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

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WP2 Partners

Short name of participant	Person/months per participant	Role
DTI	12	WP leader;
		Task leader of T2.1, (T2.2.1), (T2.2.2), (T2.3.2)
		Task partner of (T2.2.3), (T2.2.4), (T2.3.1)
ECN	20	Task leader of T2.4, (T2.4.1), (2.4.3), T2.5
		Task partner of T2.1
DLO	43	Task leader of (T2.4.4,) (T2.3.3)
		Task partner of (T2.4.2), (T2.4.3), T2.5
AVT	26	Task leader of (T2.4.2)
		Task partner of (T2.4.1), (T2.4.3)
SIOEN	5	Bridging WP1 & WP2
MATIS	21	Task leader of (T2.2.3), T2.3, (T2.3.1)
		Task partner of (T2.4.3)
FEX	12	Task leader of (T2.2.4)
		Task partner of (T2.2.1), (T2.2.2)

Objectives

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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.

Tasks

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- Task 2.1: **Conditioning and storage of macro-algae** (DTI, ECN; M3-36)

  Start: March, 2016

  - Task 2.1.1 **Dewatering** will be tested by **screw pressing** each **green, red and brown seaweed species**, harvested at the time of the year that they have contain high levels of carbohydrate molecules. The *species* will be dewatered to a dry matter content of 30 %.
  - Task 2.1.2 **Drying** tested in lab and pilot scale using low-cost and large-scale drying methods to be developed and implemented in this task. The quality and quantity of the stored macro-algae *species* will be monitored in time in terms of available sugars and total biomass.
  - Task 2.1.3 **Optimal logistical requirements** of large volume biomass with respect to transport from the harvesting/storage location to the bio-refinery process plant will be determined to arrive at **an optimal cost model as input for WP6.**

Tasks

- Task 2.2: **Storage and pre-treatment by biological and chemical ensiling** (DTI, FEX, MATIS; M6-44)

  Start: June 2016

  – Task 2.2.1 **Ensiling** (DTI, FEX) The fermentation effects by inoculum of homofermentative bacterias on different particle size of grinded dried algae biomass (from task 2.1) will be tested. pH change, lactic acid production, enzyme production, sugar consumption, fibre morphology change, *etc.* during the tested ensiling process will be monitored and investigated.

  – Task 2.2.2 **Combined ensiling and acid addition** (DTI, FEX). Based on the results from task 2.2.1, optimal storage will be tested using combined ensiling and acid addition (e.g. lactic acid) **to reduce loss of sugars**. pH change, lactic acid production, enzyme production, sugar consumption and fibre morphology change during this combined process will be monitored and investigated. **Treatment time will be optimized to arrive at an acceptable loss of sugars determined in collaboration with WP6.**

Tasks

- Task 2.2: **Storage and pre-treatment by biological and chemical ensiling** (DTI, FEX, MATIS; M6-44)

  Start: June 2016

  – Task 2.2.3 **Biological pre-treatment** (MATIS, DTI) **Robust and efficient alginate degrading bacteria strains will be constructed by MATIS** for use in the pre-treatment of seaweed prior to biofuel production (WP3) and to **instigate alginate hydrolysis during storage and the downstream pre-treatment steps.** Three different microbial systems will be established and examined for this purpose. They are based on *Bacillus subtilis*, *Lactobacillus sp.,* and the thermophilic marine bacterium *Rhodothermus marinus*. The organisms will be optimized for maximum sugar liberation and a minimal viscosity.

  – Task 2.2.4 Long term and pilot scale ensiling (FEX, DTI). Raw material seaweed handling and storage will be **demonstrated** (envisioned quantity on the order of tonnes wet seaweed) at **Fermentation Experts over a year based on the most promising results from** task 2.2.1-2.2.3, with on-line monitoring of pH, CO_2, water content, lactic acid bacteria, enzymes and temperature

Tasks

- Task 2.3: **Enzymatic degradation of macro-algal polysaccharides** (MATIS, DTI, DLO; M3-M36)

  Start: March 2016

  - Task 2.3.1 (MATIS , DTI) **Development and test of robust recombinant enzymes** of thermophilic and marine origins, including **alginate lyases, laminarinases, cellulases** and **xylanases**. These aforementioned enzymes will be produced in a 10 litre fermentor and tested on the properly conditioned seaweed feedstocks. Auxiliary sulfatases will also be developed to aid the release of fermentable mono-sugars from sulphated galactans and the polyuronate ulvan. The effect of the relevant **commercial enzymes** (i.e. mixtures of cellulases, β-glucanases, xylanases, galactanases, *etc.*) from Novozymes A/S (Denmark) will also be **investigated and compared** by DTI.

  - Task 2.3.2 (DTI) A pre-process fractionation stage will be developed using **most robust and salt tolerant enzymes** for **reducing viscosity, and hydrolysis of polysaccharides to fermentable mono-sugars.** Optimal design and construction of equipment will be developed to treat high dry matter algal feed stock in lab and pilot scale.

Tasks

- Task 2.4: **Fractionation and mild chemical treatment (**ECN, DLO, AVT; M6-36)
  Start: June 2016

Liquefaction of seaweed species by hydrolysis, to produce sugar syrups for ABE fermentation (WP3) and pre-cursors for furanic based fuels (WP4)

Main operating strategy:

- Process developing will be started by focusing on one selected seaweed species, with a later-stage application into all the species of project interest.

- DLO→ WP3 tasks (ABE fermentation)
  ECN &AVT→ WP4 tasks (Converting seaweed fraction and isolated sugars into furanics based fules. e.g. alginate and xylose-derived furfural, furanics based fuels from laminarin and rhamnose)

Tasks

- Task 2.4: **Fractionation and mild chemical treatment (**ECN, DLO, AVT; M6-36)

  Start: June 2016

  – Task 2.4.1 (ECN, AVT) **Liquefaction** of seaweed by **light (organic) acid or base hydrolysis** with optimization of temperature, time, catalyst and biomass loading. For fermentative purposes, the carbohydrate molecules need to be hydrolysed to their monomers. Using **organic acids** which are needed for fermentation, such as acetic acid, will be used to **produce sugar syrups suitable for fermentation** aiming at a concentration of 60 g/l. **Base hydrolysis** on the other hand can yield pre-cursors for **furanic based fuels**. For that approach we aim for sugar syrups that can be concentrated to 10% sugars. The syrups will be used in WP3 & 4.

  – Task 2.4.2 (AVT, DLO) **Mineral acid hydrolysis** of seaweed for liquefaction and partial hydrolysis will be tested at various temperature, time, acid and biomass loading. In some cases, organic acids are inhibitors for fermentation or thermochemical conversions. We aim to produce sugar syrups that contain 60 g./l. of sugar suitable for both fermentation as well as thermochemical conversion.

→ **T 2.4.1 + T2.4.2 (ECN, AVT, DLO)** Liquefaction of seaweed by hydrolysis, to produce sugar syrups and pre-cursors for furanic based fuels

Tasks

- **Task 2.4: Fractionation and mild chemical treatment** (ECN, DLO, AVT; M6-36)
  Start: June 2016

  – Task 2.4.3 (ECN, MATIS OHF, DLO, AVT) Based on results from the tasks above**, combinations of chemical and enzymatic treatments** will also be explored as well as sequential mild aqueous extractions **combined with mechanical treatment such as shredding, reactive extrusion etc.** for full hydrolysis of seaweed to monomeric sugars and processable oligomeric sugar streams. By using chemically enhanced enzymatic hydrolysis, we aim to further reduce the amount of process water by less than 50 % of the amount present in seaweed, while still obtaining sugar syrups containing 60 g/l of processable sugars.

  – Task 2.4.4 (DLO) **mechanical treatment** to yield sugar solutions for WP 3 and 4 will be **optimized** using among **others screw presses, stirred reactors, extruders and similar equipment** to yield solutions which contain sugars that are suitable for fermentation and or thermochemical conversions. Insights from literature on the structural components will guide this optimisation. **Optimal protein recovery** both in terms of fermentable and convertible sugar as well as protein value (WP 5) will be developed. **Data for WP5 for the protein in terms of solubility and emulsifying properties** will be generated.

Tasks

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

  Start: December 2016

  – The sugar solutions derived from Task 2.3 and 2.4 will be **upgraded by purification** (e.g. ion-exchange) and **further concentrated** (e.g. membrane filtration or evaporation of water) to develop an **intermediate sugar syrup** with properties suitable for fermentation (WP3) and thermochemical conversion (WP4), with a minimal concentration of 60 g/l (if not already achieved) for fermentations or 10-20 % for thermochemical conversions.

WP2 involved milestones

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Milestone number	Milestone name	Related work package(s)	Estimated date	Means of verification
M1.1	Yield of 25 kg ww/m^2.year demonstrated on 200 m^2 scale	WP1,2,6,7	M24	Report shortly describing how this milestone was met
M1.2	Confirmation of successful crop rotation of seaweed cultivation	WP1,2,6,7	M36	Report shortly describing how this milestone was met
M2.1	Safe storage method of macroalgae	WP2,6,7	M18 June 2017	Less than 5% sugar loss
M2.2	Efficient hydrolysis of algal sugar polymers	WP2,6 7	M24	Hydrolysis efficiency of 85% of all fermentable sugars
M2.3	Method for concentrated algal sugar syrup	WP2,6,7	M24	Less than 3 % sugar loss
M3.1	First results on fermentation to ethanol and to ABE from seaweed fractions	WP2,5,6,7	M24	Report, with acceptance of the results
M3.2	First results on Butanol production by anaerobic *species* by fermentation	WP2,5 6,7	M12 December 2016	Report, with the acceptance of the results

WP3: Biochemical fuel production
WP6: Techno-economic and sustainability assessment
WP7: Dissemination, communication and exploitation

Deliverables

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Deliverable (number)	Deliverable name	Work package number	Short name of lead participant	Type	Dissemination level	Delivery date
D2.1	Optimized ensiling process for seaweed storage	WP2	DTI	R	CO	M36
D2.2	Developed combined ensiling and acid addition process for seaweed storage	WP2	DTI	R	CO	M36
D2.3	Constructed microbial systems for biological pre-treatment on seaweed	WP2	MATIS OHF	R	CO	M36
D2.4	Efficiency of enzymes applied in the pre-treatment of seaweed	WP2,3	MATIS OHF	R	CO	M36
D2.5	Chemical conversion of seaweed to monomeric and oligomeric sugars	WP2,3,4	ECN	R	CO	M36
D2.6	Demonstration of method for algal sugar syrup production for thermochemical conversion and fermentation	WP2,3,4	ECN	Other	N/A	M24
D2.7	Production of proteins suitable for evaluation	WP2,5	DLO	Other	N/A	M28
D2.8	Demonstration of long-term and pilot-scale ensiling treatment for storage of seaweed	WP2	DTI	R	CO	M42

Dec, 2017

1st WP2 meeting

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- Skype meeting
- Late February/Early March, 2016

- Informing cultivation status
- Collecting biomass requirement
- Selecting the starting seaweed species
- Other feedbacks

Acknowledgement

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