4th WP2 meeting

WP2 Conditioning, pre-treatment and storage

Wageningen, the Netherlands

10th January, 2017

Objectives

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To follow up the WP2 progress (M6-M12) and discuss common interests and/or possible problems

Biomass status

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Biomass for year 2016 has been delivered to all WP2 partners.

e.g. DTI 2016 received:

- Air-dried *Saccharina latissima* (cultivated & harvested by SAMS in Scotland from 16th Aug, 2016): 2 kg
- Frozen *Saccharina latissima* (cultivated & harvested by SAMS in Scotland from 16th Aug, 2016): 5 kg
- Frozen *Palmaria Palmata* (cultivated & harvested by SAMS in Scotland from 16th Aug, 2016): 2 kg
- Frozen *Ulva lactuca* (cultivated & harvested by SAMS in Scotland from 16th Aug, 2016): 1 kg
- Frozen *Fucus vesiculosus* (Wild type collected by AU in Denmark from 16th Jul, 2016): 3.5 kg
- Frozen Gracilaria vermiculophylla (Wild type collected by AU in Denmark from 5th Jul, 2016): 1.5 kg

Biomass request 2017

Biomass request from WP2_2017		DTI			ECN			DLO			AVT			MATIS			FEXP		
		Biomass amount (note down kg-FW or kg-DW)*	Transport requirement	Delivery time	Biomass amount (note down kg-FW or kg-DW)*	Transport requirement	Delivery time	Biomass amount (note down kg-FW or kg-DW)*	Transport requirement	Delivery time	Biomass amount (note down kg-FW or kg-DW)*	Transport requirement	Delivery time	Biomass amount (note down kg-FW or kg-DW)*	Transport requirement	Delivery time	Biomass amount (note down kg-FW or kg-DW)*	Transport requirement	Delivery time
Brown seaweed	*Alaria esculanta* (SAMS)	5 DW + 5 FW	Air-dried for dried material; Frozen for wet material	>May	50 FW	TBD	>May	70 DW	Air-dried to >80% dw	Direct after harvest				5 DW	Air-dried for dried material; Frozen for wet material	>May	*5 kg FW*	*Fresh frozen*	*When possible*
Brown seaweed	*Sacharina latissima* (SAMS)	10 DW + 10 F		>May	100 FW	TBD	>May	60 DW			10 DW of the same type of seaweed we received earlier in 2016, namely Sacharina latissimi (SAMS) -- Comment from Martijn			15 DW + 10 FW		>May	*5 kg FW*	*Fresh frozen*	*When possible*
Brown seaweed	*Fucus vesiculosus* (AU)	5 DW + 5 FW		>May	50 FW	TBD	>May	5 DW						5 DW		>May	*5 kg FW*	*Fresh frozen*	*When possible*
Red seaweed	*Gracilaria vermiculophylla* (AU)	2 DW + 2 FW			10 FW	Fresh		2 DW									*5 kg FW*	*Fresh frozen*	*When possible*
Red seaweed	*Palmaria palmata* (SAMS)	2 DW + 2 FW			No (lab), may-be for task 2.5			5 DW						10 DW*		when possible	*5 kg FW*	*Fresh frozen*	*When possible*
Green seaweed	*Ulva lactuca* (SAMS)	2 DW + 2 FW			No (lab), may-be for task 2.5			None						10 DW*		when poss	*5 kg FW*	*Fresh frozen*	*When possible*
					FW	Depends on outcome comparison tests.								*If possible					
	*FW=Fresh Weight DW=Dry Weight				Minimum														

- This request has been sent to WP1 on 5th January 2017
- The estimation of biomass amount requirement for year 2018:

  Should be ready by September/October 2017, before WP1 (SAMS) deployment

Update of WP1 status

- **SAMS has deployed *Saccharina* and *Alaria* on different SIOEN's substrates (ribbons and nets) in October to December, for the bulk biomass required for year 2017.**
- **Due to a slow growth rate, *Palmaria* activities have been reduced within a Macrofuels context. SAMS is however, continuing to develop *Palmaria* seeding methods through the activities of a Masters student.**
- **SAMS has begun lab seeding trials using *Ulva zoospores* with the view to deploy seeded material later this year.**
- Other underway trials: the development of growing systems that will allow easier harvest; optimisation of the time for deploying seeded material and to investigate how coppicing could be used to increase yield per annum.

Task 2.1: Conditioning and storage of macro-algae

- Task 2.1.2 **Drying** tested in lab and pilot scale using low-cost and large-scale drying methods. The quality and quantity of the stored macro-algae *species* will be monitored in time in terms of available sugars and total biomass.
- Contribution ECN: carbohydrate analysis for seaweeds dried at DTI.
- Plan 2017: milestone 2.1 (M18, Safe storage method for macroalgae). Less than 5% sugar loss.

Task 2.1 Conditioning & Storage of macro-algae by de-watering, silaging and drying (DTI, ECN)

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- **Oven drying effects**

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Saccharina latissima (SAMS) harvested on August 2016, frozen transported

Unfreeze & Separate

Heavily fouling part

Less fouling part

Duplicate/oven

105°C | 70°C | 55°C

Carbohydrate analysis — ECN

Task 2.1 Conditioning & Storage of macro-algae by de-watering, silaging and drying (DTI, ECN)

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- **Oven drying effects**

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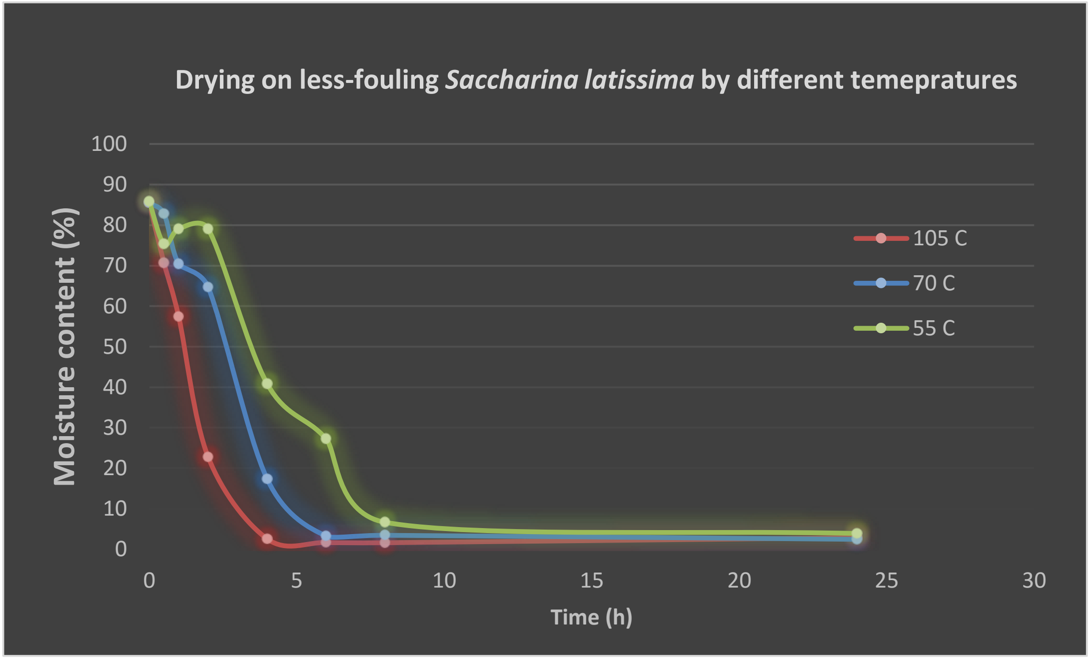

Drying temperature	moisture (%)		Hours for reaching steady weight
105°C	3.0	2.2	4
70°C	3.2	3.4	8
55°C	4.5	3.4	between 8~24 hours, missed the point

Task 2.1 Conditioning & Storage of macro-algae by de-watering, silaging and drying (DTI, ECN)

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- **Oven drying effects**

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ECN

	Temperature (°C)	Glucose (% DM)	Xylose (% DM)	Galactose (% DM)	Fucose (% DM)	Mannose (% DM)	Mannitol (% DM)
Less-fouling *S. latissima*	105	5.9	-	0.78	1.90	-	6.57
	70	6.7	-	0.80	2.23	-	7.05
	55	7.0	-	0.79	2.21	-	7.63
Heavily-fouling *S. latissima*	70	2.63	-	0.60	0.98	-	4.48
Un-separated *S. latissima*	air-dried by SAMS	3.55	-	0.52	1.49	-	3.15

Moisture Content Dry Seaweeds

Sample	Moisture (%)
P palmata fresh aug2016 (freeze-dried @ECN)	6.3
P palmata air-dried aug2016 (further dried in oven @ECN)	6.7
U lactuca fresh aug2016 (freeze-dried @ECN)	10.6
U lactuca air-dried aug2016 (further dried in oven @ECN)	10.4
S lattisima fresh aug2016 (freeze-dried @ECN)	4.9
S lattisima air-dried aug2016 (further dried in oven @ECN)	5.9

Observations:

- Each seaweed seems to have its own equilibrium moisture content when it is dry.
- Moisture contents ECN are higher than those determined by DTI after drying for task 2.1.

Seaweed Composition

- Composition of MacroFuels seaweed batches 2016 determined with methods presented at Reykjavik meeting.
- Spread within consortium Dec 2nd 2016.
- More detailed information on status method development available upon request.
- Planned activities 2017:
  - Optimization analytical hydrolysis for various seaweeds with NREL protocol (i.e., 2nd step @ 121°C).
  - Scientific publication.

Task 2.1 Conditioning & Storage of macro-algae by de-watering, silaging and drying (DTI, ECN)

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- **Screw-Pressing**

Defrost less-fouling *Saccharina*, *Palmaria*, *Ulva*

Lab-scale Screw Press

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Liquid fraction

- Keep in -20 °C
- Mapping composition
- Bioactivity test

Solid fraction

- Keep in -20 °C
- Mapping composition

Task 2.1 Conditioning & Storage of macro-algae by de-watering, silaging and drying (DTI, ECN)

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- **Screw-Pressing**

	DM (%)	Ash content (% DM)
Saccharina fresh	13.2	37.0
Solid fraction after screw-pressing	14.6	34.1
Palmaria fresh	22.9	18.9
Solid fraction after screw-pressing	29.3	14.0
Ulva fresh	17.7	31.8
Solid fraction after screw-pressing	26.2	29.2

Liquid fraction from	Total amount recovered after screw-pressing (% w/w)	DM(%)	Ash content (%)
Saccharina	7.7	7.2	56.4
Palmaria	19.3	11.5	42.6
Ulva	39.2	7.5	69.9

	Total recovery (%)[a]	Total recovery (%)[b]
Saccharina	72.4	76.0
Palmaria	62.7	61.2
Ulva	57.1	57.2

a. Based on ash content
b. Based on weight

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Task 2.1 Conditioning & Storage of macro-algae by de-watering, silaging and drying (DTI, ECN)

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- **Screw-Pressing**

✓ Dewatering to a DM content of 30% by Screw Pressing is possible for *Palmaria* and *Ulva* by Screw Pressing, a bit challenging to *Saccharina*

✓ Salts together with water are partly removed by Screw Pressing, which might benefit the following biological process e.g. enzymatic hydrolysis and fermentation

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Task 2.1 Conditioning & Storage of macro-algae by de-watering, silaging and drying (DTI, ECN)

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- **Plan for next 6 months (M12-M18)**

➢ Mapping the composition of liquid and solid fraction after screw pressing

➢ Bioactivity test of liquid fraction to explore its potentials as by-products of biofuel production

➢ Further investigation on the solid fraction (e.g. the suitability as feedstocks for ensiling & enzymatic hydrolysis)

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Task 2.2 Storage and pretreatment by biological and chemical ensiling (DTI, FEXP)

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- **Lab-scale ensiling**

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❑ **Investigation of possible easy monitoring for lactic acid production during ensiling process: CO_2 loss – lactic acid production correlation**

- **Mixed culture (homolactic *Pediococcus pentosaceus* +homolactic *Pediococcus acidilatici* + heterolactic *Lactobacillus plantarum*)**

✓ provided by FEXP (commercial product Pig Stabilizer 600)

✓ Total bacterial concentration: 2 x 10^{10} bacteria per gram

- **Fermentation flasks (200 mL) with yeast locks**

Task 2.2 Storage and pretreatment by biological and chemical ensiling (DTI, FEXP)

MACROFUELS

- **Lab-scale ensiling**

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❑ **Investigation of possible easy monitoring for lactic acid production during ensiling process: CO_2 loss – lactic acid production correlation**

- ✓ **Modified deMan-Rogosa Sharpe (MRS) medium ($g \bullet L^{-1}$):** casein peptone (10); yeast extract (5), glucose (20), K_2HPO_4 (2), sodium acetate (5), diamonium citrate (2), $MgSO_4 \bullet 7H_20$ (0.2)
- ✓ **35ºC**
- ✓ **No light**
- ✓ **pH=6.2-6.5**
- ✓ **No shaking**

- ✓ **Inoculum concentration: 5 % (v/v) liquid culture with OD(570 nm)=2.9**

- ✓ **Weight loss (due to the production of CO2) measured by balance**

✓

Task 2.2 Storage and pretreatment by biological and chemical ensiling (DTI, FEXP)

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- **Lab-scale ensiling**

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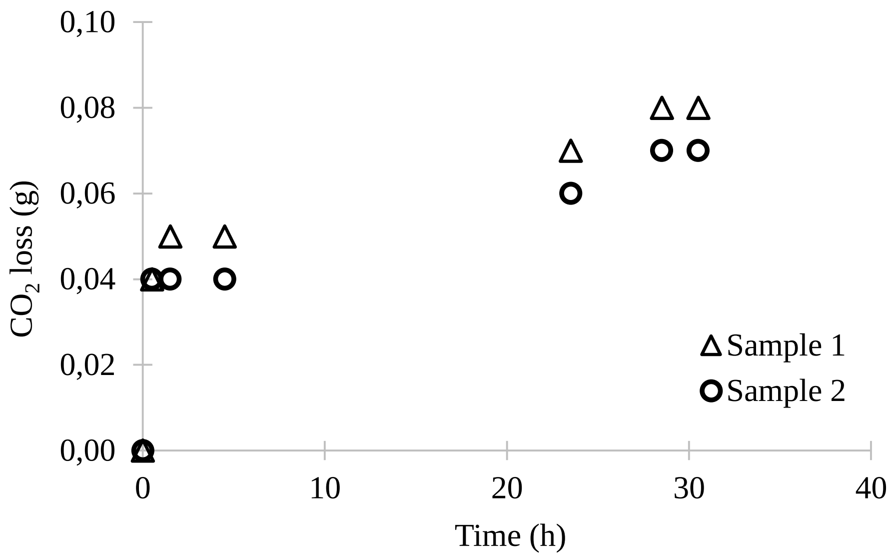
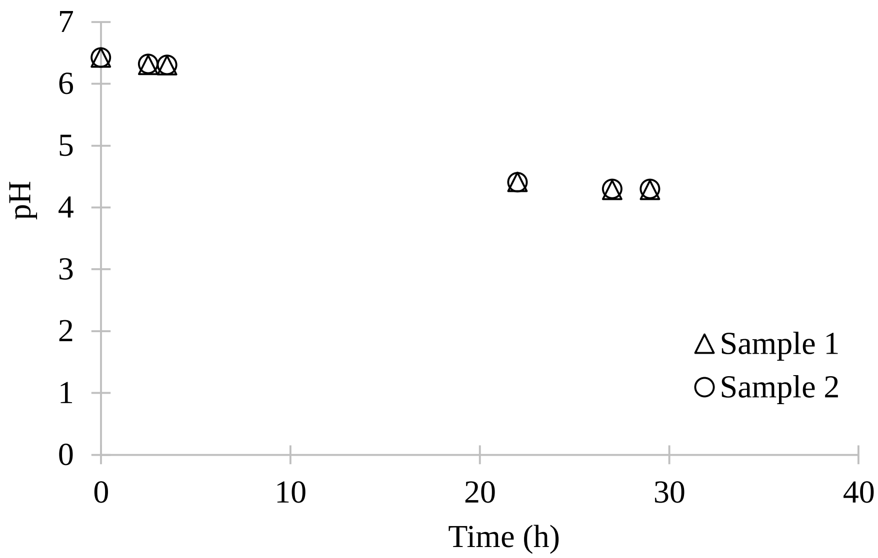
❑ **Investigation of possible easy monitoring for lactic acid production during ensiling process: CO_2 loss – lactic acid production correlation**





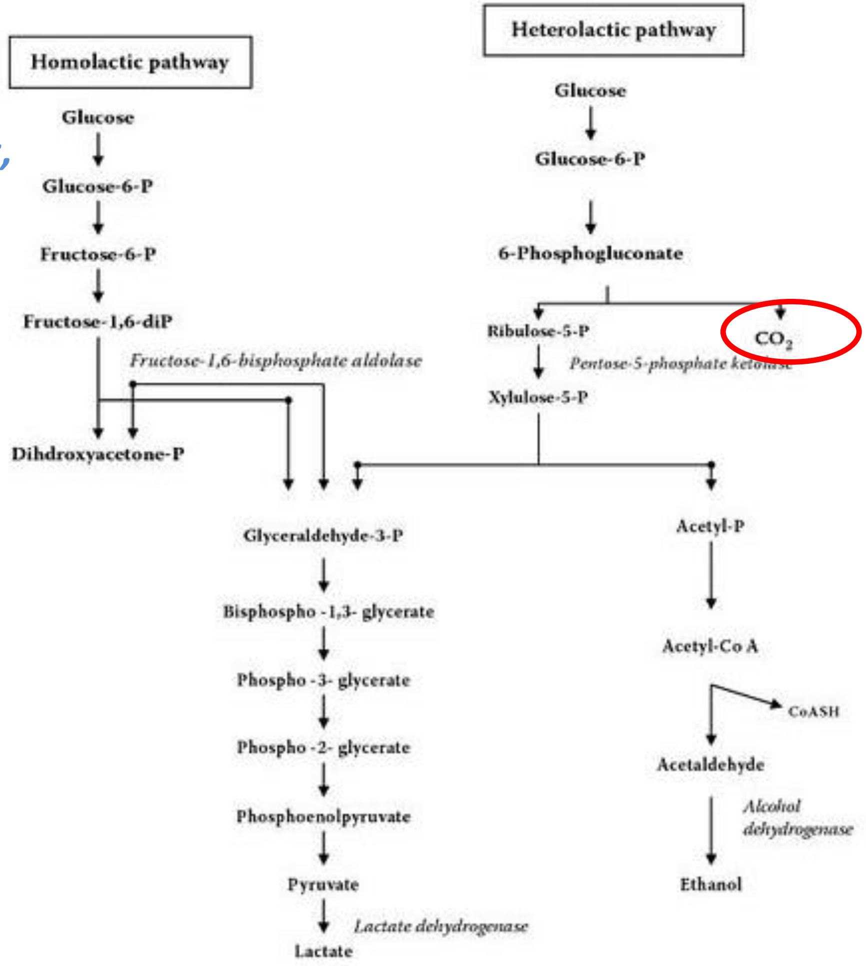
Weight loss (CO_2 production) from MRS fermentation by the mixed culture is 20 % from the maximum theoretical CO_2 production from heterolactic fermentation. Possible reasons

Task 2.2 Storage and pretreatment by biological and chemical ensiling (DTI, FEXP)

- **Lactic acid fermentative pathways**

Pediococcus pentosaceus, Pediococcus acidilatici

Lactobacillus plantarum



Task 2.2 Storage and pretreatment by biological and chemical ensiling (DTI, FEXP)

- **Lab-scale ensiling**

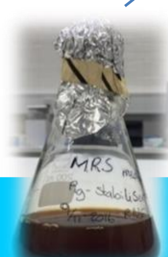
❑ **Protocol establishment for seaweed biomass ensiling**

+ MRS (- glucose)

Natural ensiling

Homogenized sugar-rich *Saccharina latissima*

+ MRS (+ glucose)



Sugar+microbe enriched ensiling

Task 2.2 Storage and pretreatment by biological and chemical ensiling (DTI, FEXP)

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- **Lab-scale ensiling**

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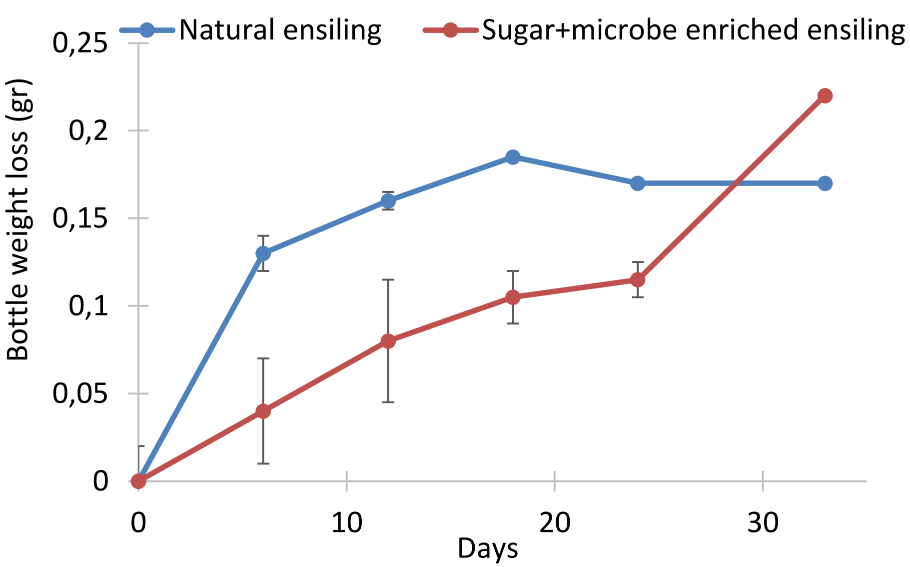
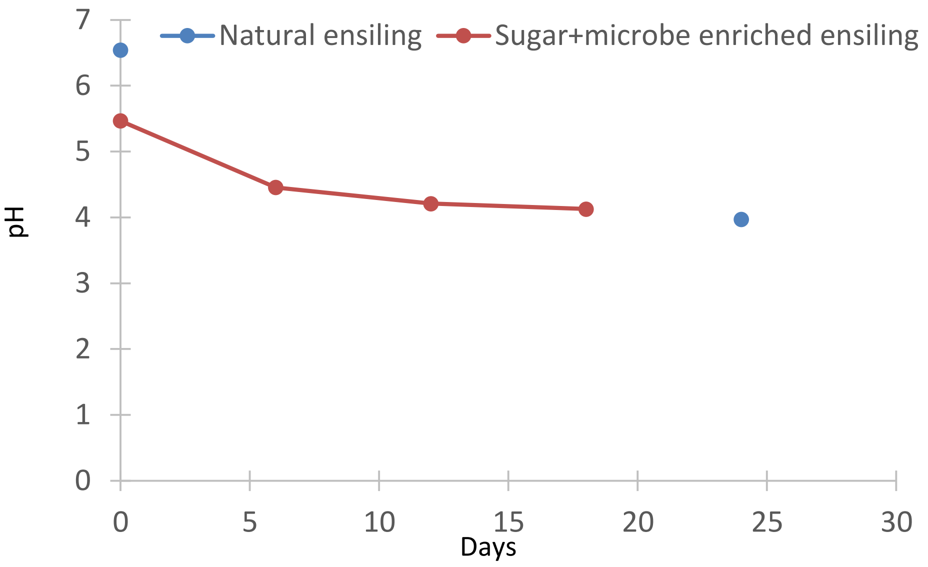
❑ **Protocol establishment for seaweed biomass ensiling**





Waiting for HPLC results for sugars and lactic acids

Task 2.1 Conditioning & Storage of macro-algae by de-watering, silaging and drying (DTI, ECN)

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- **Lab-scale ensiling**

✓ Weight loss (resp. CO_2 production) was correlated with pH drop probably due to lactic acid production

✓ lactic acid production is waiting for being proven by HPLC results

✓ Natural ensiling was observed

✓ Protocols for seaweed ensiling is established

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Task 2.1 Conditioning & Storage of macro-algae by de-watering, silaging and drying (DTI, ECN)

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- **Plan for next 6 months (M12-M18)**

- ➢ Alternative ways of addition of lactic acid bacteria (e.g. spraying the seaweed with fresh bacterial culture) for better mass transfer and more efficient fermentation rates
- ➢ Ensiling effects by pure heterolactic e.g. *Lactobacillus plantarum* VS natural bacteria. The possible synergistic effects of natural bacteria and the inoculum will be investigated.
- ➢ Effects of substrate concentration (water content)

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Task 2.3 Enzymatic hydrolysis of macroalgal polysaccharides (MATIS, DTI, DLO)

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Development and test of robust recombinant enzymes of thermophilic and marine origins (MATIS)

- Seaweed hydrolysate (pilot scale, DTI) has been received by MATIS on 15th September
- No success of asking for free enzymes from Novozymes A/S

Task 2.4 Fractionation and mild chemical treatment (ECN, DLO, AVT)

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Task 2.5 Purification and concentration of algal sugar syrups (ECN, DLO)

Free Discussion

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- 1st Year Scientific Report of WP2

Next WP Meeting

- Together with the next project meeting?
- Or: Mobilemeeting?

Acknowledgement

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Thank you for your attention!

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