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

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

MacroFuels 3rd Project meeting, Bruges, Belgium

4th July 2017

Pipeline

- **Objectives**
- **Deliverables**
- **Progress (M12-M18) & Future Plan (M19-M24)**

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.

Deliverables

	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

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	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**

M12-M18 Progress

M12 – M18:

- **4th WP2 meeting:** 10th Jan 2017, 10:00 – 12:00, Wageningen, the Netherlands
- **5th WP2 meeting**: 18th Jan 2017, 13:30 – 15:00, Skype
- **6th WP2 meeting:** 3rd July 2017, 14:00 – 16:30

M12-M18 Progress

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

- **To de-water the seaweed species to a dry matter content of 30%, by Screw-Press**
- **To stable store seaweed biomass with less than 3% sugar loss**

M12-M18 Progress

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

Screw Pressing test :

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

Defrost *Alaria*

- ✓ Composition mapping is waiting

Future Plan M19-M24

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

- 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)
- Further develop/optimize the screwpressing e.g. by diluted acid (0.1 M HCl) pretreatment or salt grinding.

M12-M18 Progress

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

- **Cost-effective**

M12-M18 Progress

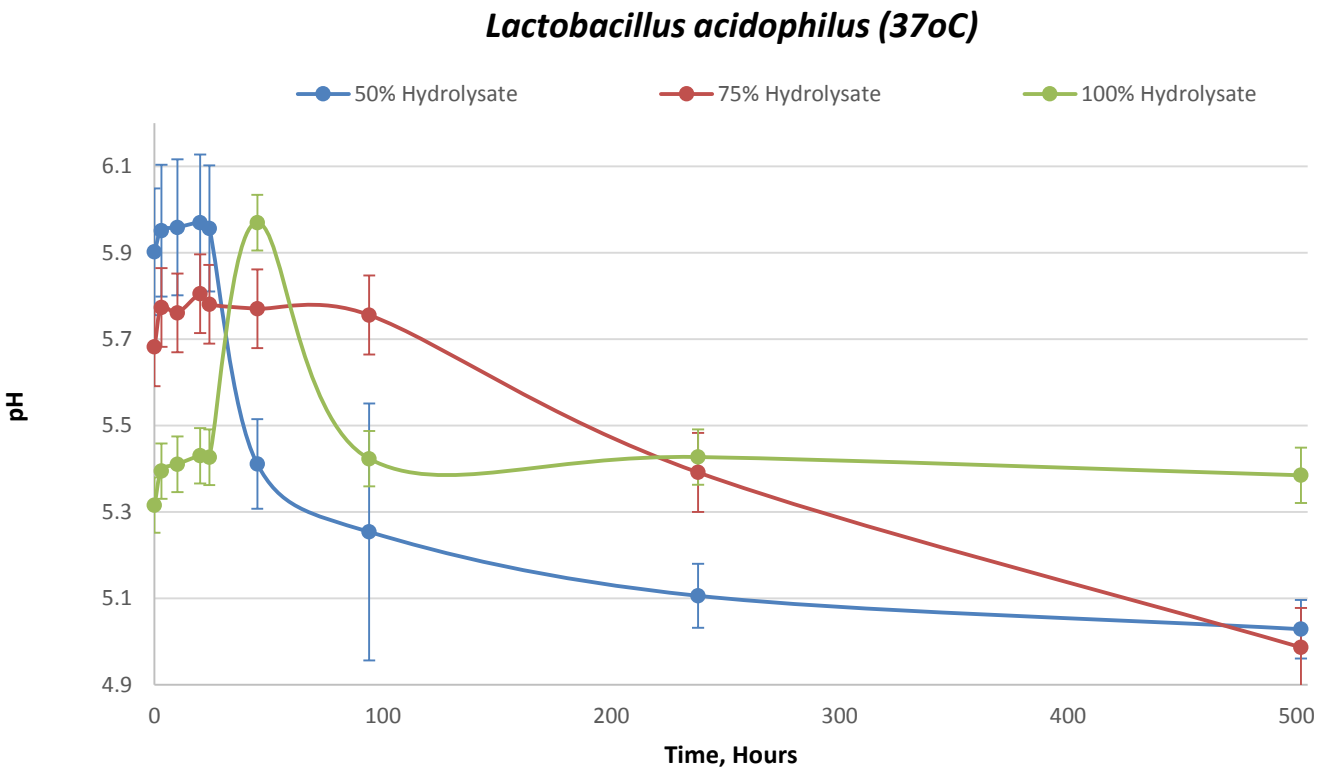
Task 2.2: **Storage and pre-treatment by biological and chemical ensiling** (DTI, FEX, MATIS; M6-44)

Lab-scale ensiling:

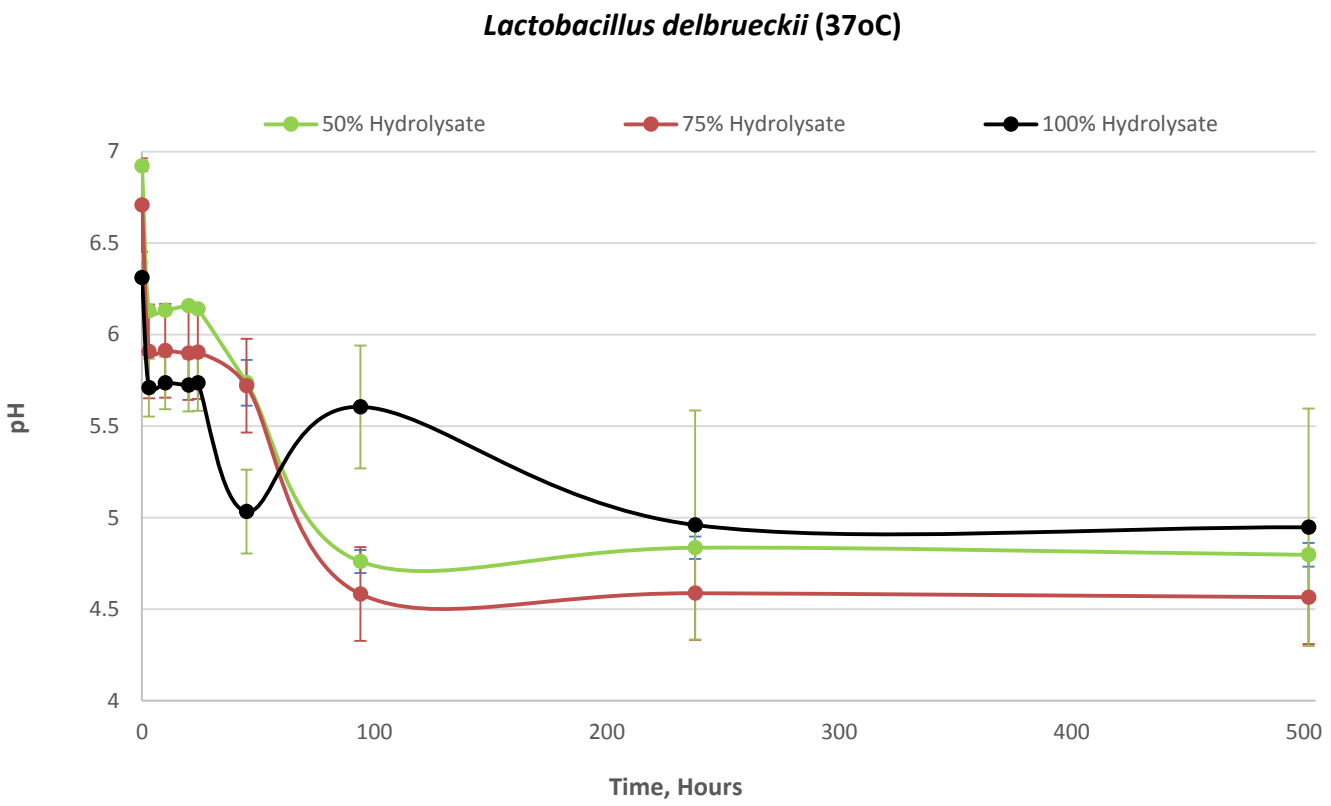
✓FEXP has provided DTI with one freeze dried pure culture & one commercial product of mixed culture incl. 3 lactic acid bacteria strains

✓*Lactobacillus delbrueckii* showed best performance for ensiling of hydrolysate, lowest pH drop (4.5) obtained after 4-5 days

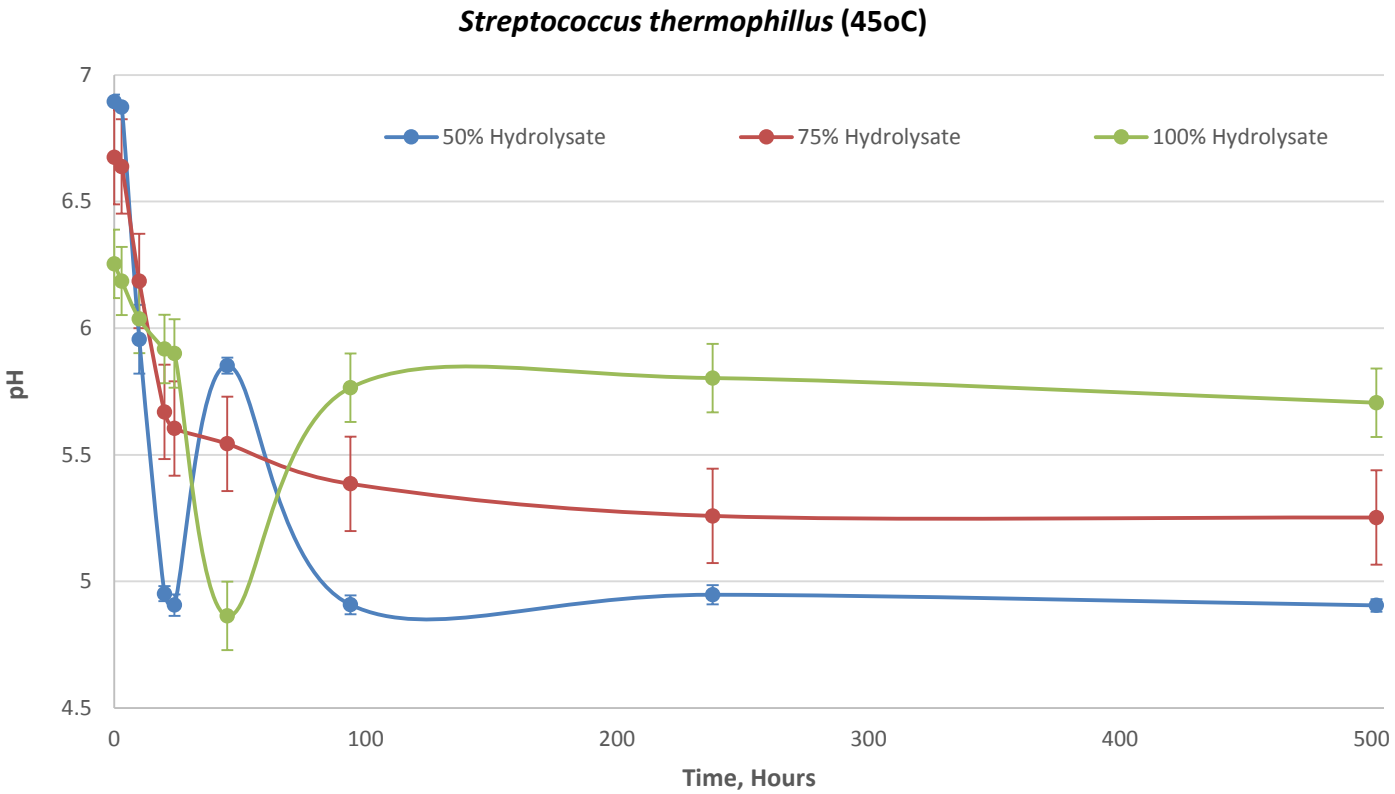
Biological ensiling results of DTI:
seaweed hydrolysate



Biological ensiling results of DTI:
seaweed hydrolysate



Biological ensiling results of DTI:
seaweed hydrolysate



M12-M18 Progress

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

✓One **thermophilic alginate degrading** strain (*Rhodothermus marinus*) and one recombinant *Bacillus subtilis* strain producing thermostable alginate lyase under the control of mannitol promoter are ready for the purpose of pre-treatment.

✓The construction of the other two systems for alginate degrading i.e. the recombinant *Lactobacillus reuterii* and *Bacillus subtilis* is under going.

Future Plan M19-M24

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

- Effect of mixed culture (FEX) fermentation on the process performance (pH drops, lactic acid production, sugars profile) will be investigated.
- Combination of biological and chemical ensiling (by addition of lactic or formic acid) will be investigated.
- The three strains with alginate lyases activities (native *Rhodothermus marinus* and recombinant *Lactobacillus reuterii* and *Bacillus subtilis*) will be investigated for their behaviors on brown seaweed with respect to temperatures and aerobic/anaerobic conditions.

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Progress task 2.4 – part ECN

Wouter Huijgen (ECN)

MacroFuels Progress Meeting, Brugge
4th July 2017

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Task 2.4: Fractionation & chemical treatment

- **<u>Goal: Liquefaction of seaweed species by hydrolysis, to produce sugar syrups for ABE fermentation (WP3) and pre-cursors for furanic based fuels (WP4)</u>**

- 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**.

- 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.

→ **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

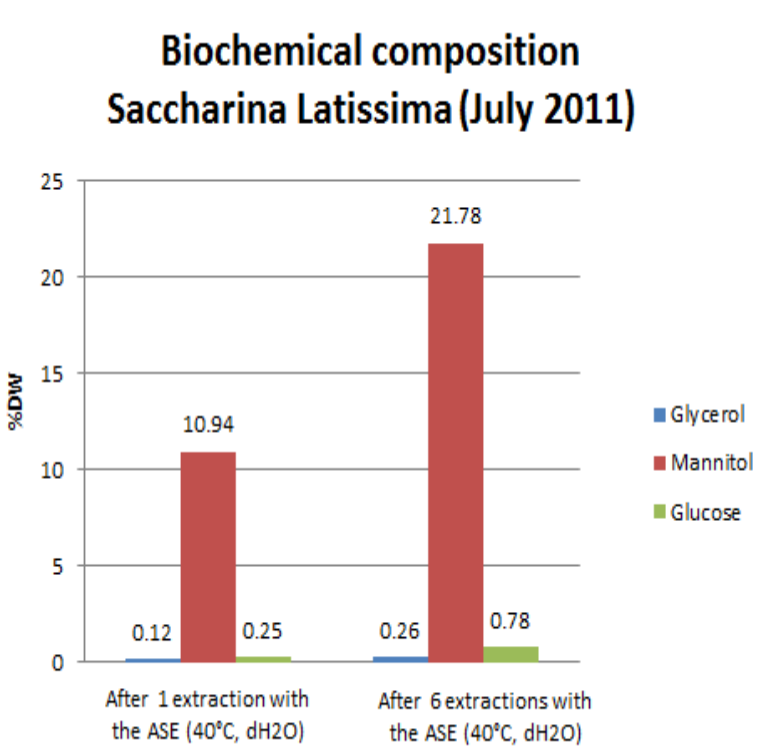
- Task 2.4.3 (ECN, MATIS OHF, DLO, AVT) Based on results from the tasks above**, combinations of chemical and enzymatic treatments** will also be explored as well as sequential mild aqueous extractions **combined with mechanical treatment such as shredding, reactive extrusion etc.** for full hydrolysis of seaweed to monomeric sugars and processable oligomeric sugar streams.

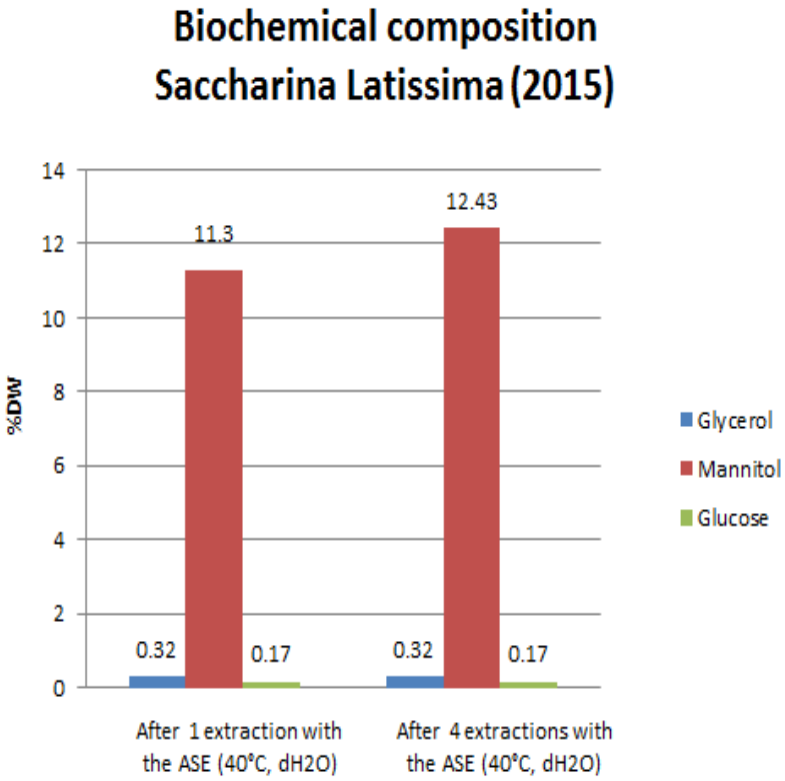
- Task 2.4.4 (DLO) **mechanical treatment** to yield sugar solutions for WP 3 and 4 will be **optimized** using among **others screw presses, stirred reactors, extruders and similar equipment** to yield solutions which contain sugars that are suitable for fermentation and or thermochemical conversions.

Seaweed Composition

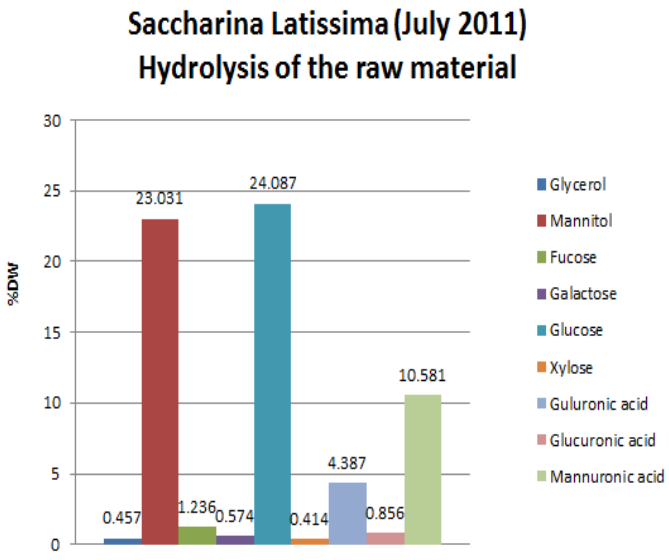
- Composition of MacroFuels seaweed batches 2016 determined with methods presented at Reykjavik meeting.
- Activities 2017:
  - Publication (book chapter).
  - Further completion of composition:
    - Pre-extraction.
    - Alginate determination.
  - Optimization analytical hydrolysis for various seaweeds with NREL protocol (i.e., 2nd step @ 121°C).

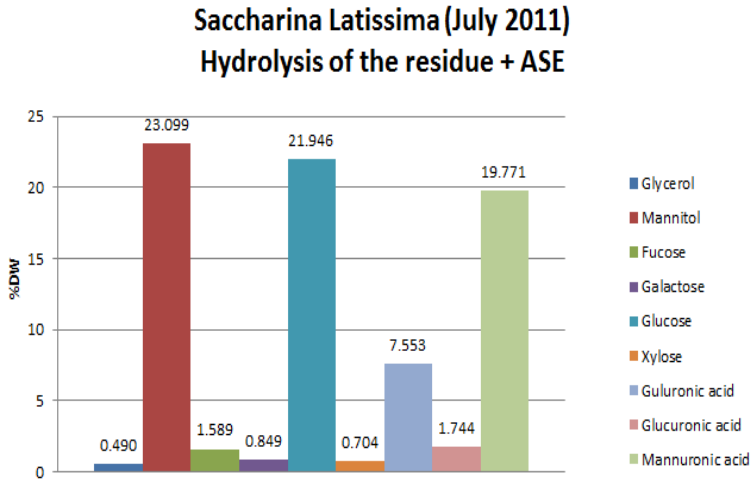
ASE


Biochemical composition
Saccharina Latissima (July 2011)
%DW
25
20
15
10
5
0
10.94
21.78
0.12
0.25
0.26
0.78
After 1 extraction with the ASE (40°C, dH2O)
After 6 extractions with the ASE (40°C, dH2O)
Glycerol
Mannitol
Glucose


Biochemical composition
Saccharina Latissima (2015)
%DW
14
12
10
8
6
4
2
0
11.3
12.43
0.32
0.17
0.32
0.17
After 1 extraction with the ASE (40°C, dH2O)
After 4 extractions with the ASE (40°C, dH2O)
Glycerol
Mannitol
Glucose

Balances


Saccharina Latissima (July 2011)
Hydrolysis of the raw material
%DW
30
25
20
15
10
5
0
23.031
24.087
10.581
4.387
0.457
1.236
0.574
0.414
0.856
Glycerol
Mannitol
Fucose
Galactose
Glucose
Xylose
Guluronic acid
Glucuronic acid
Mannuronic acid


Saccharina Latissima (July 2011)
Hydrolysis of the residue + ASE
%DW
25
20
15
10
5
0
23.099
21.946
19.771
7.553
0.490
1.589
0.849
0.704
1.744
Glycerol
Mannitol
Fucose
Galactose
Glucose
Xylose
Guluronic acid
Glucuronic acid
Mannuronic acid

Saccharification Palmaria

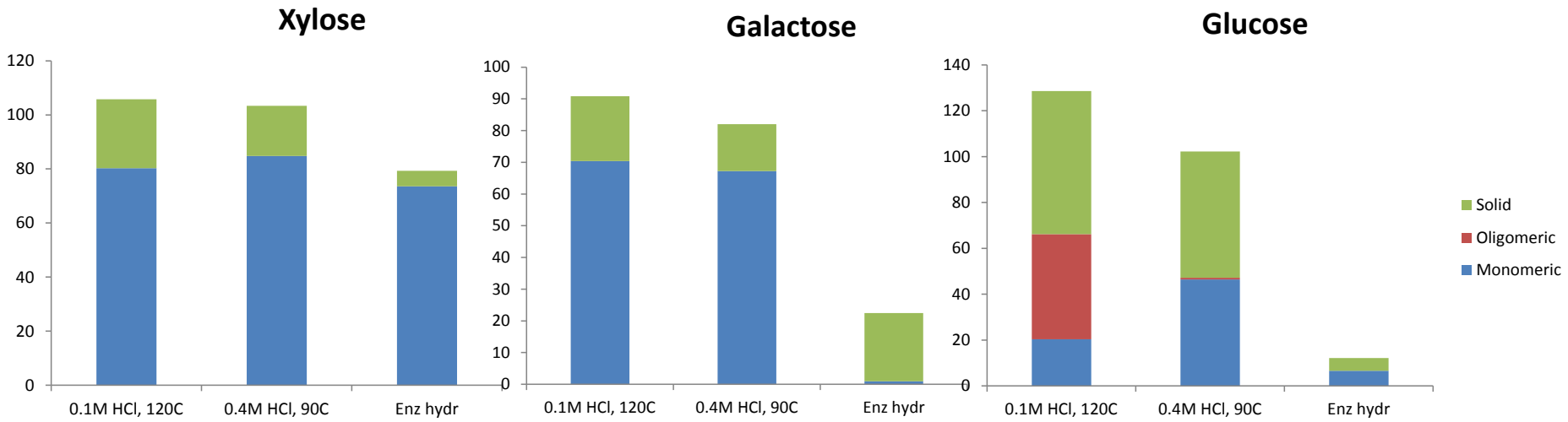
- Hydrolysis tests performed with fresh seaweed (1:1 w/w):
  1. 120 °C, 0.1M HCl, 2hr (optimum previous project*).
  2. 90 °C, 0.4M HCl, 3hr (optimum literature)
  3. Enzymatic hydrolysis (Accellerase TRIO/XY), 24hr.

- Product liquors delivered to:
  - WUR-FBR, ABE fermentation (WP3): 1 & 3.
  - ECN, furfural conversion (WP4): 1 & 2.

** W.J.J. Huijgen, A. Lopez Contreras, J. van Doorn & J.W. van Hal (2017) Xylose production and fermentation from red seaweed (Palmaria palmata) (in preparation).*

Yields & Mass Balances

- Residual solid: 33-36 dw%.
- Mass balances adequately closed for HCl-catalyzed processes, except Glc.
- Yield monomeric Xyl up to 85% and Gal up to 70%.
- Both HCl-catalyzed processes perform equally for Xyl and Gal.
- Xylan-backbone *P palmata* hydrolysable with commercial enzymes. However, no hydrolysis activity for floridoside and glucobiose (no cellulose?).



Planned activities 2017

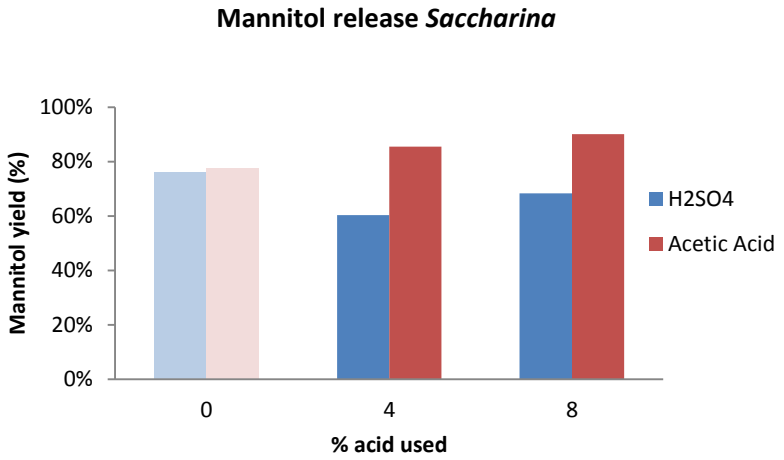
- M2.2 (M24, Efficient hydrolysis of algal sugar polymers). Goal: Hydrolysis efficiency of 85% of all fermentable sugars.
- Effect of feedstock storage method on biorefining (testing frozen and air-dried seaweeds).
- Alkaline liquefaction and combined chemical-enzymatic saccharification of Kelps.
- Construction and testing of new seaweed processing line (incl upscaling from 20L (current volume) to 100L).

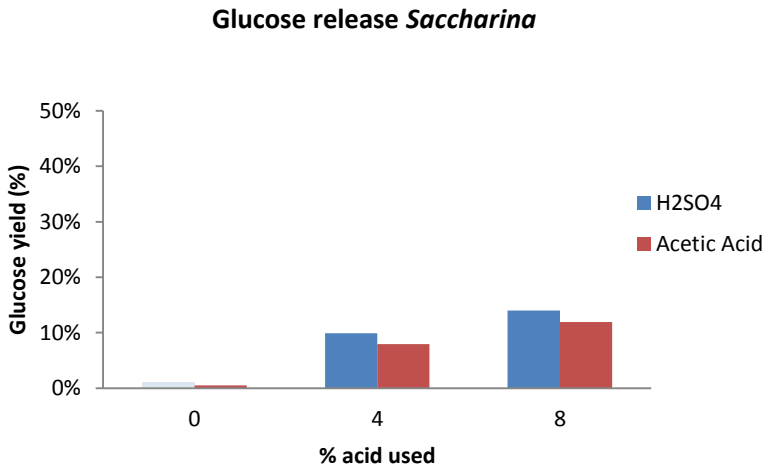
Acid hydrolysis (WUR)

- Cut *Saccharina latissima* and *Palmaria palmata*
- Hydrolysis at 100 or 130 ^{o}C for 60 minutes
- Sulfuric acid or acetic acid
  - 4 or 8% (w/dw) H_2SO_4
  - 1:10 biomass:liquid ratio
- 100 ml scale

Weak acid hydrolysis *Saccharina* (liquid phase) (WUR)

- Same approach as with strong acids
  - 4 or 8% acetic acid, 100 ^{o}C, 1 hour
- Higher mannitol release, similar glucose



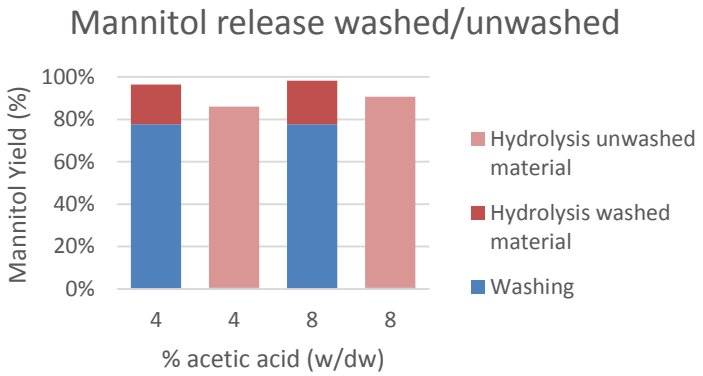


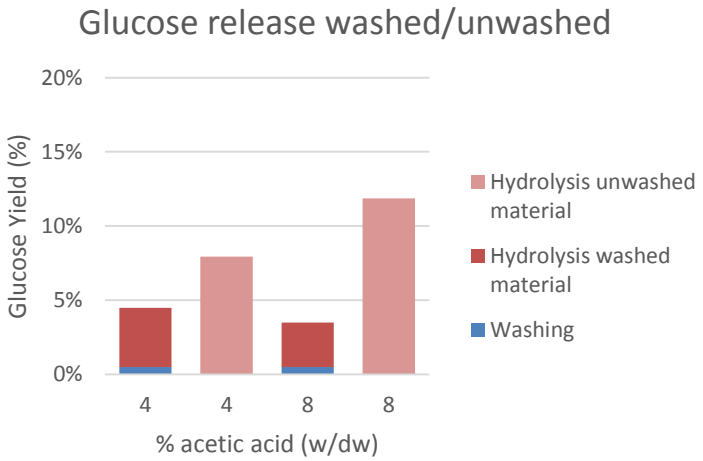
Hydrolysis of washed material (WUR)

- Cut *Saccharina latissima* and *Palmaria palmata*
- Wash (stirred) with fresh water (1:25 dw/w)
- Hydrolysis washed material at 100 ^{o}C for 60 minutes
- Sulfuric acid or acetic acid
  - 4 or 8% (w/dw) H_2SO_4
  - 1:10 biomass:liquid ratio
- 100 ml scale

Washing effect (WUR)

- Washing has similar effect on weak and strong acid
- More glucose extracted without washing





Conclusions (WUR)

Saccharina latissima

- Easy mannitol release
- Low glucose release
  - Similar for both acids
  - Washing reduces effect of acid hydrolysis

Future work (WUR)

- Repeat experimental setup
  - New biomass
  - Lactic acid
  - (other organic acids optional)

Introduction

- Applying Zambezi technology in the hydrolysis of biomass. Comparing hydrolysis experiments with raw and pelletized material.
- These comparative experiments were performed with grass and seaweed.
- The reason to use grass instead of wood was based on its physical properties, more similar to seaweed. Also, bulk density of both dried materials are roughly the same.
- The commision, recipe and method development were carried out with grass and then applied to seaweed samples. Available amounts of seaweed for experimentation was lower.

Miller and pelletizer

Recipe development:

- First step: Mill the raw material.

Material	Remarks
Weide Hooi/Grass (Tijssen) Barn-I Ruwvoeder	More difficult to mill than higher density materials. Raw material is homogeneous and dry. (wc = 6.03 %dw)
Brown seaweed / *Saccharina* (SAMS)	Even more difficult to mill. The raw material looks heterogeneous and not completely dried. Some parts are dry and brittle, others are elastic and robust. (wc = 8.50 %dw)

Grass milled material

Seaweed milled material

Miller and pelletizer

Recipe development:

- Final results

Left: Seaweed pellets. Right: Grass pellets.

Miller and pelletizer

Recipe development:

- Conclusions and observations:
  - First consistents pellets were obtained, following the recipe pellets can be easily produced but the recipe can be improved.
  - In comparison with other pellets they are still brittle. In case of grass pellets they are not shiny.
  - Bulk density achieved after pelletization is 6 times higher comparing it with the raw milled material.

Material	Bulk density (g/mL)
Milled grass	0.0768
Grass pellets	0.4856
Milled seaweed	0.0948
Seaweed pellets	0.6420

Comparative Hydrolysis experiments

- Quick screening.
- Comparative hydrolysis experiments.
- These experiments don’t follow optimum hydrolysis conditions, and therefore it is only indicative of how these materials would perform using the Zambezi technology.

Comparative Hydrolysis experiments

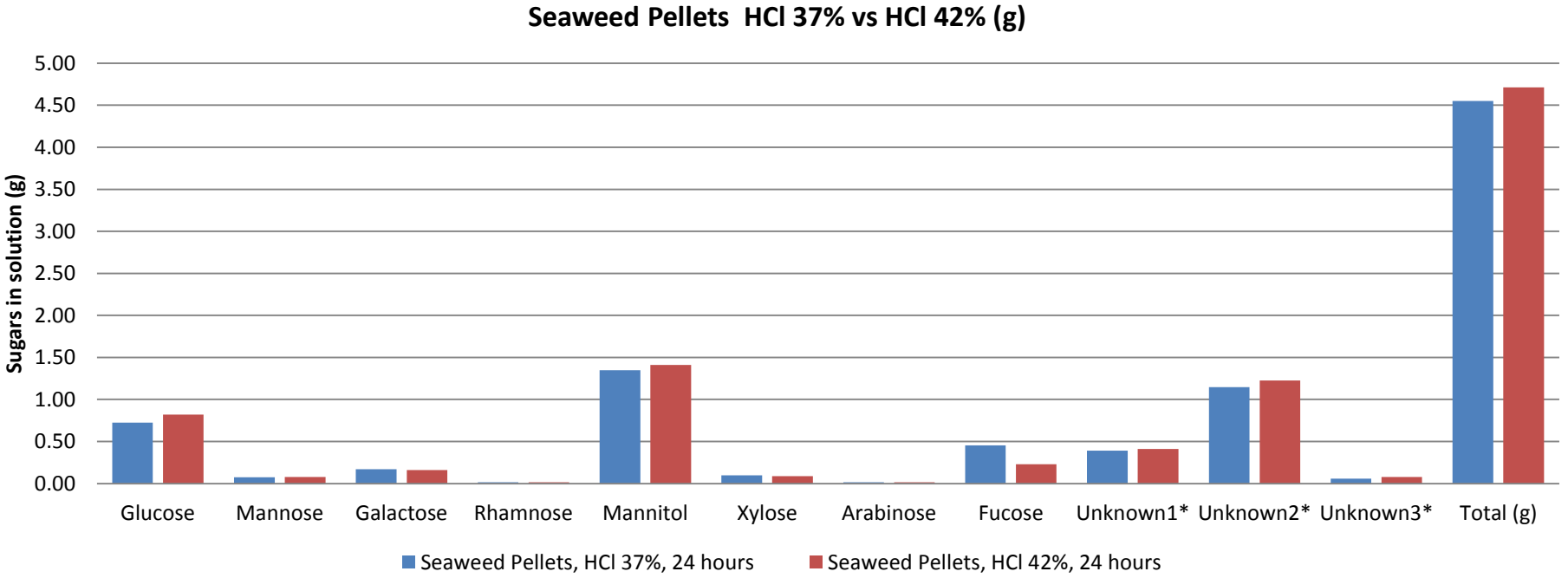
Method:

- Biomass:
  - Milled Grass and Seaweed (D=1mm)
  - Pelletized Grass and Seaweed
- Mineral acid: Hydrochloric acid solution 37%wt. and 42%wt.
- Ratio biomass:HCl 1:10
  - Biomass fed: 30g
  - HCl aqueous solution: 300g
- Room Temperature.
- No stirring.
- 24h Contact time.

Macrofuels: Seaweed Pellets hydrolysis

Conclusions:

- No significant differencies using HCl aqueous solution 37% or 42%wt.



Composition analysis Seaweed Pellets (wt%). RSD: 1%											
Glucosan	Mannan	Galactosan	Rhamnan	Mannitol	Xylan	Arabinan	Fucosan	Guluronic acid	Mannuronic acid	Glucuronic acid	Total
5.21	0.35	0.67	0.05	5.39	0.36	0.07	1.32	4.15	7.23	0.54	25.34

MACROFUELS: WP2, TASK 2.4

Conclusions and future work:

- A consistent recipe was developed for the production of grass and seaweed pellets. It might be improved lowering the water sprayed after steam formation.
- Small differences in composition after pelletization can be explained by the heterogeneity of the seaweed.
- No significant differencies using HCl 37 or 42% with seaweed.
- Uronic acids missing. Unknowns 1-3 were found in the analysis of uronic acids but they couldn't be positively identified as guluronic, mannuronic and glucuronic acids. Further work should be developed to study them.

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Progress task 2.5

Wouter Huijgen (ECN)

MacroFuels Progress Meeting, Brugge
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Task 2.5 Purification and Concentration of Algal Sugar Syrups

- Upgrading of sugar solutions from Task 2.3 and 2.4 **by purification** (e.g. ion-exchange) and **further concentration** (e.g. membrane filtration or evaporation of water).

- Goal: an **intermediate sugar syrup** with properties suitable for fermentation (WP3) and thermochemical conversion (WP4), with min. 60 g/l sugars for WP3 or 10-20 % for WP4.

Salt Targets (feedback WP3)

- WUR-FBR, ABE fermentation:
  - 5 g/L K^+ or Na^+ causes 50% inhibition.
  - Target: <2 g/L K^+ or Na^+.
- DTI, EtOH fermentation
  - Baker's yeast: 10 psu (10 g/kg of total ions)
  - Thermophilic bacteria: up to 4% NaCl (Thermoanaerobacter pentosaceus)*.
- No additional requirements received from WP4, since simply unknown.

→ Target set at <2 g/L.

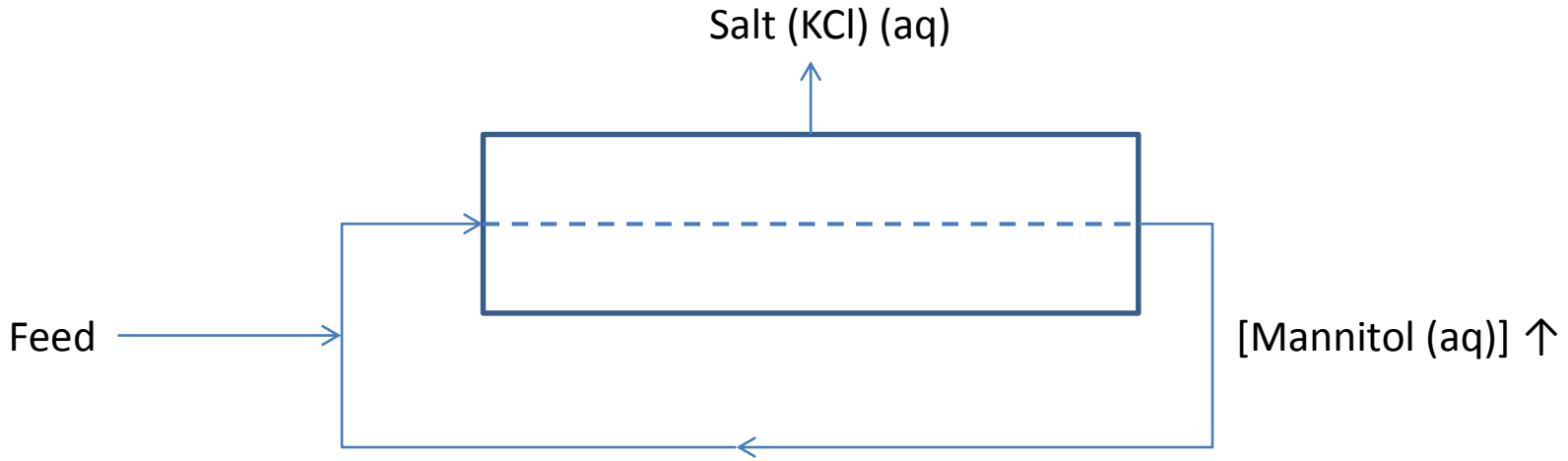
*) Tomas et al, 2003, Int J Syst Evol Microbiology. 63, 2396-2404

ECN: Membrane filtration

- ECN seaweed membrane-unit:
  - Year of construction 1995
  - Retrofit 2016
  - ±6 m^2
  - 4 m^3/h crossflow
  - 4..10 bar
  - Max 300 L/h permeate

- Flexible set-up:
  - Removal of salt (diafiltration)
  - Mannitol-laminarin separation (Kelps) (ultrafiltration)
  - Concentration of sugars (nanofiltration)
- Status: unit adapted & technically operational.

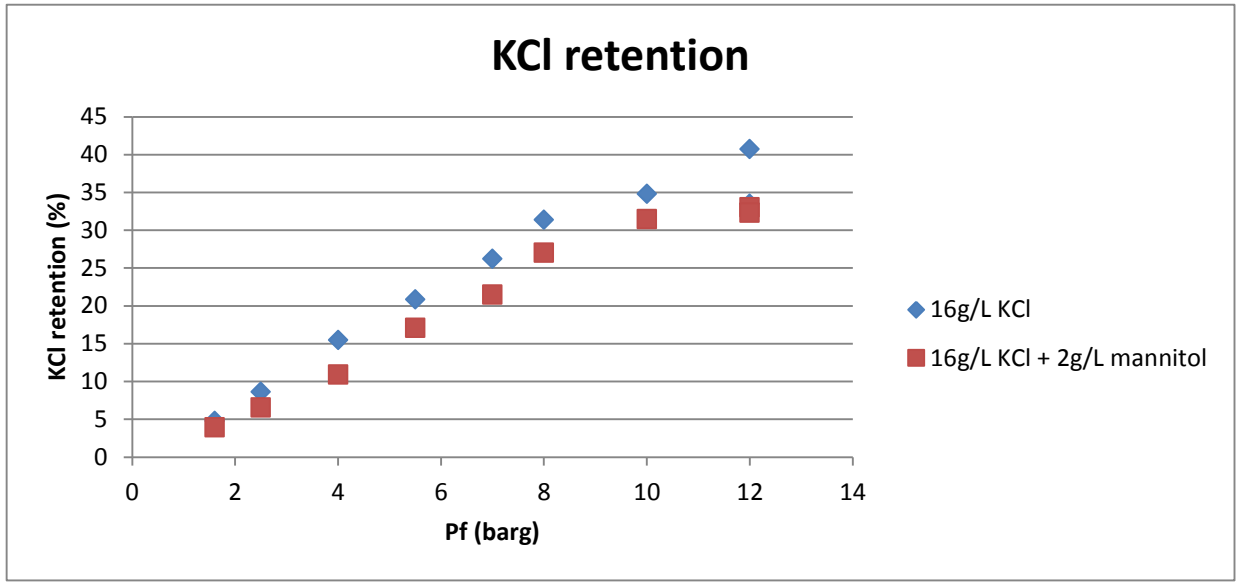
Nanofiltration Set-Up Tested



- Start with membrane performance tests using nanofiltration (membrane same as diafiltration).
- If required, later water addition and testing diafiltration.
- Aim:
  1. Removing salts: <2 g/L KCl in product liquor.
  2. Concentrating sugars: >60 g/L sugar in product liquor & <3% sugar losses.

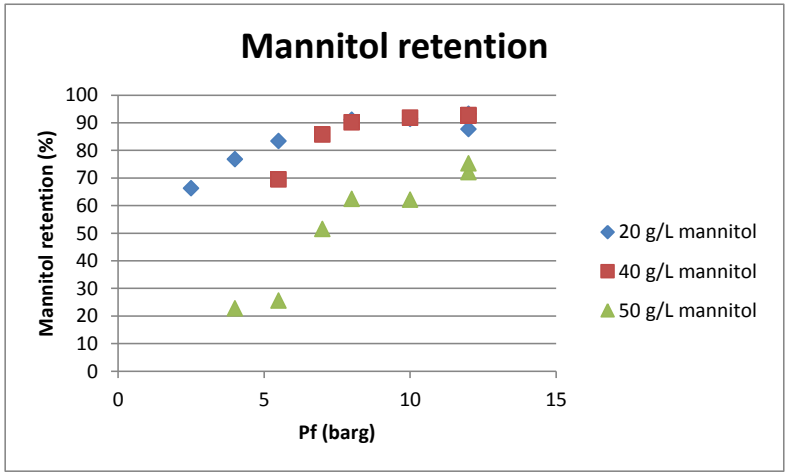
KCl retention

- KCl retention lower at lower operating p.
- (Small) beneficial effect of presence mannitol on KCl retention.



Mannitol retention

- Mannitol retention higher at high p.
  - Optimum pressure between retention KCl and mannitol.
  - Defined tolerable sugar loss very low → high p and multiple passes over membrane probably required.
- Results 50 g/L seem correct.
  - Cause for change under study.
  - Need for diafiltration?



Approach for Kelps

Performed:

- Technical testing of system.
- Model system mannitol-KCl:
  - Effect of operation conditions.
- Model system glucose-(mannitol)-KCl.

Ongoing / future:

- Influence alginate (and protein) on model systems.
- Testing Saccharina extract (demineralised water extract containing mannitol, salt, …).
- If required, testing microfiltration or ultrafiltration as ‘pre-cleaning step’.
- Diafiltration.
- Setting-up overall membrane/concentration system concept in e.g. Aspen.

Screening purification methods (WUR)

- Size exclusion chromatography
- Active carbon
- Mixed bed ion exchange
- Filtration

Screening purification methods (WUR)

- Size exclusion chromatography
- Active carbon
- Mixed bed ion exchange
- Filtration

Conclusions and future work (WUR)

- Size exclusion possibly technically suitable as alternative to filtration
  - Scalable?
- Sugar loss in mixed bed too high
  - Confirm initial results
- Compare to filtration (as performed by ECN)

Acknowledgement

Thank you for your attention!

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