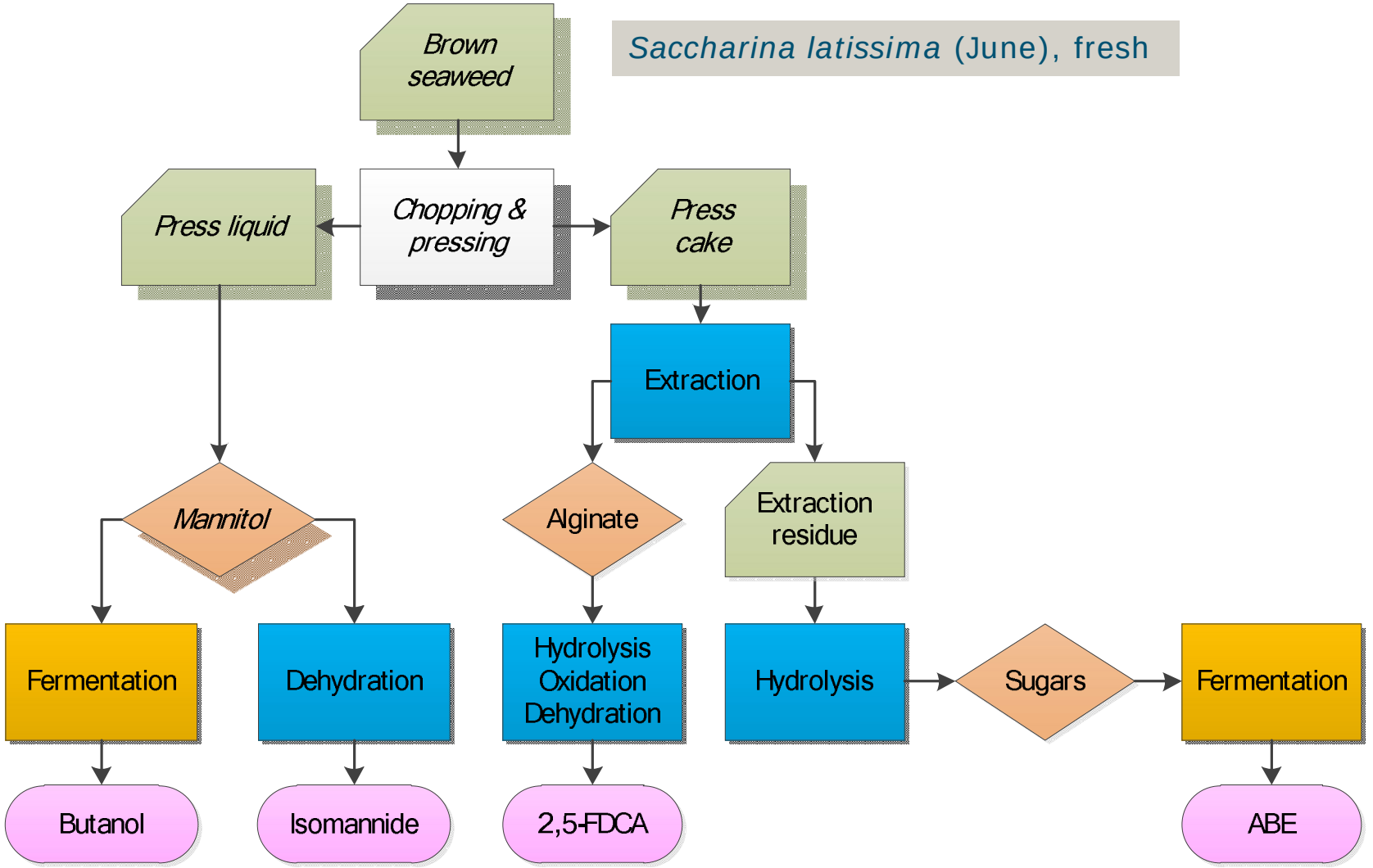
Ref:Van der Werken *et al* (2008), Bennett & San (2001)

Summary & conclusions (I)

- 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. beijerinkii*, resulting in ABE and 1,2-PD formation.
- *C. beijrerinckii* utilized rhamnose, and in control cultures with high concentration of this sugar, 1,2-propanediol (9.7 g/L) was produced.

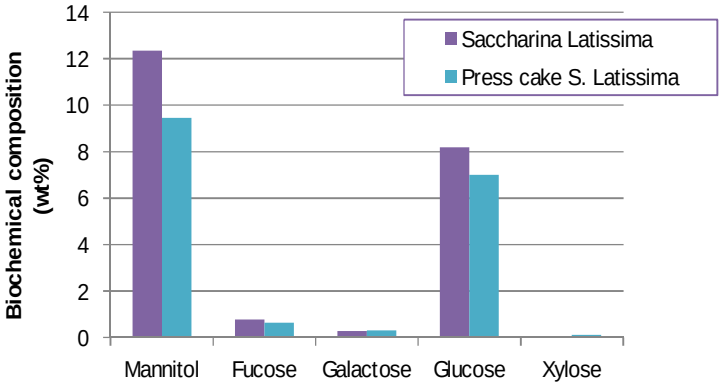
Biorefinery of brown seaweeds



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

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Biorefinery of brown seaweeds

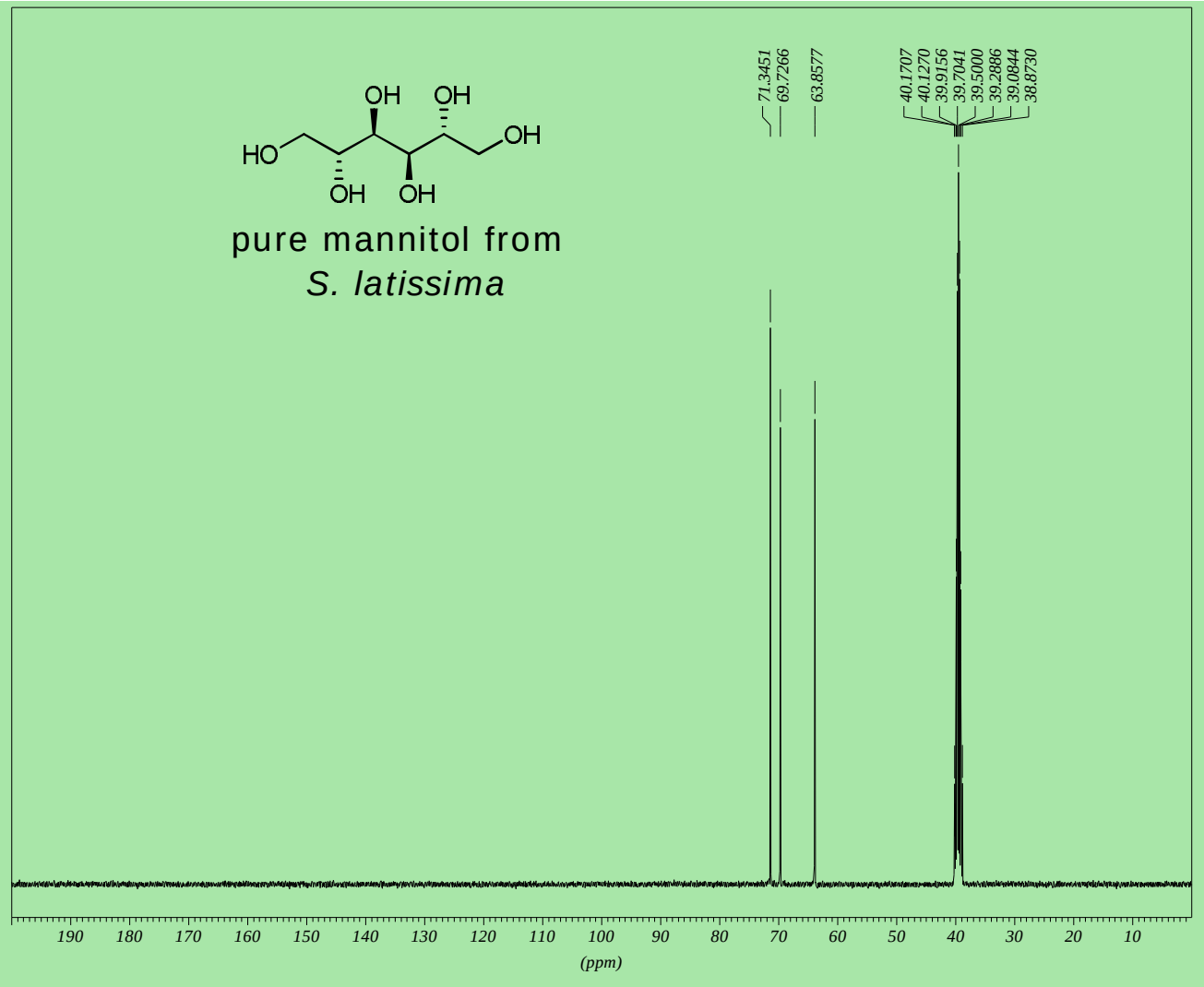
Extraction and purification of mannitol

- 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):



Biorefinery of brown seaweeds

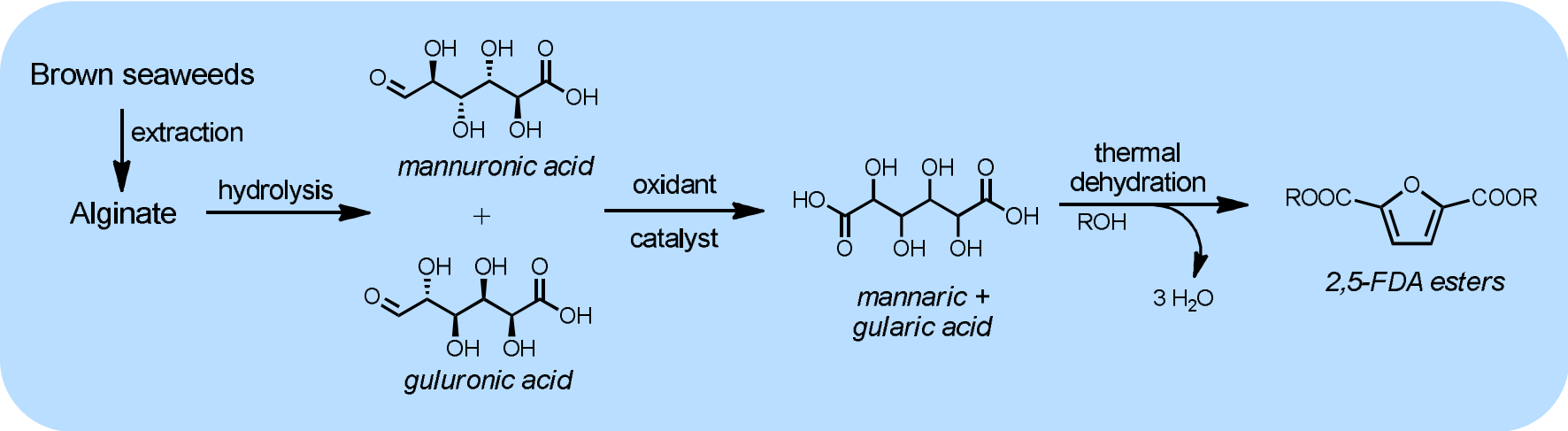
Extraction of alginate from *Saccharina latissima* press cake[1]

- Acidified press cake (160 g) suspended in 4% Na_2CO_3 (800 mL) for alginate extraction → increasing viscosity. Centrifugation, supernatant stored overnight at 4°C
- Addition of H_2SO_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

[1] Vauchel et al. *Food Bioprocess Technol* **1**, 297–300 (2008)

Biorefinery of brown seaweeds

- Conversion of alginate to bio-based chemical building blocks

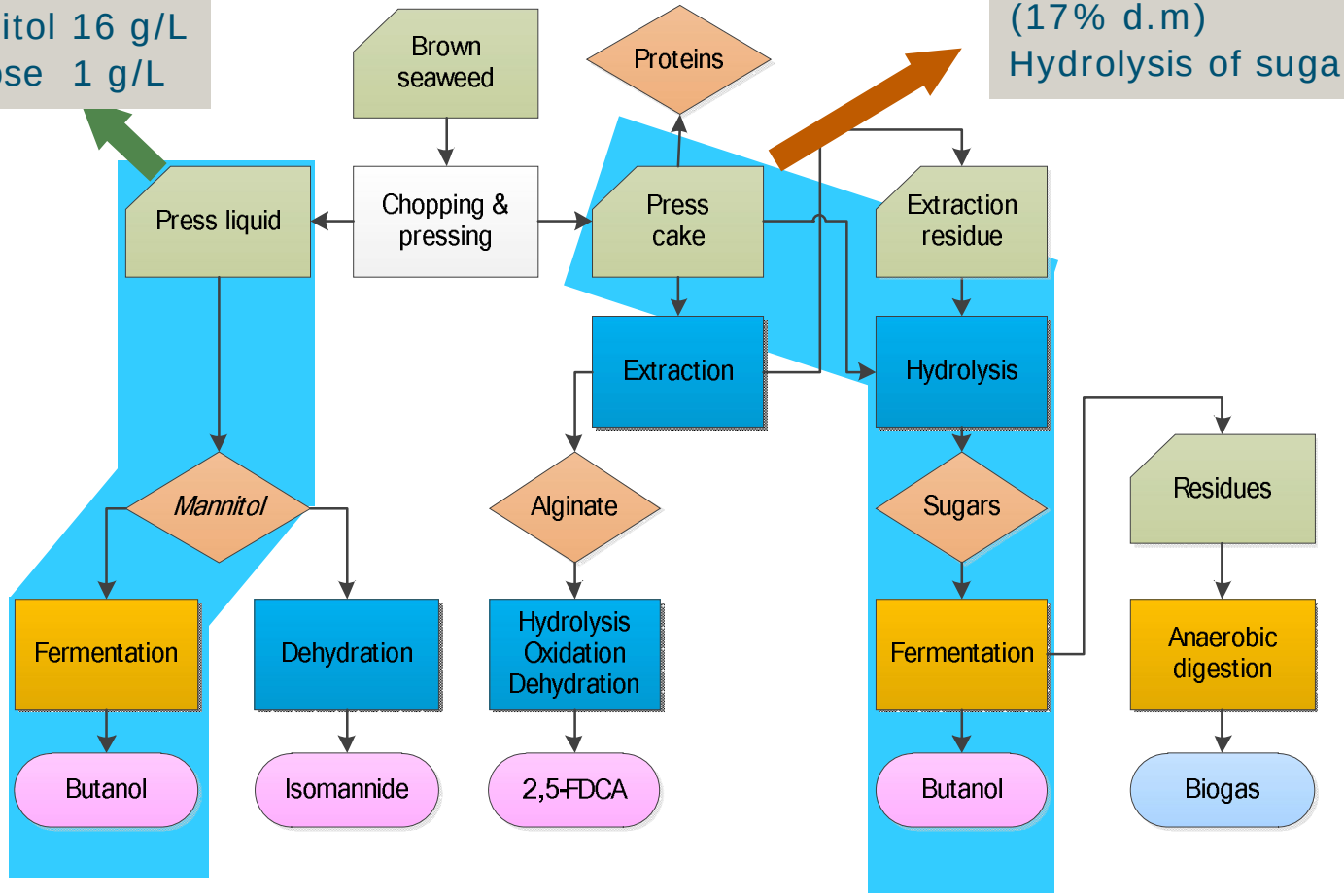


- Challenge: alginates are notoriously difficult to fully hydrolyse to monomeric uronic acids

Fermentation of *Saccharina latissima* fractions

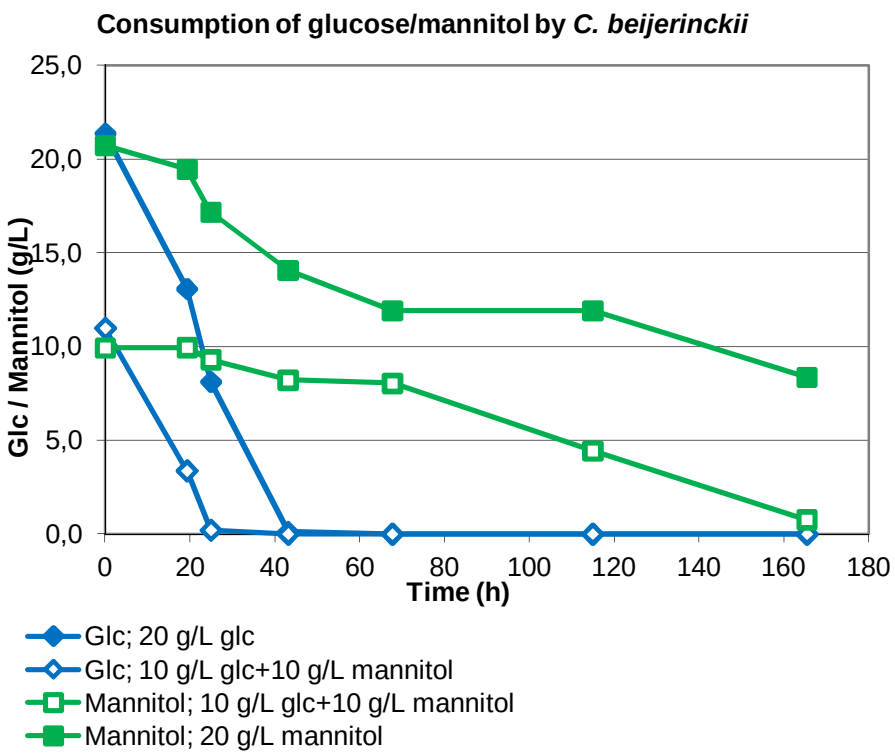
Very viscous
- Mannitol 16 g/L
- Glucose 1 g/L

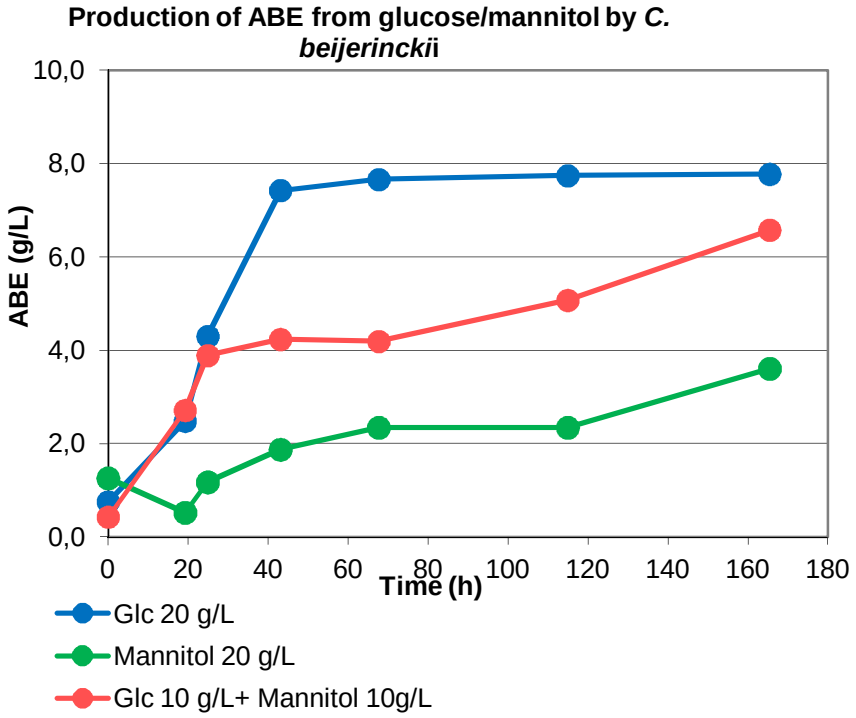
Approx. 70% of original seaweed (17% d.m)
Hydrolysis of sugar polymers



Fermentation of *Saccharina latissima* fractions

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





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Fermentation of *Saccharina latissima* fractions

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

Fermentation products from press cake(PC) hydrolysate by *C. beijerinckii*.

Products (g/L)	PC hydrolysate	PC Hydrolysate 2x dil.
ABE	0 (no growth)	3.8
Butyric acid	0	1.9

Toxicity of the hydrolysate may be due to:

- High salt concentration?
  - in hydrolysate, conductivity approx. 20x higher than in control medium.
- Other?

Press cake

Milling

Enzymatic hydrolysis GC220, 50°C, 16 h

Centrifugation

Seaweed hydrolysate
42 g/L mannitol
17 g/L glucose

Summary and Conclusions (II)

- Because of diversity, a species-dependend 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

Biorefinery and natural fibre Group

Steef Lips

Jacqueline Donkers

Bioconversion Group

Hetty van der Wal

Bwee Houweling-Tan

Pieternel Claassen

Sustainable Chemistry Group

Jan Stoutjesdijk

Marinella van Leeuwen

PRI-WUR

Julia Wald

ECN

Ron van der Laan

Arjan Smit

Wouter Huijgen

Ocean Harvest

Stefan Kraan

Declan Haniffy

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Agentschap NL
Ministerie van Economische Zaken, Landbouw en Innovatie

Thank you!

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