WP3 progress

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1st Macrofuels progress meeting

Matis, Iceland, 28-06-2016

WP3: Biochemical fuel production

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Objectives:

1. **Improved ethanol from seaweed syrups**

    1.a Conversion of all sugars (C5, C6, Mannitol, uronic acids..)

    1.b Improved tolerance to inhibitors (salts, furanics, metals, protein)

    1.c Study Simultaneous Saccharification and Fermentation (SSF) vs Separate Hydrolysis and Fermentation (SHF)

1. Improved ethanol from seaweed syrups- Progress

1.1 Mesophilic ethanol production (T 3.1, DTI)

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Activities start in Sept. 2016, new scientist has been appointed

1.2 Thermophilic ethanol production (T3.2, Matis)

matis

Thermoanaerobacterium AK17: Thermophilic anaerobe (T_{opt}: 60°C, pH_{opt}: 6)

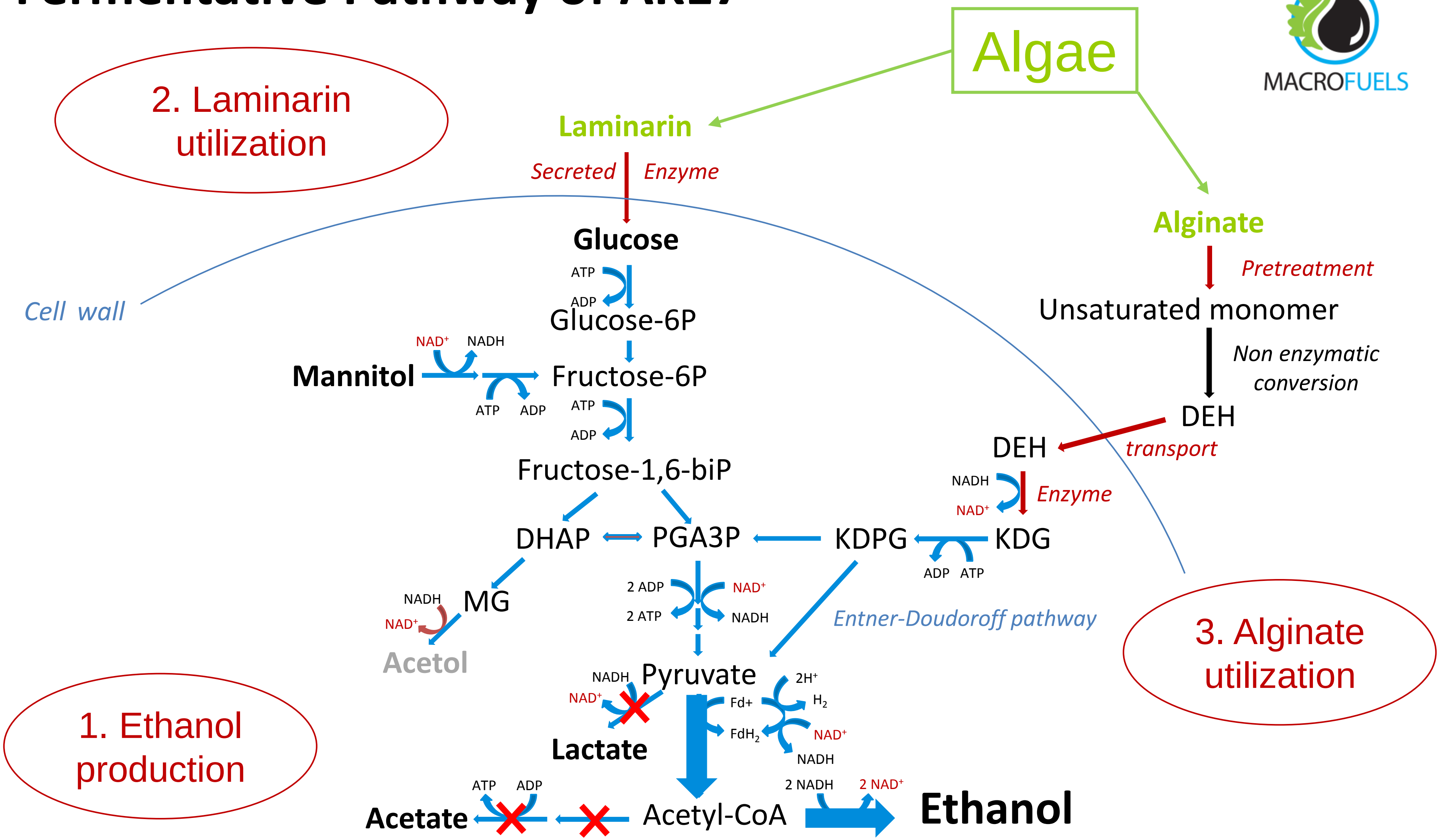
Efficient fermentation capacities: ethanol, acetate, lactate as products

Broad substrate range (C5, C6, di-saccharides) and Genetically accesible

Aim: Engineer AK17 for macroalgal carbohydrate utilization

Víti in Krafla volcano

Fermentative Pathway of AK17



Engineering plan 1 : Ethanol production

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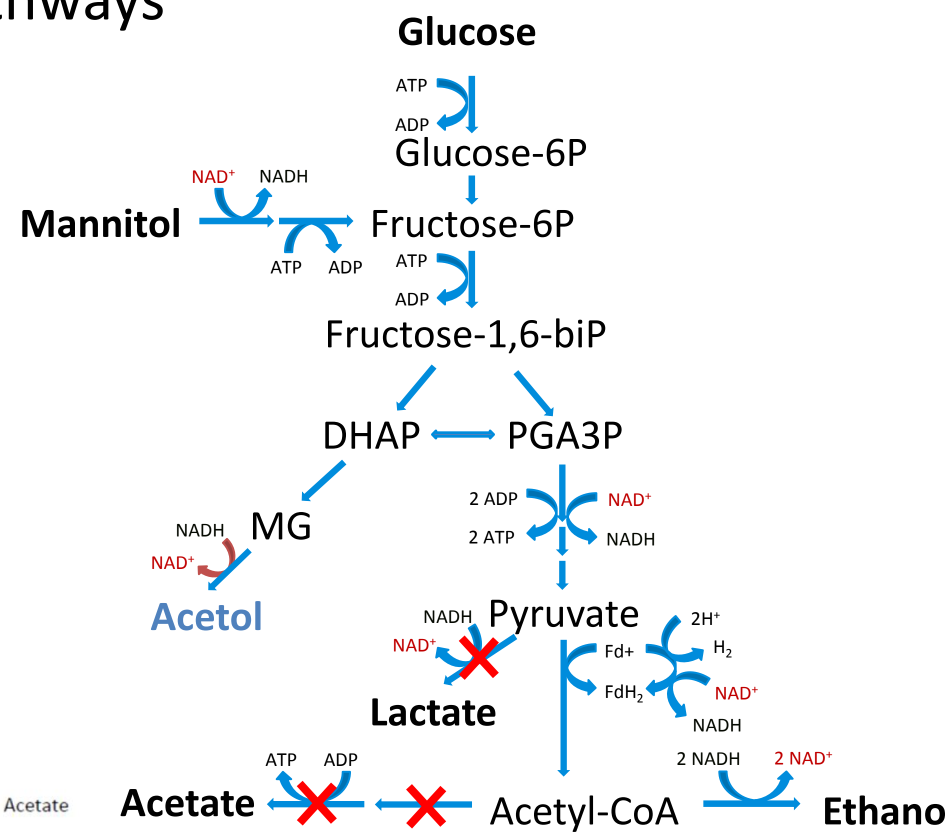
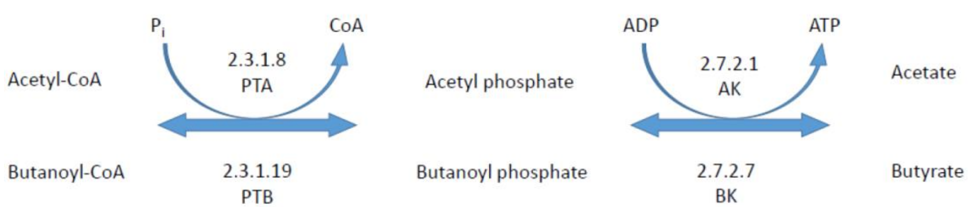
Requirements: Knocking out pathways

- Knocking out Lactate pathway
- Knocking out Acetate pathway
- Knocking out Butyrate pathway

Re-production of acetate by an adapted double mutant:

- Following continuous adaptation to increased ethanol tolerance
- By the butyrate pathway (enzymatic assays)
- Phosphotransbutyrylase knockout





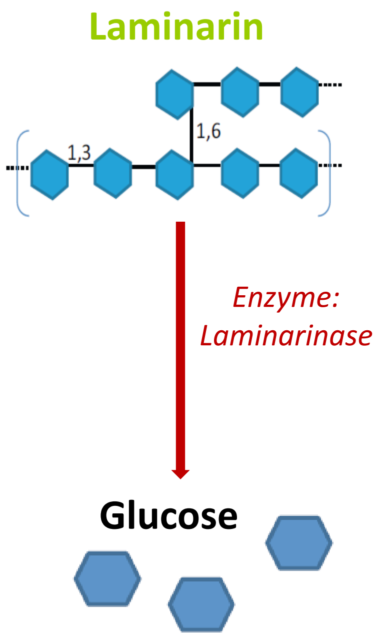
Engineering plan 2 : Laminarin utilization

Requirements: secreted thermostable laminarinase

- Expression of a thermostable β-glucosidase
- Secretion of the enzyme

Progress

- Successful expression of thermostable laminarinase in AK17
  - Insertion only obtained when amo78 cloned without signal peptide
  - Intracellular laminarin degrading activity verified
  - No secretion observed
- 3 different CBM have been selected and cloned upstream Amo78
  - Clones obtained with one CBM, expression and secretion on going



Engineering plan 3 : Alginate utilization

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Requirements:

- Thermostable alginate lyases (pre-treatment)
- Mono-uronate transport system
- Thermostable DEH reductase

Progress

- Successful insertion into AK17 and expression verified (kanamycin resistance)
- Intracellular DEH reductase activity not verified
  - Inadequate expression
  - Low sensitivity assay
- Expression by replicative plasmid ongoing
- 4 promoters have been selected and constructed with 2 reporters
  - Constructs with fluorescent reporter have been cloned, but no fluorescence observed
  - Constructs with a β-glucosidase ongoing

Alginate

↓ *Pretreatment*

Unsaturated monomer

↓ *Non enzymatic conversion*

DEH

↓ *transport*

DEH

↓ *Enzyme* (NADH → NAD^+)

KDG

2. Improved Acetone, Butanol and Ethanol production

2.a Conversion of all sugars (C5, C6, Mannitol, uronic acids) in seaweed syrups into ABE. Study tolerance and nutrient requirements

2.b Consolidated Bioprocessing of (fractions of) seaweeds. Fermentation and degradation of polymers by one organism. Reduction of enzyme use in the pre-treatment.

2. Improved Acetone, Butanol and Ethanol production- Progress

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Tolerance to salts in seaweeds & hydrolysates

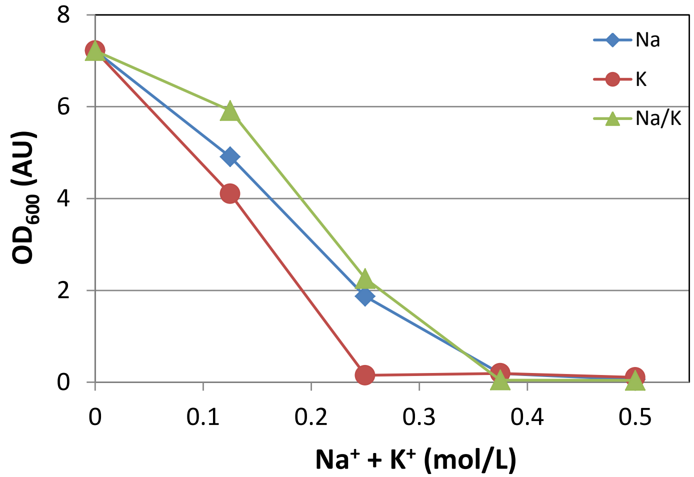
- High content of K^+ and Na^+ in seaweed biomass and hydrolysates prepared from seaweed (data from previous studies)

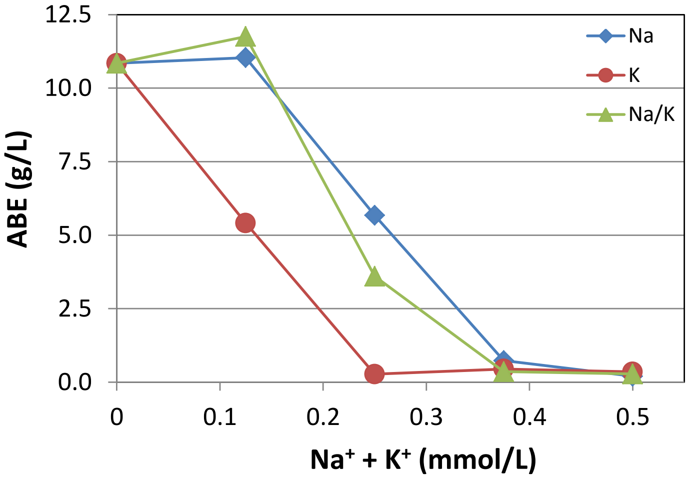
	Alaria	Gracilaria	Saccharina	Alaria	Gracilaria	Saccharina
	Seaweed biomass			Hydrolysate from seaweed		
	% of dry matter			g / 10 g sugar (monosacch., Man-ol)		
K	14 – 37	11 – 14	14	2.8	0.9	2.3
Na	12 – 20	9 – 14	7	4.4	1.4	1.2
Ca/Mg	0.8 – 2.5	0.2 – 0.6	n.d.	0.8	0.2	n.d.

Salt tolerance of *C.beijerinckii*

- Growth and ABE production are inhibited by K- and Na-salts

	Growth	ABE production
	IC_{50} (g/L)	
Na	4.2	6.0
K	5.2	5.0





Direct hydrolysis and fermentation of seaweeds and seaweed fractions **(Consolidated bioprocessing)**

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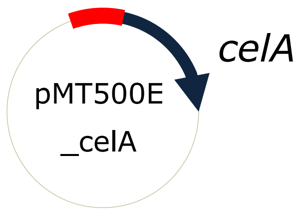
Approach:

- Develop tools for genome editing in ABE-producers: CRISPR/Cas9
- Insert a laminarinase, *CelA*, from *Neocallimastix* into Clostridia.

Laminarin is present in mainly in brown seaweeds

- **Expression of *celA* in *C. beijerinckii***



Transformed in
Clostridium

Cellulolytic activities :
- Lichenan
- CMC

➔ **Selection pressure (antibiotic) required to maintain the plasmid**

- **Insertion of *celA* in the genome of *C. beijerinckii***

➔ **Stable mutant obtained**

Characterization in progress
- Hydrolase activities on polymers and on seaweed fractions
- Fermentation performances on control and seaweed fractions

- **Next step: other enzymes and systems (cellulosome) to be inserted**

3. Digestion of residues from fractionation and fermentation to biogas (T3.4, SAMS, start M12)

SAMS can start already with assessment of the residues from:

WP2: pre-treatment residues, pellets, etc

WP3: broth after removal of fuels, solid streams, etc

Agreed on: keep all types of residues documented, and send them to Angela Hutton of SAMS.

Amounts: for small tests, grams are enough

4. Analysis of the residues for evaluation of applications (T3.5, ECN)

Protocols set-up is ongoing, reports Wouter/Jaap

5. Production of sufficient fuel for benchmarking /blending in WP5

ECN indicated a need of 20-L of ethanol or of ABE for engine tests at DTI

Plans to realize this: First step, use hydrolysate available at DTI (approx. 100-L, approx 25 g/L sugars, fermentable)

For 20-L ABE we need approx 1000-L of hydrolysate, need to plan availability with WP2

Discussions:

- Ensiled biomass is interesting for fermentations. Appointments have been made with Fermentation Experts (Jens, Rene) for material transfer to DLO, DTI and Matis

- High salts concentrations have a negative impact on the fermentation. Important to collaborate with WP2 (ECN) on desalting, and get de-salted streams for fermentation.
  Calcium peaks in composition could be due to shell growth on the seaweed (Matis observation)

- Analysis of feedstocks and streams need to contain elemental data (ECN). Analysis of uronic acids is important as well.

Dissemination

Presentations at ISS last week by Jaap and by Anne-Belinda

Webpages at institutes

Publication on *Ulva lactuca* biorefinery (DLO, ECN), April

Book chapter in preparation

Planned:
October 2016 at Nordic Seaweed congress, DTI

Summary

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Tasks are progressing according to scheme. Progress report prepared and sent to Rita for intranet

New interactions started with Fermentation experts on ensilaged seaweeds

Strategy for upscaling to generate 20-L of fuels need to be made soon (WP2-WP3-WP5)

Thanks to:

All partners for collaboration, inputs and discussions

Matis team for organisation and hosting of this meeting

We look forward to the hiking tour!