

Active storage as a way to produce fermentable sugars all year round

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History

- Noord4Bio: opportunities for a bio-economy in Northern Netherlands. One of the ideas: carbohydrate based fermentation industry in Northern Netherlands
- Project S4F Sugar for Fermentation (Johan Sanders, Harriëtte Bos en Johan van Groenestijn), 2018

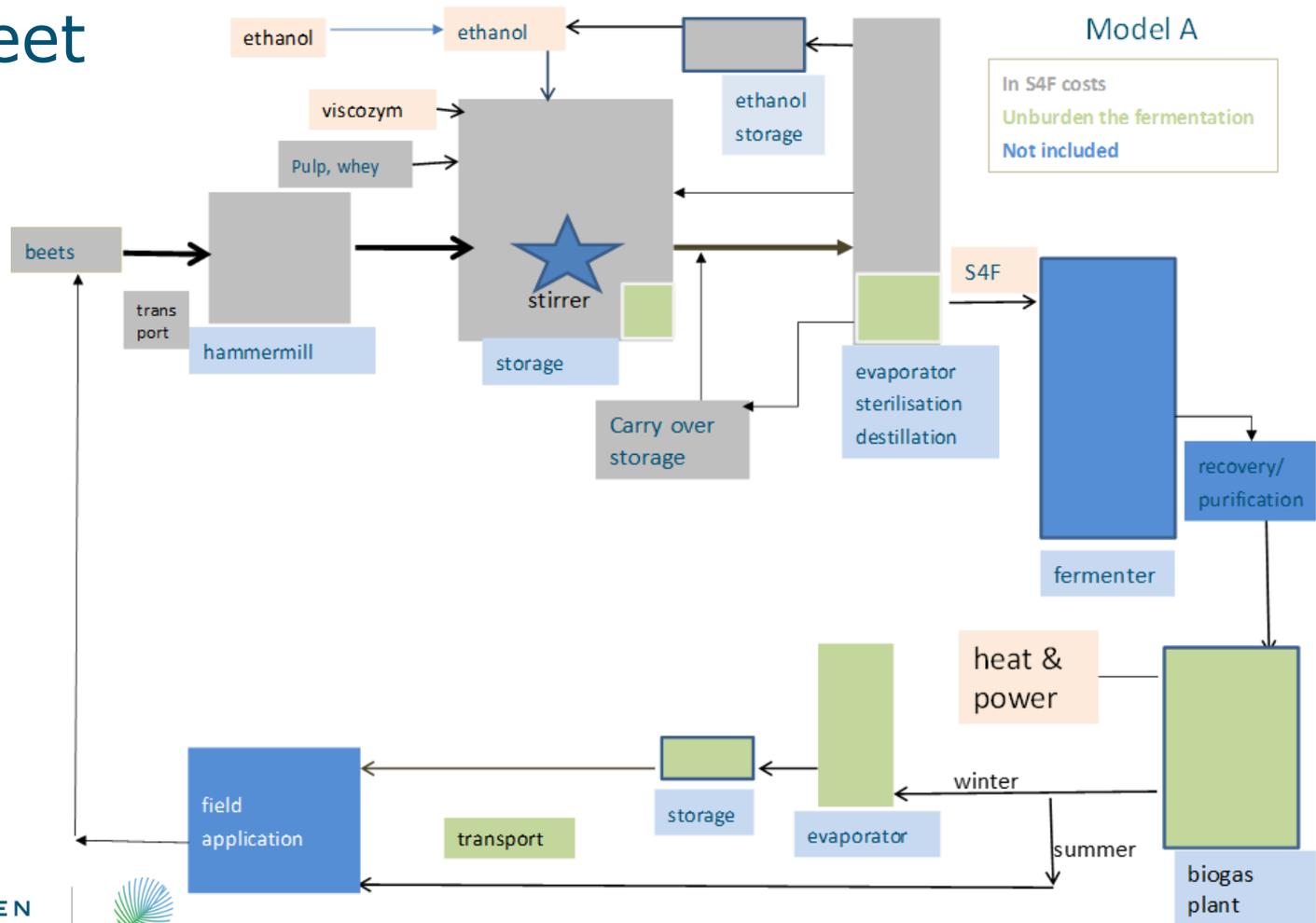
Requirements for fermentable sugars

- Sugars for fermentation do not need to be 100% pure.
- The sugar concentration does not need to be high. A concentration of 30% (w/w) will be sufficient in a fermentation medium.
- Carbohydrate-rich feedstocks: seasonal campaigns (sugar beets, potato press pulp), but the sugars should be available whole year round. Therefore, a storage and preservation method is required.
- Not more than € 250/ton sugar.

Active storage

- Method for making sugars all year round from raw materials supplied in campaigns. By means of active storage (with enzymes which convert polysaccharides into monosaccharides; reduce viscosity; with preservative; with combined sterilization, concentration and optionally ethanol recovery).
- Patent application S4F (Sanders, Van Groenestijn)

Flow sheet



EFRO project (2020-2023)

Sugar-derived Polymers Attainable by Cost-Effective Conversion of Renewable feedstock by Anaerobic Fermentation Technologies: SPACECRAFT

WP 1 Production of fermentable sugars

WP 2 Anaerobic fermentation (production of monomers)

WP 3 Production of polymers

Coordinator: RUG (Gert-Jan Euverink)

WP1 leader: Johan van Groenestijn

PM for WFBR is Rolf Blaauw

Rijksuniversiteit Groningen

Sanovations B.V.

Senbis Group B.V.

Wageningen Food & Biobased Research

Hanzehogeschool Groningen

Bioclear Earth B.V.

Participants WP1

- Bioclear Earth: Jeroen Tideman, Matthijs de Koning, Jidske Knigge, Alie Taken, Sjoerd Jan de Vries, Daan Metz
- Sanovations: Johan Sanders
- RUG: Gert-Jan Euverink, Dana Colpa
- WFBR: Johan van Groenestijn, Willem Drost, Martijn van Walsem, Jacinta van der Putten, Richard op den Kamp, Koen Meesters

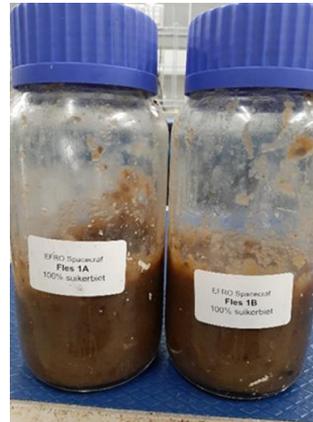
Aim WP1

- To develop new knowledge and expertise on setting up a biorefinery process for obtaining fermentable sugars from local carbohydrate-containing raw materials such as sugar beets, maize and grains, potatoes and other starch crops.
- The (combination of) raw materials is liquefied and then concentrated in evaporators in which the sterilization is also carried out.

Development of the active storage principle

- 9 months active storage
- Preservation (ethanol, low pH)
- Enzymatic hydrolysis cell walls

First experiments with 25 bottles with different feedstocks and conditions



Week 8: counting microorganismen (CFU/g)

	Biomassa	Aerobic mesophilic	Anaerobic mesophilic	yeasts	Fungi
1A/B	100% mashed sugar beets/ Ethanol	<1,000/2,000	<1,000/<1,000	<1,000/ <1,000	<1,000/ <1,000
	2A/B	75% mashed sugar beets and 25% potato press pulp/ ethanol	4,000/2,000	<1,000/3,000	<1,000/ <1,000
3A/B	33% corn and 67% mashed beets/ ethanol	<1,000/3,000	2,000/<1,000	<1,000/ <1,000	<1,000/ <1,000
4A/B	75% mashed beets and 25% potato press pulp	1,700,000/ 410,000	15,000,000/ 4,500,000	<1,000/ <1,000	<1,000/ <1,000
	Low pH, no ethanol				
5A/B	75% mashed beets and 25% potato press pulp	3,000,000/ <3,000,000	>30,000,000/ >30,000,000	6,000/10,000	<1,000/ <1,000
	No ethanol, no enzymes				
19	100% mashed beets/ ethanol 30°C	<1,000	<1,000	<1,000	<1,000
20	100% mashed beets/ ethanol 40°C	<1,000	<1,000	<1,000	<1,000

Sugars after extraction at 80°C (g/kg wet) week 36

Bottles with ethanol and enzymes; various compositions

Sample		Fucose	Rhamnose	Arabinose	Galactose	Glucose	Mannose	Xylose	Fructose	Sucrose
1A		0.0	0.5	6.2	1.0	33.5	0.0	0.0	62.9	34.6
1B		0.0	0.5	6.1	0.9	42.6	0.0	0.0	66.4	31.6
2A		0.0	0.5	4.2	1.8	45.3	0.0	0.0	46.3	20.9
2B		0.0	0.4	3.3	1.6	43.6	0.0	0.0	48.2	22.8
3A		0.1	0.2	3.6	0.5	75.9	0.0	0.1	37.3	16.3
3B		0.1	0.3	4.3	0.7	85.6	0.0	0.1	42.4	19.4
4A	No ethanol	0.0	1.0	1.2	1.0	1.7	0.0	0.2	1.6	1.0
4B	No ethanol	0.0	1.1	1.6	0.8	0.6	0.0	0.2	0.6	0.6
5A	No ethanol	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.1	0.3
5B	No ethanol	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.2
6		0.1	0.2	3.8	0.5	90.0	0.0	0.1	37.2	15.8
7		0.0	0.7	4.7	2.1	45.6	0.0	0.0	42.7	19.3
8		0.0	0.3	3.2	1.4	41.8	0.0	0.0	54.1	25.1
9		0.0	0.5	5.0	2.0	44.2	0.0	0.1	46.6	21.4
10		0.0	0.5	5.2	1.9	51.1	0.0	0.2	53.9	25.7
11		0.0	0.6	4.6	2.0	43.3	0.0	0.0	46.0	20.6
12		0.0	0.5	5.0	1.9	40.4	0.0	0.0	54.8	25.2
13		0.1	0.2	4.0	0.6	86.7	0.0	0.1	42.9	18.0
14		0.1	0.2	4.1	0.5	77.4	0.0	0.1	42.1	18.0
15		0.1	0.2	4.5	0.6	90.2	0.0	0.1	44.5	19.9
16		0.1	0.0	2.0	0.3	72.9	0.0	0.3	38.6	17.1
17		0.1	0.3	3.9	0.6	83.5	0.0	0.1	42.3	18.6
18		0.1	0.2	4.2	0.6	87.5	0.0	0.1	40.8	18.0
19		0.0	0.0	2.4	0.3	51.1	0.0	0.0	55.0	26.9
20		0.1	0.0	1.2	0.1	33.2	0.0	0.0	35.0	19.8

Glucose balance in active storage (sugar beet and potato press pulp)

g/kg wet material	Start	Week 36
Glucose in sucrose	51.7	13.5
Glucose in starch	9.7	1.1
Glucose in cellulose	9.3	1.1
Mono glucose	0	51.1
TOTAL:	70.7	66.8

Sugars

- In all bottles, hemicellulose, pectin and starch are extensively converted after 36 weeks, usually $> 95\%$. Whereas in week 8 this conversion was usually halfway through.
- Cellulose breakdown usually around 80%.
- Correlation residual pectin and hemicellulose with viscosity.
- Low molecular weight sugars are preserved in 12% ethanol.
- More than half of sucrose is split into glucose and fructose.

Organic acids

- Without ethanol: production of lactic acid, acetic acid and ethanol.

Viscosity reduction

- Viscosity from $>15,000$ mPa.s to <100 mPa.s within a few weeks



Summary results

- Addition of enzymes are really required for liquefaction
- Polysacharides are degraded and viscosity is reduced
- Cossettes can be liquefied; more size reduction not required
- Enzymes are active in 12% ethanol
- 12% ethanol: lower number of microorganism, preservation of sugars

Production large batch 8 kg liquefied sugar beets

- After 1.5 week viscosity only 20 mPa.s. A flowing liquid with minimum cell wall debris. More than 200 g mono+disaccharides/L
- Used by WP2 team for fermentations and by a fermentation company.

Next

Process design and optimisation.

The quest for acid and enzyme producing bacteria active below pH 3.5: cost-effective alternative: *in situ* production of the enzyme and the preservative.