Meeting Minutes

MacroFuels WP3 meeting

Date: 03-03-2016

Time: 15.00-16.00

Location: Skype meeting

Participants

Name:	Company:
Ana M. López Contreras	DLO/Food & Biobased Research, WUR
Florent Collas	DLO/Food & Biobased Research, WUR
Truus de Vrije	DLO/Food & Biobased Research, WUR
Emil Wolbert	DLO/Food & Biobased Research, WUR
Xiaoru Hou	DTI, Denmark
Arlene Ditchfield	The Scottish Association for Marine Science
Bryndis Björnsdottir	Matis OH
Olafur Fridjonsson	Matis OH

Agenda

1. **Introduction to the meeting (Ana López Contreras)**

2. **Task 3.1. Fermentation of seaweed syrups to ethanol by mesophilic organisms (DTI; M1-40) -** ***Xioaru Hou***

3. **Task 3.2. Thermophilic anaerobic biorefinery organisms (MATIS OHF; M1-40) -** ***Bryndis Björnsdottir***

4. **Task 3.3 Efficient fermentation of seaweeds and seaweed fractions to ABE (DLO; M1-40)-** ***Ana López Contreras***

5. **Task 3.4 Anaerobic digestion of seaweed fractions, (SAMS; M12-48)-** ***Arlene Ditchfield***

6. **Task 3.5 Analysis for evaluation of applications of seaweed fractions after biochemical conversion (DLO, ECN, DTI, SAMS; M20-24)-** ***Ana López Contreras***

7. **Any other business, next meeting**

Minutes of meeting

1. AL gave a short summary of WP objectives: improvement of the fermentative production of three biofuels, ethanol, ABE, and biogas, from seaweeds and residues. The first deliverables and milestones will be due in month 24. The ethanol and ABE fermentations need to deliver enough biofuel for engine testing in WP5 (deliverable M36).

2. Task 3.1. The main objective of the mesophilic ethanol fermentation is to produce at least 4 – 5% of ethanol. For the consumption of C6, C5 sugars and mannitol different yeast strains will be used. Various fermentation strategies will be tested: SSF, SHF, and fed-batch. The latter one will be applied to prevent severe inhibition of fermentation with high seaweed dry matter concentrations in the medium. Fermentations will be firstly done at 100-ml fermentation flasks (for testing different yeast strains aimed for different sugars e.g. glucose, xylose, galactose, mannitol), and later upscaled to a few liter. Hydrolysates will be produced by DTI in WP2 from the brown seaweeds, e.g. *Alaria esculenta* and *Saccharina latissima* for starting up in 2016.
Discussion: At high dry matter concentrations of 15 – 20%, much of the substrate is not used possibly due to inhibiting compounds such as salts (*AL*: K+), uronic acids, heavy metals, e.g. arsenic salts.

3. Task 3.2. The thermophilic anaerobic strain *Thermoanaerobacterium* AK17 is an ethanol producer and can be genetically modified. In the first period AK17 will be adapted to utilize mono-uronic acids by introducing genes for metabolism (reductase) and transport (transport system) of mono-uronic acids. In following periods the aim is to improve the osmotolerance of AK17 by enhanced trehalose production, and to abolish the formation of side-products for improvement of the ethanol yield.
Discussion: First mono-uronates will be used as substrate, later growth on seaweed polysaccharides will be tested. Laminarin as substrate is tested in another project. For the moment there are no needs from other WPs.

4. Task 3.3.In previous projects it was noticed that growth on high sugar hydrolysates was seriously hampered by high salt concentrations and possibly other inhibitors like phenolics. Together with WP2 methods to reduce the salt content will be developed. ABE producing Clostridia are able to consume many sugars and some are able to utilize uronic acids. Fermentations will start in May – June when hydrolysates are available. Secondly, Clostridium will be genetically modified with a fungal laminarase gene for growth

on laminarin. This task has already started. Growth will be with pretreated seaweed prepared by WP2 with better accessible (for enzymes) polysaccharides. First material will be available in May. Alginates will be studied later.

5. Task 3.4. Anaerobic digestion experiments are scheduled starting from M20. But SAMS will start with an anaerobic digester already at the end of this year. At the moment a PhD student nearly finished his thesis on biogas production using amongst other fish farm sediments and anaerobic digester sludge. With different substrates the development of the methanogens was followed. This approach will also be followed with seaweed residues.
The amount of material necessary for anaerobic digestion tests will be determined in due time.

6. Task 3.5, analyses of seaweed fractions, will start in M20. AL asked all partners to store residues and side-products from fermentation experiments. The responsible partners for the different analyses need to be determined. ECN is e.g. equipped for elemental analyses, CHNO, salts, and caloric value determinations. Standard analyses of sugars, organic acids, alcohols in fermentations will be done by the partners themselves.

7. Other business
1) Alcohols for fuel testing: quality and volume.
Matis and DTI mentioned that they do not have the equipment for distillation of ethanol. AL: this and other issues regarding fuel production e.g. required quantities, will be discussed with WP5, and is already mentioned to ECN.
2) Next meeting: Monday June 27, in the morning, Reykjavik, Iceland (preliminary)

Noted by Truus de Vrije

Action Items:

Action item	WP	Person responsible	Completed by
Organize next WP3 meeting	3	Ana López Contreras	1-6-2016
Design pretreatment protocol together with WP2 DLO colleagues	2,3	Truus de Vrije	1-5-2016
Requirements of residues from fermentation experiments for biogas production	3	Arlene Ditchfield	27-6-2016
Check volume and purity of fuel needed for engine tests in WP5 (Jaap van Hal)	5	Ana López Contreras	1-6-2016
Responsible partners for analysis of components	3	ECN	1-6-2016