

WP2 Conditioning, pre-treatment and storage (M3-M44)

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DTI, ECN, DLO, AVT, MATIS, FEXP, SIOEN

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Pipeline



- **Objectives**
- **Progress (M3-M6, Mar – Jun 2016) & Agreement/Plan**
- **Milestones & Deliverables**
- **Video - FEXP**



Objectives

PROCESSING of fresh/stored seaweeds for the **production of INTERMIDATES** to be converted to fuels i.e. develop methods for conditioning, pre-treatment and storage of harvested seaweed for conversion to liquid biofuels components (e.g. ethanol, butanol, furans in WP3 and WP4); the work will focus on following area:

- **Combined storage** and in-situ (biological) **pre-treatment**;
- **Mild chemical treatment** for liquefaction and partial hydrolysis of seaweed;
- **Hydrolysis** of seaweed polysaccharides to **monomeric sugars** suitable for fermentation;
- Preparation of concentrated **sugars syrups**;
- **Optimal protein recovery** in terms of sugar yield and protein value.

Progress & Agreement



WP2 period: M3 – M 44 (Mar 2016 – Aug 2019)

- **Kick-off (1st) WP2 meeting: 23th Feb 2016, 14:00 – 15:30, Skype**
- **2nd WP2 meeting: Iceland: 27th June 2016, 17:00 – 18:30, Iceland**



Progress & Agreement

- Biomass supply** (updated on 07 March 2016)

Seaweed species	DTI	Transport requirement	Delivery time	ECN	Transport requirement	Delivery time	DLO	Transport requirement	Delivery time
<i>Alaria esculanta</i> SAMS	5 kg FW	Fresh frozen	April-June 2016	1 kg DW	Fresh cooled (not frozen)	Straight after harvest	5 kg	fresh frozen	when available
<i>Sacharina latissima</i> SAMS	20 kg FW	Fresh frozen	April-June 2016	5 kg DW	Fresh cooled (not frozen)	Straight after harvest	50 kg	fresh frozen	april-june 2016
<i>Sacharina latissima</i> DTI/AU	5 kg FW	Fresh frozen		5 kg DW	Fresh cooled (not frozen)	Straight after harvest	50 kg	fresh frozen	april-june 2016
<i>Fucus vesiculosus</i> DTI/AU	5 kg FW	Fresh frozen		1 kg DW	Fresh cooled (not frozen)	Straight after harvest	5 kg	fresh frozen	when available
<i>Gracilaria vermiculophylla</i> DTI/AU	5 kg FW	Fresh frozen		1 kg DW	Fresh cooled (not frozen)	Straight after harvest	5 kg	fresh frozen	when available
<i>Palmaria palmata</i> SAMS	20 kg FW	Fresh frozen	After summer 2016	5 kg DW	Fresh cooled (not frozen)	Straight after harvest	50 kg	fresh frozen	when available
<i>Ulva lactuca</i> SAMS	5 kg FW	Fresh frozen		5 kg DW	Fresh cooled (not frozen)	Straight after harvest	5 kg	fresh frozen	when available

Progress & Agreement



- Biomass supply** (updated on 07 March 2016)

Seaweed species	AVT	Transport requirement	Delivery time	MATIS	Transport requirement	Delivery time	FEXP	Transport requirement	Delivery time	SAMS	Transport requirement	Delivery time	
<i>Alaria esculanta</i> SAMS	In total brown seaweed; 10-15 kg DW	Dried	July - Aug 2016				No specific seaweed requirement in 2016						
<i>Sacharina latissima</i> SAMS				2 kg DW	fresh frozen OR dried and milled (from DTI)	April-June 2016							
<i>Sacharina latissima</i> DTI/AU													
<i>Fucus vesiculosus</i> DTI/AU													
<i>Gracilaria vermiculophylla</i> DTI/AU													
<i>Palmaria palmata</i> SAMS				2kg DW	fresh frozen OR dried and milled (from DTI)	After summer 2016							
<i>Ulva lactuca</i> SAMS													



Progress & Agreement

- Task 2.1: **Conditioning and storage of macroalgae** (DTI, ECN; M3-36)
- **Lab-scale screw pressing (DTI) vs Lab-scale Drying (ECN)**
 - The investigation focus will be on the sugar loss.
 - DTI and ECN will deliver INTERNALLY a small report with results and protocols, which are the components of the milestone MS3 “Safe storage method of macroalgae, with less than 5% sugar loss”.

Progress & Agreement



- Task 2.2: **Storage and pre-treatment by biological and chemical ensiling** (DTI, FEX, MATIS; M6-44)
- **Lab-scale ensiling (DTI) vs Pilot-scale ensiling (FEXP)**
 - FEXP has provided DTI with one freeze-dried *Lactobacillus* strain, which has been revived and preliminarily tested the basic properties in DTI.
 - The investigation focus will be on the sugar loss.
 - The effects of sugar content in seaweed biomass and temperature will be investigated.
 - FEXP will send some ensiled biomass to DLO, DTI and MATIS for the pre-test e.g. lactic acid to ABE fermentation, enzymatic hydrolysis efficiency on the ensiling (treated) biomass.



Progress & Agreement



- Task 2.2: **Storage and pre-treatment by biological and chemical ensiling** (DTI, FEX, MATIS; M6-44)
- **Biological pretreatment (alginate hydrolysis)**
- MATIS: One thermophilic **alginate degrading** strain (*Rhodothermus marinus*) and one recombinant *Bacillus subtilis* strain producing thermostable alginase under the control of mannitol promoter are ready for use in the pre-treatment of seaweed prior to biofuel production and to instigate **alginate hydrolysis** during storage. The construction of the third alginate degrading system is based on *Lactobacillus reuteri* is ongoing.



Progress & Agreement



- Task 2.3: **Enzymatic degradation of macro-algal polysaccharides** (MATIS, DTI, DLO; M3-M36)
- **Recombinant enzymes (MATIS) vs Commercial enzymes (DTI)**
- MATIS will test two robust **recombinant alginate lyases, laminarinase (BglII), pustulanase (β -1,6,-glucanase)** on properly conditioned seaweed feedstocks in WP2. Also **auxiliary sulfatase** will also be developed to aid the release of fermentable mono-sugars from sulphated fucoidans, galactans and the polyuronate ulvan.
- MATIS has already produced three of these enzymes (**alginase – Alg3, pustulanase, and laminarinase Bgl110**) in 10 liter bioreactors, yielding few gr of each enzyme. Production of Alg3, Alg4 and laminarinase Bgl110 in 100 liters fermentor is ongoing. Production of sulfatase SulA in 10 liter fermentor is planned soon.



Progress & Agreement



- Task 2.3: **Enzymatic degradation of macro-algal polysaccharides** (MATIS, DTI, DLO; M3-M36)
- **Recombinant enzymes (MATIS) vs Commercial enzymes (DTI)**
- DTI will send dried and milled seaweed substrate to MATIS
- DTI has contacted Novozymes A/S for possible commercial enzymes supply → waiting for their reply
- DLO will focus first on *Saccharina Latissima* for the up-scaling trials for the production of sugars for ABE fermentation. Later on *Palmaria palmata* will be used. These trails will start when DLO is informed by the results from MATIS and DTI.



Progress & Agreement



- Task 2.4: **Fractionation and mild chemical treatment** (ECN, DLO, AVT; M6-36)
- **Providing hydrolyzed sugar-rich seaweed liquid fraction, to produce sugar syrups for ABE fermentation (DLO's responsibility, WP3) and precursors for furanic based fuels (ECN & AVT's responsibility, WP4)**
- Since ECN is the task leader of this task and also the leader of WP4, ECN will take the responsibility for ensuring the supply from this task to WP4.



Progress & Agreement



- Task 2.4: **Fractionation and mild chemical treatment** (ECN, DLO, AVT; M6-36)
- **Providing hydrolyzed sugar-rich seaweed liquid fraction, to produce sugar syrups for ABE fermentation (DLO's responsibility, WP3) and pre-cursors for furanic based fuels (ECN & AVT's responsibility, WP4)**
- DLO has finished preparative work for the production of sugar syrups for WP3 (i.e. method has been selected). DLO will design biorefinery schemes for brown, red and green seaweeds obtained from SAMS (i.e. *Saccharina*, *Palmaria* and *Ulva*), taking into account the type of sugars that can be fermented to ABE and possible inhibitory compounds (e.g. salts). Subsequently, DLO will perform experiments on lab scale and bench scale for isolation of sugars for ABE fermentation.
- AVT has already started preparations for pelletisation studies on dried seaweed, but do not anticipate supplying hydrolysates to WP4 in 2016, as the work in 2016 will be mainly on the effects of “synthetic” sugar mixtures.



Progress & Agreement

- Task 2.5: **Purification and concentration of algal sugar syrups** (ECN, DLO; M12-24)

Start: December 2016

- ECN has started to adapt a bench-scale membrane-based separation unit for the purification and concentration of seaweed-derived sugar syrups, so that the membrane-based separation unit can be ready to process seaweed-derived sugar streams from Month 12.
- DLO has performed a literature survey on the requirements of sugars for ABE and possible inhibitory compounds in the sugar syrup. It is expected that (high) salt concentrations, and especially potassium salts, may cause problems during ABE fermentation. For purification of the sugar syrups, focus will be on salt removal

WP2 Involved Milestones



FUELS

	Title	WP Number	Lead Beneficiary	Due Date (in months)	Means of verification
MS1	Yield of 25 kg ww/m ² ·year demonstrated on 200 m ² scale	WP1, WP2, WP6, WP7	6 - SIOEN	24	Report shortly describing how this milestone was met
MS2	Confirmation of successful crop rotation of seaweed cultivation	WP1, WP2, WP6, WP7	5 - SAMS	36	Report shortly describing how this milestone was met
MS3	Safe storage method of macroalgae	WP2, WP6, WP7	1 - DTI	18	Less than 5% sugar loss
MS4	Efficient hydrolysis of algal sugar polymers	WP2, WP6, WP7	10-MATIS, OHF	24	Hydrolysis efficiency of 85% of all fermentable sugars
MS5	Method for concentrated algal sugar syrup	WP2, WP3, WP4, WP6, WP7	2-ECN	24	Less than 3 % sugar loss
MS6	First results on fermentation to ethanol and to ABE from seaweed fractions	WP2, WP3, WP5, WP6, WP7	1 - DTI	24	Report, with acceptance of the results
MS7	First results on Butanol production by anaerobic species by fermentation	WP2, WP3, WP5, WP6, WP7	3 - DLO	12	Report, with the acceptance of the results



Deliverables



ELS

	Deliverable Title	WP Number	Lead Beneficiary	Type	Dissemination Level	Due Date (in months)
D2.1	Optimized ensiling process for seaweed storage	WP2	1 - DTI	Report	Confidential, only for members of the consortium (including the Commission Services)	36
D2.2	Developed combined ensiling and acid addition process for seaweed storage	WP2	1 - DTI	Report	Confidential, only for members of the consortium (including the Commission Services)	36
D2.3	Constructed microbial systems for biological pre-treatment on seaweed	WP2	10 - MATIS OHF	Report	Confidential, only for members of the consortium (including the Commission Services)	36
D2.4	Efficiency of enzymes applied in the pretreatment of seaweed	WP2	10 - MATIS OHF	Report	Confidential, only for members of the consortium (including the Commission Services)	36
D2.5	Chemical conversion of seaweed to monomeric and oligomeric sugars	WP2	2-ECN	Report	Confidential, only for members of the consortium (including the	36



Deliverables



	Deliverable Title	WP Number	Lead Beneficiary	Type	Dissemination level	Due Date (in months)
D2.6	Demonstration of method for algal sugar syrup production for thermochemical conversion and fermentation	WP2	2-ECN	Other	Confidential, only for members of the consortium (including the Commission Services)	24
D2.7	Production of proteins suitable for evaluation	WP2	3 - DLO	Other	Confidential, only for members of the consortium (including the Commission Services)	28
D2.8	Demonstration of long-term and pilot-scale ensiling treatment for storage of seaweed	WP2	1 - DTI	Report	Confidential, only for members of the consortium (including the Commission Services)	42



Video - FEXP



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Thank you for your attention!



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