Meeting Minutes

MacroFuels WP3 meeting

Date: 18-05-2017

Time: 13.00-14.30

Location: On line, Skype for business

Participants

Name	Company
Xiaoru Hou	DTI
Dimitar Karakashev	DTI
Bryndís Björnsdóttir	Matís
Guðmundur Óli Hreggviðsson	Matís
Antoine Moenaert	Matís
Ólafur Friðjónsson	Matís
Ana M. López Contreras (chairperson)	WFBR
Truus de Vrije	WFBR

Agenda

1. General matters WP3 (Ana)

- Minutes from last meeting at Wageningen, progress on action items
- Reporting M13-M18.

2. Task 3.1. Fermentation of seaweed syrups to ethanol by mesophilic organisms (DTI) – *Dimitar* / *Xiaoru*

3. Task 3.2. Thermophilic anaerobic biorefinery organisms (MATIS) – *Bryndís* / *Guðmundur*

4. Task 3.3 Efficient fermentation of seaweeds and seaweed fractions to ABE (DLO)- *Truus* / *Ana*

5. Task 3.4 Anaerobic digestion of seaweed fractions (SAMS) - *Arlene*

6. Upscaling of fermentation in WP5 - *Ana / Dimitar / Xiaoru*

7. General Discussions

Minutes of meeting

1. General matters

- Minutes from Project meeting 10-01-2017, Wageningen

Action items (minutes WP3 meeting Wageningen January 2017)

#	Action item	Responsible
1	Preparation of 1st 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 *L. digitata* hydrolysate at next Skype meeting	DTI, Matís, WFBR
5	Upload conference and meeting presentations on web site, via Rita Clancy	All

1. Preparation of 1st year Progress report is done.
2. This skype meeting was shifted from April to May.
3. No residues available yet from pretreatment/hydrolysis by WFBR. DTI will first prepare the best protocol for treatment of fresh material.
4. *Laminaria* hydrolysate of DTI is tested by WFBR and Matís, and results will be discussed in this meeting.
5. AL emphasizes the importance of updating dissemination activities (via Rita Clancy). Publications should be in Open Access journals (required by EC). If chosen otherwise please explain why.

- Reporting M13-M18. The Progress report for the period M1 -12 needs to be updated with results from period M13 -18. XH mentioned that a new format will be sent around next week to WP leaders (week 21) which is slightly different from the current one. The deadline for preparation is the end of June. TV will send around in June the latest, but new version, of the previous WP3 report (M1-M12) to all for updates.

2. Progress in Task 3.1

- The hydrolysate from *Laminaria digitata* (wild harvest) contains circa 19 g of glucose and glucan per L and 3 – 4 g of mannitol per L.
- Two thermophilic strains are selected for ethanol production: *Defluviitalea phaphylia* (60 °C) and *Thermoanaerobacter pentosaceus* DTUO1T (70 °C). *D. phaphylia* is from the Japan Collection of Microorganisms and has the advantage of simultaneous usage of glucose, mannitol and alginate, and

no need for pretreatment of brown seaweed (Ji et al., 2016). *T. pentosaceus* does not consume alginate.

- Disadvantage of using these thermophiles for ethanol production is the low ethanol content and/or low substrate tolerance.
- No toxic effect of hydrolysate and good growth in fermentations with both thermophiles (in medium with 100% of hydrolysate). No results yet on substrate consumption and ethanol production.
- Plans for next period: Effect of pH, T and macronutrients on growth and ethanol production.

Discussion: XH: toxic effects by salts are not expected with this hydrolysate because the salt content is very low (conductivity of 4.8 mS/cm, measured by WFBR). With *Saccharina* toxic effects from inhibitors and salts are expected. XH: yeast fermentations with more than 10% dry matter of *Laminaria* results in unused substrate. XH: Two-stage fermentation (yeast followed by thermophilic fermentation) is not necessary if *D. phaphylia* is able to consume all substrates from hydrolysates.

3. Progress in Task 3.2

The thermophile *Thermoanaerobacterium* AK17 (60 °C) is used for high yield ethanol production from brown seaweeds

- A triple mutant (ace- lac- but-) does no longer produce acetic and lactic acid
- Sea salt and oxygen tolerance was tested
- Ethanol is produced in medium with *L. digitata* hydrolysate to 100%, supplemented with yeast extract, vitamins, reducing agent, and consumption of glucose and mannitol.
- Ethanol production and yield is higher by the triple mutant than by the wild type strain (less by-products with mutant strain).

Discussion: XH: consumption of other components in *L. digitata* hydrolysate, i.e. glycerol, lactic acid? To be determined. AL/GH: good growth with 100% hydrolysate, but substrate consumption of 35% may be improved by better pH regulation.

4. Progress in Task 3.3

- ABE and IBE production from *L. digitata* hydrolysate 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 from *L. digitata* hydrolysate by *C. acetobutylicum* strains ATCC824 with ABE yields lower than obtained with *C. beijerinckii* strains, but with higher substrate consumption of more than 90% on hydrolysate only. Consumption of glucose and glucan and about half of the mannitol.

In conclusion: all tested ethanol and A/IBE producers grew well on medium with a high content of *L. digitata* hydrolysate (to 100%). As far as determined glucose and mannitol were consumed, and with *Clostridia* spp. also glucan was used. More than 3 and 7 g/l of ethanol and ABE, resp., were produced in small scale systems. *L. digitata* is an “easy” substrate because of its low salt content and absence of other inhibitors, apparently in contrast to substrates from *Saccharina*.

5. Progress in Task 3.4
SAMS did not join the meeting. (*Note from Arlene Ditchfield*, "they were busy with sampling")

6. Upscaling of fermentation in WP5
AL: DTI informed the WP5 partners earlier this year that it cannot perform the upscaling of the ethanol fermentation as described in WP5. Therefore we are looking for solutions for this. One option is to perform the upscaling at WFBR, in which DTI takes the lead in the Tasks using the facilities and operators/scientists in Wageningen. Other option is to look for an external party to perform the ethanol fermentation upscaling (such as BPF in NL or other in DK). Decision will be taken in September.

XH: Matís is mentioned as the lead participant of deliverable D3.5 "Fuel samples for engine testing". Guðmundur commented that it is not appropriate. To whom this lead beneficiary should be changed needs to be discussed . AL suggests to do this as part of the first reporting period, together with other changes.
XH/DK: strains used at DTI for upscaling of ethanol production are either a wild type thermophile (plan A) or yeast (plan B), not a combined two-stage procedure. It will depend on the sugar composition of *Saccharina* harvested this summer. DK: it is allowed to use *Defluviitalea phaphylia* for research purposes.

XH: Adrian Macleod (SAMS) sent the following update on the harvest and composition of *Laminaria* and *Saccharina*: harvest is scheduled for the first week of June weather dependent. Mannitol levels (free form) are rising and vary from 8-25% and 6-20% per dry matter, resp., determined by a quick method. Biomass is free from fouling and is looking healthy.

7. General discussion
- *Dissemination activities*, done and planned in next 6 months, please communicate these to Rita.

World Expo 2017 "Future energy", Astana, Kazakhstan, from June 10 – September 10, for which we are asked to present the MacroFuels project. For this a short movie about the key features is prepared by Rita and others. AL delivered a video on the use of seaweeds for biofuel prepared within another project.

Seagriculture 2017 - 6th International Seaweed Conference, the Hague, the Netherlands, 7 and 8 November 2017. AL will attend the meeting and present MacroFuels.

EUBCE 2017 – 25th European Biomass Conference & exhibition, Stockholm, Sweden, June 12 – 15. Anne-Belinda Bjerre will attend.

World Seafood Congress 2017, Reykjavík, Iceland, September 10-13. Matís will attend and present other projects than MacroFuels.

- *Next meeting*. In Bruges few time is available for WP meetings. AL suggested to meet on line or by e-mail with task leaders only at the end of June for the preparation of the WP3 presentation and other deliverables.

XH: WP2 partners will meet on line. XH will not attend the Bruges meeting. She will be on maternity leave from July – March 2018. There will be two replacements, for management tasks and for WP2 coordination.

Noted by Truus de Vrije; Revisions by Xiaoru Hou.

Action Items

#	Action item	Responsible
1	Preparation of Progress report M1-18 (periodic reporting). Deadline end of July, depending on the time of opening periodic reporting process in ECAS. A new template from EC will be sent around together with an instruction for updating.	DTI, WFBR, All
2	Next WP3 meeting, end of June 2017. On line or by e-mail.	WFBR
3	Storage of all residues of hydrolysis/fermentation experiments (without alcohols) for anaerobic digestion	WFBR, All
4	Delivery of a second batch of *L. digitata* hydrolysate to Matís (2 liter)	DTI
5	Upload conference and meeting presentations on web site, via Rita Clancy	All