MACROFUELS

WP3 Biochemical fuel production

Macrofuels plenary meeting
Wageningen Food and Biobased Research
Wageningen, The Netherlands
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Task 3.1 Fermentation of seaweed syrups to ethanol

Xiaoru Hou, Dimitar B Karakashev, Randi Neerup, Anne-Belinda Bjerre (DTI)

- Aim: To achieve final ethanol concentrations on seaweed syrups higher than 4%

Seaweed Biomass

- Sugar composition of *Sacch*

	Tempera ture (°C)	Glucose (% DM)	Xylose (% DM)	G (%			
Less-fouling *S. latissima*	105	5.9	-	0			
	70	6.7	-	0			
	55	7.0	-	0.79	2.21	-	7.63
Heavily-fouling *S. latissima*	70	2.63	-	0.60	0.98	-	4.48
Unseperated *S. latissima*	air-dried by SAMS	3.55	-	0.52	1.49	-	3.15

Sugar content of Saccharina is too low

Use sugar-rich Seaweed hydrolysate from previous project

Seaweed Hydrolysate

Dried and grinded *L. digitata*

30 kg ≈ 150 kg fresh *L. digitata*

Enzymatic hydrolysis

800L reactor with 600L working volume

T= 45°C

Liquid fraction

Stored at T= -20°C

Glucose (g/L)	Glucan (g/L)	Mannitol (g/L)
9.27	5.76	3.32

This hydrolysate was sent to DLO and to Matis. Used for fermentations at DTI and DLO.

Task 3.1.1 Mesophile yeast fermentation

Strain: *Saccharomyces cerevisiae* (Quick Yeast, Doves Farm Foods Ltd.)
Medium: the seaweed hydrolysate
Temp: 30°C
Horizontal rotation rate: 120 rpm

Final Ethanol Yield: 73.3 (± 2.1)% theoretical max.
Final Ethanol Conc.: 3.47(±0.12) g/L

- Fast and efficient fermentation (almost finished after 24 hours)
- No un-fermented monomer glucose was detected
- No obvious lag phase observed (according to CO_2 production monitored by weight loss)
- Glucan and mannitol were not used

Task 3.1.2 Thermophile anaerobacter fermentation.

- Hydrolysate toxicity test

- 10 % hydrolysate + 90 % BA medium
-25 % hydrolysate +75 % BA medium
- 50 % hydrolysate + 50 % BA medium

Preliminary results:
-Excellent bacterial growth, no inhibition observed

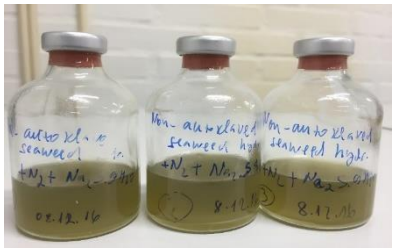
- Waiting for HPLC results of sugars and ethanol

Basic anaerobic (BA) medium containing 2 g xylose/L (Angelidaki and Sanders 2004)
pH: 6.5-7.0
Temp: 70°C
Fermentation hours: 48-72 h
Strain: thermophile *Thermoanaerobacter pentosaceus DTUO1T , uses both C5, C6 sugars*

Task 3.1.2 Thermophile anaerobacter fermentation.

- Preliminary fermentation on the seaweed hydrolysate
  - ✓ +/- autoclavation
  - ✓ +/- strict anaerobic conditions *(N_2 flush versus N_2 flush +Na_2S•9H2O)*

- **pH** 6.5
- **Temp:** 70°C
- 12 days fermentation (samples collected every 4 days)



Before fermentation

Preliminary results:

-Excellent bacterial growth, no inhibition observed

- Waiting for HPLC results of sugars and ethanol

After fermentation

Task 3.2. Thermophilic anaerobic biorefinery organisms

Bryndís Björnsdóttir, Guðmundur Óli Hreggviðsson, Ólafur H. Friðjónsson, Antoine Moenaert

Engineering of thermophilic strain AK17 for ethanol production and fermentation of laminarin and alginate

Thermoanaerobacterium sp. str. AK17

Robust thermophilic anaerobe

- $T_{optimal}$: 60 °C
- $pH_{optimal}$: 6,0

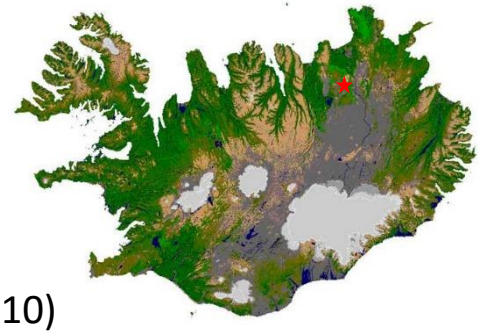

Víti in Krafla volcano

Efficient fermentation capacities

- Good ethanol producer
- Acetate and lactate

Diverse metabolic activities and broad substrate range

- Pentose : xylose, arabinose
- Hexose : glucose, galactose, glucuronic acid and mannitol
- Diose : cellobiose

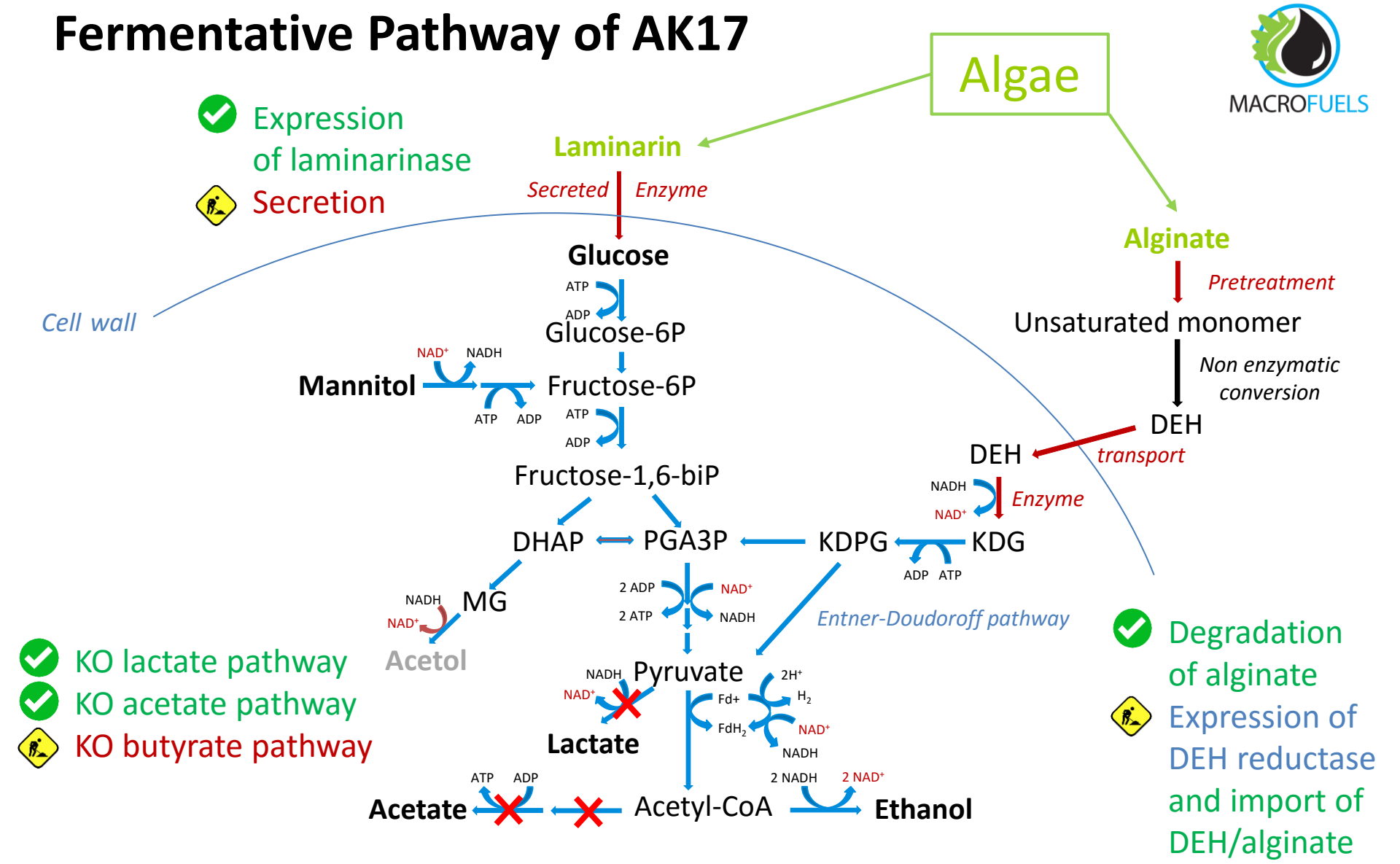


Susceptible to genetic manipulations

- Natural competence for Thermoanaerobacterium genus (Shaw et al, 2010)

Aim: Engineer AK17 for macroalgal carbohydrate utilization

Fermentative Pathway of AK17



Expression of laminarinase

Secretion

KO lactate pathway

KO acetate pathway

KO butyrate pathway

Degradation of alginate

Expression of DEH reductase and import of DEH/alginate

Task 3.3 Efficient fermentation of seaweed to acetone, butanol and ethanol

Truus de Vrije, Miriam Budde, Hetty van der Wal, Emil Wolbert, Florent Collas, Ana López Contreras

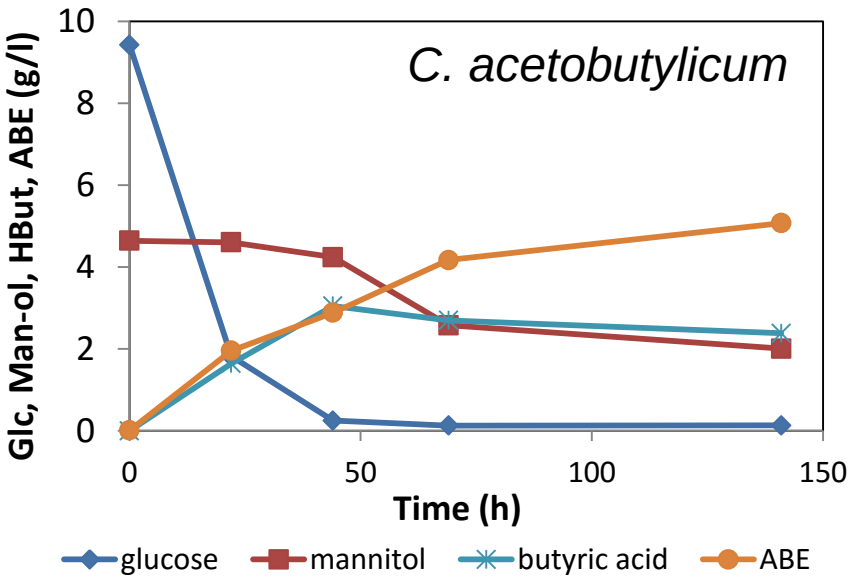
Seaweed extracts and hydrolysates for fermentation

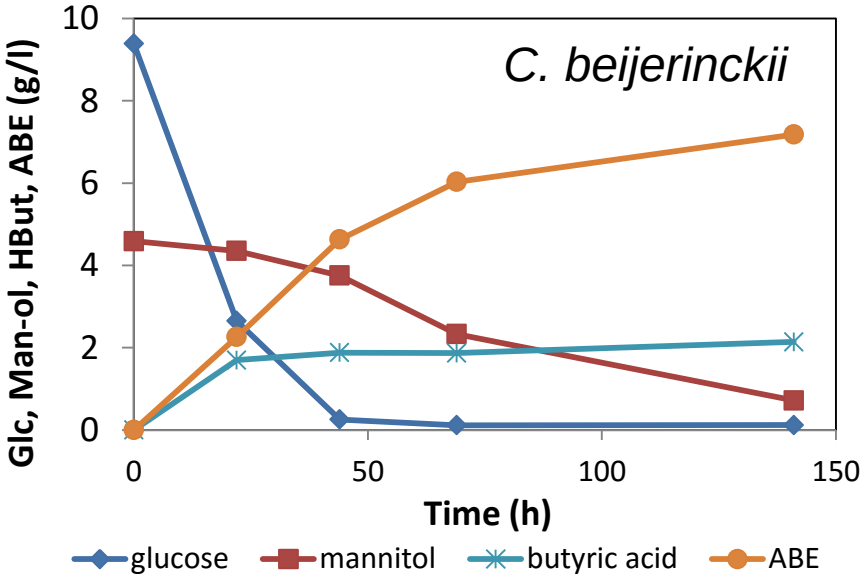
source of seaweed → preparation of extract/ hydrolysate

- Brown seaweed
  - *Saccharina latissima* — SAMS → WFBR
  - *Laminaria digitata* — in other project by DTI
- Red seaweed
  - *Palmaria palmata* — SAMS → ECN

Clostridium spp. with *L. digitata* hydrolysate

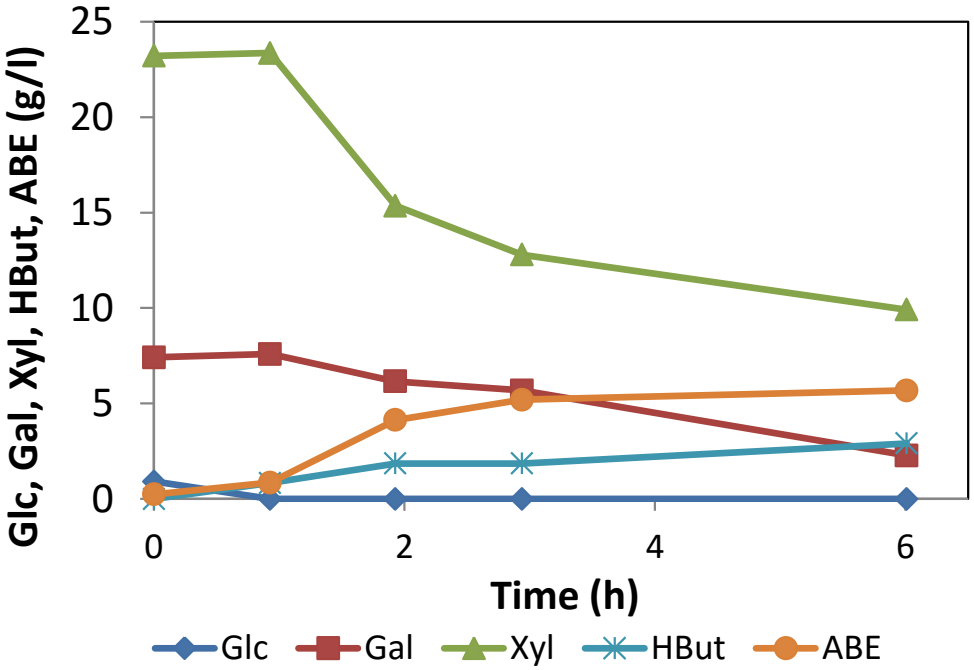
- Preferred consumption of glucose over mannitol
- More ABE produced by *C. beijerinckii*
  - growth with HS-medium, without nutrients





Growth of C. beijerinckii on *P. palmata* hydrolysate

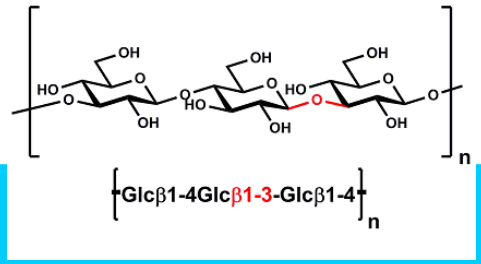
- Preferred consumption of glucose over galactose & xylose



Direct hydrolysis and fermentation of seaweeds and seaweed fractions

Approach:

- Tools for genome editing in ABE-producers: CRISPR/Cas9
- Insert enzymes for polymer degradation:
  - - Model: CelA (*N. patriciarum),* Family GH6, Binding module CBM1. Active on avicel, CMC and lichenan (Icelandic moss). Under development



Results & Targets: ABE production

	Aim	*Laminaria digitata*	*Palmaria palmata*
Consumed sugars Sugar type	All sugars	Glc, Man-ol Glucan	Glc, Gal, Xyl oligomeric Glc, Gal Xylan
[Sugars], g/l	40 - 60	25	33
Sugar consumption, %	≥ 90	87	60
[ABE], g/l	15 - 20	7.5	5.7
ABE yield, g/g	0.3 – 0.4	0.36	0.28

Task 3.4 Anaerobic digestion of seaweed SAMS

- Microcosms used to test a range of different conditions
  - Different inoculums (sludge/sediment)
  - Fresh seaweed
  - Pretreatments
  - By products
- Analytical & molecular methods to monitor performance
  - Analytical: methane, total solids, volatile solids, volatile fatty acids
  - Molecular: diversity (gel fingerprint analysis) & quantification (quantitative amplification)
- Scale up best conditions to 10litre
- What do we need to know from you
  - How much sample, when and in what form, composition?

Microcosms

10 litre vessel

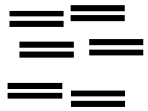
Analytical

CH_4, Total & volatile solids, VFA's

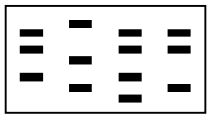
Molecular

Extract DNA

Amplify DNA & Quantify



Microbial community analysis



Plans for next 6 months

- **DTI:** Two-stage fermentation of C5-C6-rich seaweed hydrolysate:
  - 1st stage: C6 conversion to ethanol by mesophilic yeast
  - 2nd stage: C5 conversion (effluent from 1st stage after ethanol removal) to ethanol by thermophilic bacterium
- Effect of pH, sugar loading and macronutrients (N, P sources) on thermophilic fermentation

- **MATIS:** Further work on AK17 strain.
  - Fermentation of *Laminaria* hydrolysate
  - Enzyme optimisation
- **DLO:**
  - Fermentation of *Saccharina* hydrolysate
  - Improve conversion of polymers to ABE
- **SAMS:** start preparations for AD of seaweeds and fractions

Dissemination

- Manuscripts in preparation by Matis, DLO
- Congresses: CBM 2017 (MATIS), Biotech2017 (DLO)

Next meeting WP3

Skype, in April