



Meeting Minutes

MacroFuels WP3 meeting

Date: 10-01-2017

Time: 13.40-15.40

Location: Hotel “de Wageningsche Berg”, Wageningen, The Netherlands

Participants

Name	Company
Anne-Belinda Bjerre	DTI
Xiaoru Hou	DTI
Michele Stanley	SAMS
Arlene Ditchfield	SAMS
Bryndís Björnsdóttir	Matís
Guðmundur Óli Hreggviðsson	Matís
Jens Legarth	FEXP
Rene Schepens	FEXP
Jan Wilco Dijkstra	ECN
Ana M. López Contreras (chairperson)	WFBR
Truus de Vrije	WFBR
Jelle van Leeuwen	WFBR
Miriam Budde	WFBR

Agenda

- 1. Welcome and general matters WP3 (Ana López Contreras)**
- Minutes from last meeting at Matís, progress on action items
- 2. Task 3.1. Fermentation of seaweed syrups to ethanol by mesophilic organisms (DTI) – Xiaoru Hou**



3. **Task 3.2. Thermophilic anaerobic biorefinery organisms (MATIS) – Bryndís Björnsdóttir**
4. **Task 3.3 Efficient fermentation of seaweeds and seaweed fractions to ABE (WFBR) – Truus de Vrije & Ana López Contreras**
5. **Task 3.4 Anaerobic digestion of seaweed fractions (SAMS)- Arlene Ditchfield**
6. **General Discussions**
 - Action list
 - Dissemination activities, done and planned in next 6 months
 - Next meeting: April 2017 on skype

Minutes of meeting

1. Action items

- Minutes from Project meeting 27-06-2016, Reykjavik

Action items

1. Organize WP3 skype meeting, October 2016. Not done because of no need for discussions (AL)
2. Upload conference and meeting presentations on web site. Done by Rita Clancy.
3. *Laminaria* hydrolysate of DTI sent to WFBR (2 L) and Matís (1 L), together with pretreatment / enzymatic hydrolysis protocol. Done by XH, in September.
4. *Laminaria* solid residue of hydrolysate preparation by DTI send to SAMS (2 – 3 grams dry matter). Not possible because there were no residues left (XH)
5. Storage of all residues of hydrolysis/fermentation experiments for anaerobic digestion. Will be done.

2. Progress in Task 3.1. Dimitar Karakashev is the new Task leader at DTI responsible for future work.

- *Saccharina* cultured by SAMS and harvested in June 2016 was not used for experiments because the material was fouled and did not contain enough sugars.
- A hydrolysate from *Laminaria digitata* (wild harvest) prepared by DTI in another project was available for fermentations. It contains in total 18 g of sugars/l in a mixture of glucose, glucan, and mannitol.
- Efficient fermentation of *L. digitata* hydrolysate (without additions) and high ethanol yield was achieved with mesophilic yeast *S. cerevisiae*. No toxic effect of hydrolysate and good growth in thermophilic fermentations with *Thermoanaerobacter pentosaceus* DTUO1T (70 °C).



- Plans for next period: Two-stage fermentation of C5-C6-rich seaweed hydrolysate with yeast and thermophile; medium requirements for thermophilic fermentation.

Discussion: GH: Lots of wild *Laminaria* can be bought on Iceland.

3. Progress in Task 3.2. Three approaches for high yield ethanol production from brown seaweeds by the thermophile *Thermoanaerobacterium* AK17 (60 °C):

- Knocking out butyrate pathway in ethanol-tolerant strain which produces high amounts of acetate (in acetate- and lactate-knock out strain) by butyrate pathway enzymes
- Secretion of laminarinase: new beta-glucosidases, efficient and shorter in length have been identified and will be used (from *Rhodothermus*). CBM (carbohydrate binding modules) for Clostridia has been cloned and transformed into AK17, but no transformants obtained. 2 CBMs from *T. saccharolyticum* will be cloned upstream of laminarinases. Work in progress.
- Alginate pathway: import system for DEH and expression of DEH reductase in AK17 is ongoing

Discussion: AL/XH: In *L. digitata* hydrolysate of DTI glucan is present. Test for laminarinase activity by transformed AK17? Will be done. Alginate as substrate can not be tested because of precipitation due to autoclaving of the same hydrolysate.

4. Progress in Task 3.3.

- ABE and IBE production from *L. digitata* hydrolysate (from DTI) by *C. beijerinckii* strains NCIMB8052 and NRRL B593 with high yields. Glucose, most of the glucan and some of the mannitol was consumed.
- ABE production by *C. beijerinckii* NCIMB8052 from *P. palmata* extracts and hydrolysates (prepared by ECN). Highest substrate consumption and ABE yield with extract from HCl treated-seaweed. Oligo-xylan degradation to xylose by xylanase activity of *C. beijerinckii* NCIMB8052.
- For direct hydrolysis and fermentation of seaweeds and seaweed fractions genes for enzymes for polymer degradation are inserted in Clostridium. A copy of the *celA* gene is inserted in the genome of *C. beijerinckii* by the CRISP/cas9 technology, but cellulase activity was not detected. A new construct with a stronger promoter P_{thl} (thiolase gene, Cbei3630) is in preparation.

Discussion: XH: DTI has the same result with the *L. digitata* hydrolysate for butanol production. A publication is in progress. ECN is involved in the substrate analysis. The increase in mannitol after complete hydrolysis comes from the M-block (= mannitol) on laminarin.

5. Progress in Task 3.4. Angela Hatton left SAMS but she is still involved in the project.

- Inocula collected from anaerobic digester sludge and from marine sediments will be compared for activity on seaweed biomass and residues. Marine sediments might be more suitable because of the high salinity of seaweeds.
- Residues from pretreatment and other by-products will first be tested on small scale experiments (10 L) under optimized conditions.

Discussion: JL/AD: dry solid residues are suitable for these tests.



6. General discussion

Dissemination:

- Sent info the Rita Clancy
- Acknowledge the EC in text, by logo
- Use MacroFuels templates

Planned activities:

Conferences:

- AL: BioTech 2017, June 13-17, 2017. Prague, Czech republic.
- GH: CBM12 – 12th Carbohydrate Bioengineering Meeting, April 23-26, 2017, Vienna. Presentation of 2 projects: MacroFuels and MacroCascade.

Publications:

- GH: manuscript on new enzymes, for both projects
- AL: manuscript on Palmaria, together with ECN (former Dutch project)
- AL: proof prints of the book chapter are soon available

Design of processing plant by ECN

JWD: 3 cases will be designed: Biofuels (ABE and ethanol), and chemicals by thermochemical conversion (various products). Activities will start next months. Other partners will be involved where necessary.

AL likes to see an overview of the fermentation results by 3 different labs (DTI, Matís, WFBR) with the same substrate, i.e. the *L. digitata* hydrolysate of DTI. To be discussed during the next Skype meeting.

- Next meeting: in April 2017 on skype
- Action list, see table

Noted by Truus de Vrije; Revisions by

Action Items

#	Action item	Responsible
1	Preparation of 1 st year Progress report. 6M report will be sent around for updating.	WFBR, All
2	Organize next WP3 skype meeting, April 2017. Doodle will be started.	WFBR
3	Residues from pretreatment/hydrolysis by WFBR sent to SAMS; and for all partners storage of all residues of hydrolysis/fermentation experiments (without alcohols) for anaerobic digestion	WFBR, All
4	Discussion on results with <i>L. digitata</i> hydrolysate at next Skype meeting	DTI, Matís, WFBR
5	Upload conference and meeting presentations on web site, via Rita Clancy	All