Application Centre for Renewable Resources
ACRRES
een Wageningen UR initiatief

Direct processing of sugar beet using Betaprocess

Chembeet WP1 and WP2

A.M.J. Kootstra

ACRRES - Wageningen UR, October 2017 Report 744

Direct processing of sugar beet using Betaprocess

Chembeet WP1 and WP2

A.M.J. Kootstra

ACRRES, Wageningen University & Research

This study was carried out by the Wageningen Research Foundation (WR) business unit Applied Arable and Vegetable Research, team ACRRES (Application Centre for Renewable Resources) and was financed by ERA-NET Bioenergy. [Project Chembeet (Biofuels and green chemicals from sugar beet through direct processing), project number TEMW115001]

WR is part of Wageningen University & Research, the collaboration of Wageningen University and Wageningen Research Foundation.

Wageningen, October 2017

Report WPR-744

Kootstra, A.M.J., 2017. *Direct processing of sugar beet using Betaprocess; Chembeet WP1 and WP2.* Wageningen Research, Report WPR-744

This report can be downloaded for free at https://doi.org/10.18174/425474

Summary
Direct processing of sugar beets in fermentation, without first having to go through sugar extraction and refinery, potentially lowers feedstock related costs for fermentative products. By combining a heat treatment with a vacuum explosion, the Betaprocess treatment aims to open the sugar beet cells, and it is claimed that it hereby improves the sugar availability, subsequently speeding up the fermentation process. This report describes the results of Work Packages 1 and 2 in the Chembeet project: the adjustments made to the pilot plant fermentation facility at ACRRES (Wageningen Research, Lelystad, the Netherlands) as well as the trials performed on direct fermentation with and without the Betaprocess treatment. The results show that no effect of the Betaprocess treatment was found under the tested conditions. Direct processing of sugar beet was performed successfully, including fermentation, reaching 83 % to 85 % ethanol yields. 80 % to 90 % of all ethanol was produced in the first 24 hours of fermentation and peak ethanol production was approximately 4 g/L per hour.

Keywords: Direct processing, sugar beet, ethanol, Betaprocess

Project partners in the Chembeet project are:
- Wageningen Research Foundation - ACRRES (the Netherlands)
- Dutch Sustainable Development BV (the Netherlands)
- VAM Watertech BV (the Netherlands)
- University of Warmia and Mazury (Poland)

Report WPR-744

Contents

	Summary	**5**
1	**Introduction**	**7**
2	**Work Package 1: Adjustments to ACRRES bioethanol pilot plant**	**9**
3	**Work Package 2: Betaprocess pilot trials**	**13**
4	**Conclusions**	**21**
	References	**23**

Summary

Direct processing of sugar beets in fermentation, without first having to go through sugar extraction and refinery, potentially lowers feedstock related costs for fermentative products. By combining a heat treatment with a vacuum explosion, the Betaprocess treatment aims to open the sugar beet cells, and it is claimed that it hereby improves the sugar availability, subsequently speeding up the fermentation process. This report describes the results of Work Packages 1 and 2 in the Chembeet project: the adjustments made to the pilot plant fermentation facility at ACRRES (Wageningen Research, Lelystad, the Netherlands) as well as the trials performed on direct fermentation with and without the Betaprocess treatment. The results show that no effect of the Betaprocess treatment was found under the tested conditions. Direct processing of sugar beet was performed successfully, including fermentation, reaching 83 % to 85 % ethanol yields. 80 % to 90 % of all ethanol was produced in the first 24 hours of fermentation and peak ethanol production was approximately 4 g/L per hour.

1 Introduction

As of 2017, the sugar beet production quota in the EU has been lifted, and sugar beet in North-West Europe is expected to become an important source of fermentable sugar to be used for the production of building blocks for the chemical industry [Deloitte, 2014]. Fermentative production of chemical building blocks traditionally uses fermentable raw materials originating from corn, as well as from sugar cane and sugar beet, such as thick juice, molasses, and refined sugar. Focussing on sugar beets, if these could be used directly for fermentation, without first having to go through sugar extraction and refinery, feedstock related costs for fermentative products could potentially be lowered.

DSD Betaprocess is a company that markets Betaprocess equipment. The Betaprocess treatment uses a heat treatment followed by a vacuum explosion and aims to open the sugar beet cells. By freeing up the sugar in the cells, the sugar availability for the subsequent fermentation is thought to improve, hereby speeding up the fermentation process compared to when Betaprocess is not applied.

In the trials described in this report, performed as part of the Chembeet project, sugar beet is directly fermented to ethanol using activated yeast. Ethanol production was chosen for two reasons. Firstly, ethanol is a common building block for the chemical industry. Secondly, sugar-to-ethanol fermentation is relatively straightforward and can relatively easily be performed at pilot scale in order to assess an effect of Betaprocess, albeit that directly fermenting sugar beets instead of a liquid will necessitate some adjustments. If an effect can be shown for the fermentative production rate of ethanol, it is likely this will also translate to the fermentative production of higher value products than ethanol, such as e.g. lactic acid and succinic acid.

For Work Package 1 (WP1) in the Chembeet project, the pilot scale Betaprocess was integrated with the bioethanol production pilot plant located at ACRRES in Lelystad, the Netherlands. The bioethanol plant was adjusted to be able to run with sugar beet mash, and to monitor the fermentation by measuring the produced gas flow. For WP2, two trials of pilot runs were performed. The first in May/June 2016, and the second between November 2016 and March 2017. These trials have two goals: 1) to aid in the scale up of the direct processing of sugar beet, and 2) to assess the effect of the Betaprocess on the fermentation rate at pilot scale.

2 Work Package 1: Adjustments to ACRRES bioethanol pilot plant

In order to integrate the pilot scale Betaprocess (Figure 1 and Figure 2) with the existing ACRRES bioethanol pilot plant located at Lelystad, the Netherlands, some adjustments were made in order to run the trials of which the results are described in the next chapter. Some of these adjustments are straight forward, while others were more complex and sometimes correct settings had to be found through trial and error.

Heat exchanger

The hot water supply of the heat exchanger was connected to the Combined Heat & Power unit (CHP) present on location.

Washing

During sugar beet harvest, some soil inevitably remains attached to the beets, with the amount depending on soil type and harvest conditions. As soil contains microorganisms that may negatively affect the sugar-to-ethanol conversion by the added yeast during fermentation, it needs to be removed by washing. At first, an automated drum washer was installed and tested. As the resulting soil removal was seen as inadequate, subsequent runs were fed with beets washed in a different tumbler washer system, equipped with water nozzles. The beets were fed to and removed from the washer manually. This required more labour, but the resulting beets were reasonably clean, although some soil usually remained in the longitudinal groove of the beets.

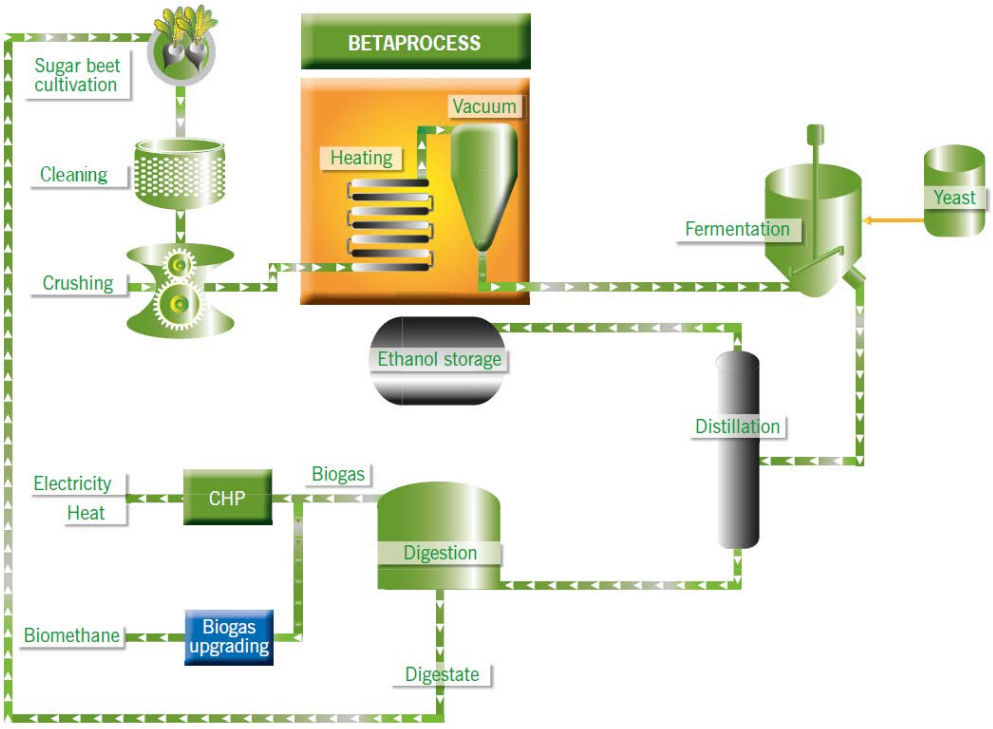

Figure 1 *Schematic representation of Betaprocess integration with bioethanol pilot at ACRRES.*

Figure 2 *The pilot scale Betaprocess.*

Crushing

To crush the beets, a Smicon SMIMO15 mill was used, equipped at first with a 5 mm sieve, which was later changed for a 7 mm sieve. The 7 mm sieve was used for the trials described in the next chapter. In order for the resulting mash to be 1) pumped through the heat exchanger, and 2) mixable in the fermenter, ~20% (expressed as fraction of the final mass of the mash) water was added in the crushing step. In principle, as little water addition as possible is highly preferable, as added water leads to increased distillation costs for the ethanol downstream.

Betaprocess

Adjusting the settings of the Betaprocess was sometimes challenging, as these also depended on the mash viscosity, available heat from the CHP, etcetera. Finding the right temperature settings and flow rates took some attention.

Fermentation: mixing

Several options were tested to replace the off-centre propeller mixer originally present in the fermenters of the bioethanol plant. In the final setup, a centrally placed cross-shaped stirrer was used (90 cm diameter, 30 rpm, blades push down). The original 0.75 kW stirrer engines were also replaced by 2.2 kW versions (Jongia).

Fermentation: rising

During fermentation CO_2 is produced and, probably due to the viscosity related causes, this did not quickly bubble to the surface but was included in the mash, resulting in rising of the mash. If the mash rises beyond stirrer reach rising may worsen and cause serious issues. For safety reasons, a release valve was installed, equipped with a break seal. Also, the fermenter was filled only for 50 %, or approximately 700-750 kg. Furthermore, a second cross stirrer was installed, identical to the first, but placed approximately 10-20 cm above the starting surface of the fermenter content.

Fermentation: pH control & gas flow measurement

An automated system for pH adjustment and maintenance was used (pumps: Emec, VACL 15-1.5). Due to the mixing issues mentioned above, there was a time lag between addition of either HCl or NaOH and their effect, potentially resulting in over-application of both. This necessitated careful

programming of the system. To monitor the gas flow, a mass flow meter (M+W, D6360-HGD-CC-S-E, Bronkhorst) was installed on each of the fermenters.

Fermentation: lactic acid formation

In order to reduce the contamination of the beet mash with lactic acid bacteria, their subsequent growth and the unwanted production of lactic acid, several efforts were made. Firstly, as the main source of contamination was likely to be the soil remaining on the beets after harvest, the beet were washed quite thoroughly. Secondly, the fermenters were cleaned before and after running. Thirdly, microbial stabilisers were added. After first trying a hops extract, fermentation seemed more stable when using thoroughly washed (see above) beets and Lactoside 247 as a stabiliser.

Distillation

The distillation column present was not suited for distillation of the fermented sugar beet mash. As it was designed for particle-free liquids, it quickly clogged when mash was used. Designing and building a new distillation column was not part of the scope of this project. However, the installation was adjusted to inject the mash in to the heating vessel at the bottom of the column, instead of the normal entry point, at the top of the column. In this manner, a distilled product of approximately 70 vol% ethanol was produced.

General discussion:

The adjustments as described above resulted in the combination of the Betaprocess and the bioethanol plant to be able to successfully directly process sugar beets to ethanol, while monitoring the fermentation. Water addition was needed, but needs to be avoided in future scale up as much as possible, as any added water adds to the distillation costs per amount of ethanol. The 20 % of water addition in the setup described here was necessary because adding less would cause issues with pumping the mash through the heat exchanger and also with mixing in the fermenter. Mixing the contents of the fermenters was needed to maintain the pH and to promote a more constant gas flow from the fermenting mash to be able to monitor the fermentation. Ideally, both the heat exchanger as well as the mixer should be redesigned to process mash without having to add water. Another point of interest is the distillation of the fermented mash. A distillation column able to process the thick fermented mash is needed. Alternatively, the fermented mash could be separated into a solid and liquid fraction, after which the liquid fraction could be more easily distilled. This would however lead some loss of ethanol that remains in the solid fraction.

3 Work Package 2: Betaprocess pilot trials

3.1 Introduction to the trials

After the adjustments to the pilot plant (Work Package 1) were performed, as described in Chapter 2, two trials were performed, the first in May/June 2016, and the second between November 2016 and March 2017. The goal of these trials is twofold: 1) to aid in the scale up of the direct processing of sugar beet, and 2) to assess the effect of the Betaprocess on the fermentation rate at pilot scale.

3.2 Materials and Methods

3.2.1 Materials

The sugar beets used for the experiments described in this report were cultivated on site at the Wageningen Research location Practical Arable and Vegetable Research in Lelystad, the Netherlands. The beets were harvested in November 2015 for the May/June '16 runs, October 2016 for the October/November '16 runs, and December 2016 for the March '17 runs. Harvested beets were stored indoors at +/- 5 °C until used.

3.2.2 Methods

3.2.2.1 Processing

Washing and milling

A drum washer equipped with water nozzles was used to wash the beets, which were fed to and taken from the washer manually. It took approximately 3 hours to wash 1500 kg beets, after which the washed beets were brought to the mill. The mill was fed with a screw feeder that crushed the beets before entering the mill. A SMIMO15 hammer mill (Smicon, the Netherlands) equipped with a 7 mm sieve wash used to grind the crushed beets to a mash. During milling, water was added to the mash (to 20 % of total mass) so that the resulting mixture could be pumped through the heat exchanger of the Betaprocess.

Betaprocess treatment

The milled mash is pumped though a heat exchanger, set at an exit temperature of 65 °C. In practice, operational exit temperature was between 60 °C and 70 °C. The heated mash exited the heat exchanger through a vacuum lock that needed +/-2 bar overpressure to be overcome, entering the vacuum chamber (-930 mbar, compared to atmospheric). Betaprocess (DSD, the Netherlands) capacity is 1 to 3 tonne/h; practical runs were performed at 1.0 to 1.4 tonne/h, heating the heat exchanger with water from the CHP present at the WUR-ACRRES pilot plant in Lelystad. The water evaporated in the vacuum vessel was condensed at the top by a water-cooled condenser. The treated mash is pumped from the bottom of the vacuum vessel to the weighing vessel, at which point the mash temperature is approximately 40 °C.

For the 'no Betaprocess' runs, the mash was still pumped through the heat exchanger, but without the vacuum lock and no application of vacuum in the vacuum vessel. The heat exchanger in this case was set at 50 °C so that the temperature of the resulting mash when it reached the weighing vessel was approximately 40 °C, just as in the 'Betaprocess' runs. The temperature of the weighing vessel is kept at approximately 40 °C, to prevent the mash from cooling.

Fermentation

The fermenters (1.5 m^3 each) were filled from the weighing vessel in two batches. Firstly, +/- 100 kg mash is let in, while adding 2 kg of 15 % HCl (Breustedt Chemie, the Netherlands) to the fermenter as well. Secondly, another +/- 600 kg is fed to the fermenter, herby mixing with the acidic mash already present, totalling +/- 700 kg. The ingredients for a typical 700 kg mash fermentation are listed in

Table 1. A microbial stabiliser (Lactoside 247, Lallemand) is suspended in 50 mL to 100 mL demineralised water and added in three batches to the weighing vessel while it is filling with the 600 kg mash.
To activate the dry yeast (Distalamax HT, Lallemand), 140 g is mixed with 150 g sucrose (store bought crystalline sugar,) in +/- 4 L tap water at 32-34 °C. After having been kept in a warm room and stirred regularly for 1 to 1.5 h, the foamy activated yeast mixture is ready to be added to the fermenter.
During yeast activation, the mash in the fermenter is constantly stirred (cross-shaped stirrer, 90 cm diameter, 30 rpm, blades push down) and the pH is set to 4.7-4.8 by addition of 15 % HCl. When a stable pH of 4.7-4.8 is reached, samples are taken for sugar analysis of the mash (= before fermentation). After the addition of extra nutrition (Distilavite GN and VM, Lallemand) and the activated yeast, the fermenter is closed. The pH is kept at 4.7-4.8 during fermentation by automatic addition (pumps: Emec, VACL 15-1.5) of 15 % HCl and 32 % NaOH (Breustedt Chemie). The temperature in the fermenter is kept at ~32 °C by cooling the mantle. Gas flow from the fermenters is measured with calibrated mass flow meters (D6360-HGD-CC-S-E, M+W Instruments, Bronkhorst,).

Table 1 *Ingredients for fermentation mixture.*

Material	Mass	Concentration (mass)
Sugar beet	560 kg	80 %
Water (milling)	140 kg	20 %
Microbial stabiliser, Lactoside 247	1.92 g	0.00027 %
Dry yeast, Distalamax HT	140 g	0.020 %
Nutrition, Distilavite GN	140 g	0.020 %
Nutrition, Distilavite VM	21 g	0.003 %

3.2.2.2 Sampling and analysis

Samples were taken before and after fermentation by pushing under a 10 L bucket, hereby collecting approximately 6 kg of material. The material in the bucket is thoroughly mixed (by stirring and folding using a 1 L grocery scoop), after which a single scoop of 200-300 g is taken and transferred in its entirety to a 500 mL wide mouthed polyethylene flask. The sample is then frozen and kept at -20 °C, until analysis. Samples were transported to the analysis laboratory (Nutricontrol, the Netherlands) using a cooling box with -20 °C ice packs.
The main analyses performed at Nutricontrol are 1) sugar content (analysis nr 10343, extraction with 40 % ethanol, High Performance Anion Exchange chromatography with Pulsed Amperometric Detection (HPAE-PAC), standard analysis includes sucrose, glucose, fructose, maltose, and lactose), analysed in the liquid fraction after centrifugation, and 2) ethanol (analysis nr 10272, enzymatic detection, Boehringer test kit), analysed in the unseparated sample. The analyses of other compounds (methanol, butanol, lactic acid, acetic acid, and succinic acid in the first trial; glycerol in the second) were also performed at the same laboratory. Protocols for analyses are available on request at Nutricontrol.
The flow of gas produced in the fermenter was measured using a calibrated mass flow meter ((D6360-HGD-CC-S-E, M+W Instruments, Bronkhorst,). By assuming all the produced gas is CO_2 resulting from ethanol production from sucrose, glucose and fructose (Eq 1 and Eq 2), measuring the gas flow allowed following ethanol formation in time.

Eq 1. From sucrose or maltose:

$$C_{12}H_{22}O_{11} + H_2O \rightarrow 2\ C_6H_{12}O_6 \rightarrow 4\ C_2H_6O + 4\ CO_2$$

100 g sucr/malt $\rightarrow$ 53.8 g ethanol + 51.4 g CO_2

Eq 2. From glucose or fructose:

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_6O + 2\ CO_2$$

100 g gluc/fruc $\rightarrow$ 51.1 g ethanol + 48.9 g CO_2

3.3 Experimental setup

The goal of treating sugar beet using the Betaprocess is to open the beet cells, hereby increasing the availability of sugar for the yeast cells present in the fermentation. The idea is that increased sugar availability will positively affect fermentation rate. Increased ethanol formation rate entails increased rate of CO_2 formation. In short, if the Betaprocess increases sugar availability, hereby positively affecting fermentation rate, this may be detected by a higher gas flow rate and/or a maximum gas flow rate occurring earlier in the process. A resulting decrease of the total fermentation time would positively influence the ethanol production business case, as a shorter throughput time means lower costs per amount of produced ethanol.
It is not expected that the Betaprocess treatment increases the final maximum ethanol yield, as no additional amount of fermentable sugar is created during the treatment. In principle however, it may be possible that part of all sugar remains in the cells during fermentation when the material is not treated, while all present sugar is released after Betaprocess treatment. In this case, the treatment may positively affect the yield. The potential ethanol formation can be calculated from the sugar content. The analysed final ethanol concentration is then used to calculate the yield. In the case of the gas flow measurements, the mass of produced CO_2 can be related to produced ethanol, in time as well as cumulatively.
In each run, one of two fermenters is filled with Betaprocess treated mash, while the other is filled with 'No Betaprocess' material (meaning no vacuum and reduced heating in the heat exchanger). The fermenters are interchanged from one run to the next, to evade any influence of the fermenter.

3.4 Results and discussion

The final ethanol yield of the May/June '16 runs (Table 2) was 70 % to 82 %, based on the sugars present before fermentation, with all sugars used up after fermentation. As was expected, no influence of Betaprocess on the final ethanol yield was found. The ethanol yields may seem somewhat low, but a few matters have to be taken into account. Firstly, it is common that yeast propagation in batch ethanol fermentation uses 5 % to 15 % of all sugars. Secondly, as the performed fermentations were not performed using washed but certainly non-sterile beets as raw material, some production of side products is inevitable. Even though the stabiliser (lactoside 247) was applied, some lactic acid was found after all runs. In fact, a wider analysis of side products was performed for the 17-5 runs, and traces of methanol, butanol, and acetic acid were found, together with a notable amount of succinic acid. Assuming a sugar requirement similar to that of ethanol formation of 2 kg sugar for 1 kg product, these products would account for approximately 5 % of all sugar present. Furthermore, samples from the 7-6 runs were also analysed for glycerol by a lab in Switzerland (results not shown), and it was found that an amount of glycerol had been formed that would account for 7 % to 8 % of all present sugar, again making the 2 kg sugar for 1 kg product assumption. All these matters add up to 17 % to 28 % of all sugar possibly being used for other purposes than ethanol production hereby closing the 18 % to 30 % gap. Thirdly, it should be taken into account that industrially optimised ethanol fermentation yields up to 90 % to 93 % ethanol [Ingledew, 2009]. Lastly, it should be taken into account that laboratory analyses combined with stirring of and sampling from large amounts of mash-type materials represent a source of inaccuracy.

The CO_2 flow measurements also show no influence of Betaprocess on the fermentation rate (Figure 3Figure 4, and Figure ***5***). Peak ethanol production takes place after 8 or 9 hours, independent on whether Bioprocess was applied or not. At the peak, around 25 L/min of CO_2 is produced, corresponding to 46 g/min ethanol, or 2.8 kg/h in the whole fermenter, or approximately 4 g/L per hour when taking 700 L as the effective reactor volume. The fermentations last approximately 30 to 35 hours starting from the addition of the activated yeast (t=0), with 80 % to 90 % of the final ethanol yield being produced in the first 24 hours. Ethanol yield following from the gas flow measurements is around 83 % to 85%, assuming 15.8 wt% sucrose in the beet. In the 17-5 and 24-5 runs, these yields correspond reasonably well to those calculated from the analysis data (Table 2). For the 7-6 runs, the yield calculated from the analysis results is somewhat lower than when using the gas flow measurements. As the yield based on gas flow is quite consistent between runs, this suggests

that the 5.0 wt% and 5.1 wt% ethanol analysis results have underestimated the actual ethanol concentration. Or, a side product may have formed that also entails CO_2 formation.
In Figure 3 (after 22 h and 26 h) and Figure 4 (after 17 h), sudden increases in gas flow were recorded. It seems these are artefacts, as are the brief and irregularly occurring drops in measured gas flow. This does not change the main results, however. Gas flow data was unavailable for the 31-5 runs.

When combining the ethanol-from-sugar yields determined from chemical analysis, and those from gas flow measurements, it is clear that the fermentations ran successfully. There seems to be approximately 5 % to 10 % room for yield improvement, keeping the 90 % - 93 % yield for fully optimized industrial fermentation systems in mind [Ingledew, 2009]. The most apparent but also challenging improvements would be the reduction of the formation of by-products, mostly lactic acid and glycerol, and yeast recycle.

Table 2 *May and June '16 runs with and without Betaprocess treatment: sugar levels and ethanol yields.*

General information								
Start date	17-5		24-5		31-5		7-6	
Betaprocess	No	Yes	No	Yes	No	Yes	No	Yes
Fermenter	R01	R02	R02	R01	R01	R02	R02	R01
Before fermentation								
Beet in fermenter (kg)	569	568	551	550	588	564	570	603
Added water in mash (%)	19	20	22	22	23	23	22	20
Non-sugar dm in beet (kg)	40	40	39	38	41	39	40	42
Fructose in mash (kg)	2.4	1.4	3.3	3.0	4.9	3.3	3.1	2.4
Glucose in mash (kg)	3.4	2.2	3.8	4.1	6.5	3.9	4.3	3.2
Maltose in mash (kg)	1.4	nd	1.6	2.0	3.6	1.4	nd	nd
Saccharose in mash (kg)	87	86	75	83	75	77	76	85
Total sugars in mash (kg)	94	90	84	92	90	86	84	91
Saccharose in beet (wt%)	15.4	15.3	13.7	15.2	12.9	13.8	13.5	14.3
Total sugars in beet (wt%)	16.7	16.0	15.3	16.9	15.4	15.4	14.8	15.2
Maximum ethanol (kg)	50	48	45	50	49	46	45	49
Maximum CO_2 (kg)	48	46	39	43	39	40	39	44
During fermentation								
Additions pH control (kg)	6.5	5.3	4.8	9.2	9.1	6.3	5.0	7.3
After fermentation								
Total mash (kg)	663	664	667	668	724	691	691	713
Total sugars in mash (kg)	nd	nd	nd	nd	nd	nd	nd	nd
Liquid fraction (kg)	623	625	628	629	682	652	651	671
Methanol in mash (kg)	0.04	0.03	na	na	na	na	na	na
Butanol in mash (kg)	0.1	0.1	na	na	na	na	na	na
Lactic acid in mash (kg)	0.5	0.9	1.0	0.7	1.4	1.4	0.6	0.7
Acetic acid in mash (kg)	0.1	0.3	na	na	na	na	na	na
Citric acid in mash (kg)	nd	nd	na	na	na	na	na	na
Succinic acid in mash (kg)	1.3	1.3	na	na	na	na	na	na
Ethanol in liquid (wt%)	6.2	6.0	5.7	6.5	5.7	5.7	5.0	5.1
Total ethanol (kg)	38	37	36	41	39	37	33	34
Ethanol yield (%)	76	77	79	82	79	80	73	70

Average values of two samples. Non-sugar dry matter in beet: estimated at 7%.

nd =not detected (below 0.1 wt% detection limit for sugars). na =not analysed.

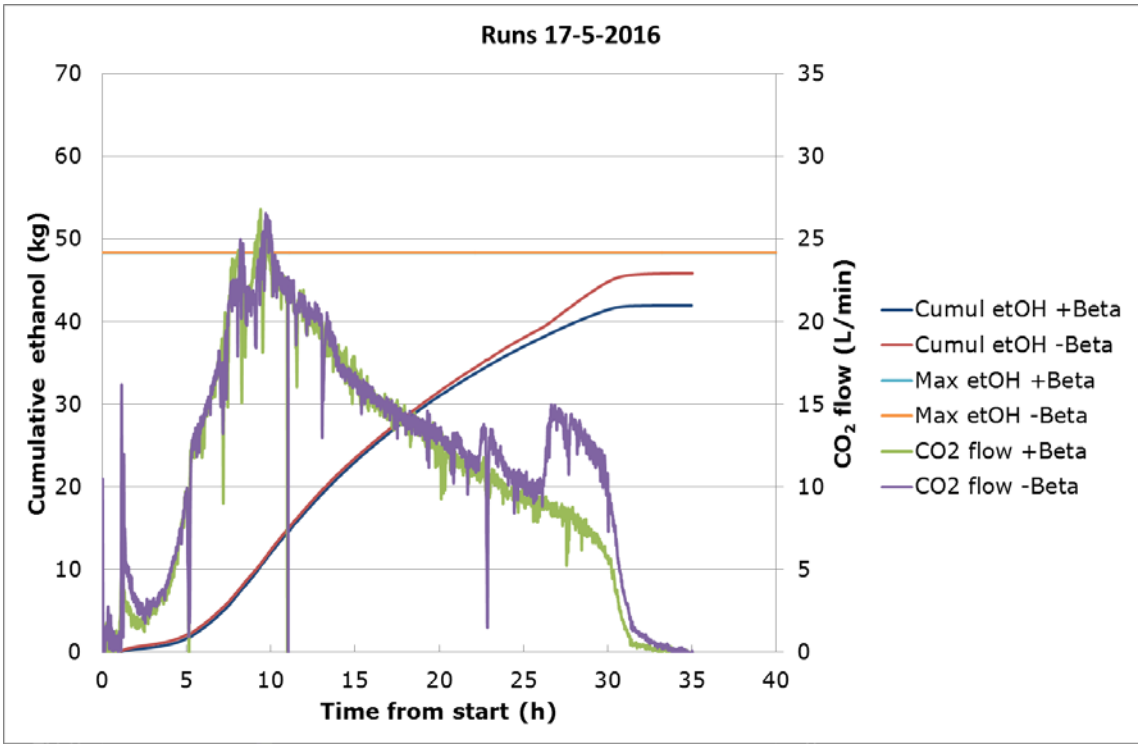

Figure 3 *CO_2 flow and ethanol formation, with and without Betaprocess; 17-5-2016 runs. 15.8 wt% sugar in beet assumed for calculation of maximum ethanol production.*

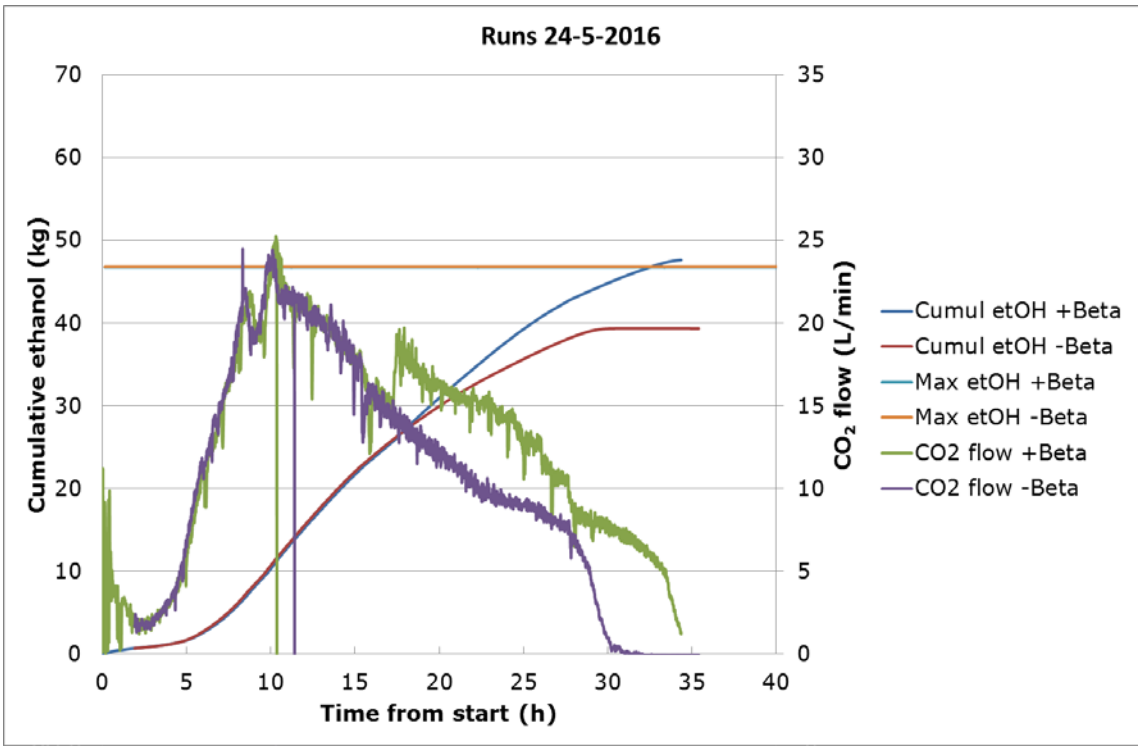

Figure 4 *CO_2 flow and ethanol formation, with and without Betaprocess; 24-5-2016 runs 15.8 wt% sugar in beet assumed for calculation of maximum ethanol production.*

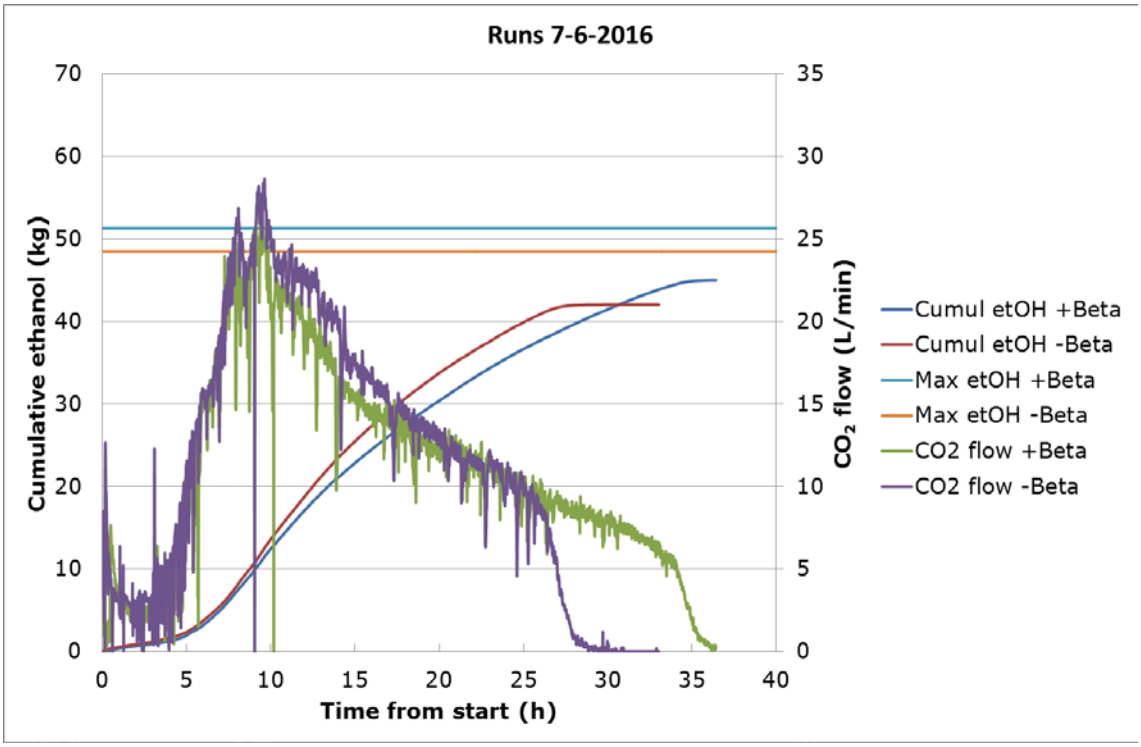

Figure 5 *CO_2 flow and ethanol formation, with and without Betaprocess; 7-6-2016 runs 15.8 wt% sugar in beet assumed for calculation of maximum ethanol production.*

Several possibilities exist to explain why no effect of the Betaprocess was observed in this trial. Firstly, the treatment may simply not have the desired effect. Secondly, 'non-treated' material was still moved through the Betaprocess equipment, but at a lower temperature of 50 °C instead of 65 °C, and without the vacuum. In principle, it is possible that this already had an effect, making it more difficult to distinguish an effect of the Betaprocess-treated material. Thirdly, it is possible that the Smicon mill already opened a large fraction of the sugar beet cells. Lumps of 2 mm to 3 mm in diameter were still clearly visible after milling, but if all or most of the material around the lumps completely disintegrated, any effect of the Betaprocess may go unobserved. Lastly, the sugar beets used for the trial of May/June 2016 had been in storage for approximately 6 months. Possibly, the long storage caused an effect of the Betaprocess to go unobserved. It was decided to repeat the trial in October/November 2016, with fresh beets harvested in October 2016.
The setup of the second trial was almost identical to the one in May/June 2016, except for the following: a) the order in which the no-treatment and Betaprocess treatment was also changed between runs, and b) glycerol analyses were included, at the expense of analysis of other side products.

Quite unexpectedly, the runs in the second trial displayed a much longer fermentation time than those in the first: 80 h to 100 h instead of the mentioned 30 h in the May/June '16 trial. It is clear that something inhibited the fermentation, but the exact cause remains unknown. Even after more than 3 full days of fermentation not all sugar was exhausted in the runs of 25-10 and 8-11 (Table 3). In fact, much more fructose was present after fermentation than before. Apparently, sugar beet sucrose is converted to fructose and glucose during the fermentation, but only (most of) the glucose was converted. A clue to the cause is that when the final run was planned (on 21-3-2017, due to weather conditions that prevented beet processing after the 21-11 runs), the sugar beets that had been in storage since October '16 showed excessive fungal growth -much more than expected after 5 months storage- and many beets had rotted as well. It seems probable that a fungal contamination present on the sugar beets caused the sugar-to-ethanol fermentation by the added activated yeast to proceed much slower. In December '16, an extra 5 tonnes of sugar beets had been harvested and stored, and these were used for the runs on 21-3-'17, as they showed much less fungal growth during storage, although they were stored in the same cooling cell. However, the resulting fermentations still lasted much longer than the 30 h in the May/June '16 trial and after 60 h, the fermentation run was stopped.

Gas flow measurements were erratic for one fermenter and not present for the other. Gas flow data was not available for the 21-11 runs. In the runs of 25-10 and 8-11, gas flow data indicates that only 30% to 50% of all saccharose seems to have been converted to ethanol after 24 h. Furthermore, while the analysis data in Table 3 seems to match with the gas flow data (albeit both pointing towards a low yield of 70 %), there is a large discrepancy between the analysis data and gas flow data in the 8-11 runs (Figure 7). The gas flow data indicate 85 % yield after 100 h of fermentation for the 'no Beta' run and 57 % for the '+ Beta' run, while analysis data point towards 60 % for both.
In short, the fermentations in the trial of October/November 2016 and March 2017 are to be considered 'failed' to the extent that these cannot be used to confirm an effect of Betaprocess. The fermentation performance itself was not good enough to draw conclusions on a Betaprocess effect. An important lesson however is that sugar beets apparently may contain compounds and/or contaminations that may seriously hamper yeast in the ethanol formation from the sucrose present. This is something to keep in mind when developing direct sugar beet fermentation processes, certainly when storage is involved.

Table 3 *October/November '16 and March '17 runs with and without Betaprocess treatment: sugar levels, glycerol yields and ethanol yields.*

General information								
Start date	25-10		8-11		21-11		21-3	
Betaprocess	No	Yes	No	Yes	No	Yes	No	Yes
Fermenter	R02	R01	R01	R02	R01	R02	R02	R01
Order	First	Second	First	Second	Second	First	Second	First
Before fermentation								
Beet in fermenter (kg)	559	561	573	550	560	539	555	577
Added water in mash (%)	21	19	19	21	20	21	21	17
Non-sugar dm in beet (kg)	39	39	40	39	39	38	39	40
Fructose in mash (kg)	1.0	1.0	1.0	0.8	1.2	1.2	1.5	1.0
Glucose in mash (kg)	1.3	1.2	1.0	1.1	1.2	1.1	2.0	1.4
Maltose in mash (kg)	nd	nd	nd	nd	nd	nd	nd	nd
Saccharose in mash (kg)	103	103	102	95	96	96	89	102
Total sugars in mash (kg)	106	105	104	97	98	98	93	104
Saccharose in beet (wt%)	18.7	18.5	18.0	17.4	17.3	17.9	16.2	17.8
Total sugars in beet (wt%)	19.1	18.9	18.3	17.8	17.7	18.3	16.8	18.2
Maximum ethanol (kg)	57	56	56	52	53	53	50	56
Maximum CO_2 (kg)	53	53	53	49	49	49	46	52
During fermentation								
Additions pH control (kg)	5.2	6.7	4.1	3.2	5.4	4.2	7.6	11.9
After fermentation								
Total mash (kg)	661	647	661	650	635	648	664	653
Liquid fraction (kg)	621	608	621	611	595	610	625	612
Fructose in mash (kg)	11	11	20	21	nd	nd	nd	nd
Glucose in mash (kg)	nd	nd	0.8	1.4	nd	nd	nd	nd
Total sugars in mash (kg)	11	11	21	22	nd	nd	nd	nd
Glycerol in liquid (wt%)	0.77	0.74	0.66	0.64	0.92	0.92	0.87	0.92
Total glycerol (kg)	4.8	4.5	4.1	3.9	5.5	5.6	5.4	5.6
Glycerol yield (%)	8	8	7	7	10	11	11	10
Ethanol in liquid (wt%)	6.5	6.4	5.5	5.0	7.0	6.9	5.7	6.4
Total ethanol (kg)	40	39	34	31	42	42	36	39
Ethanol yield (%)	71	69	61	59	79	80	72	70
Combined eth + glyc yield (%)	79	77	69	66	89	91	83	80

Average values of two samples. Non-sugar dry matter in beet: estimated at 7%. nd =not detected (below 0.1 wt% detection limit).

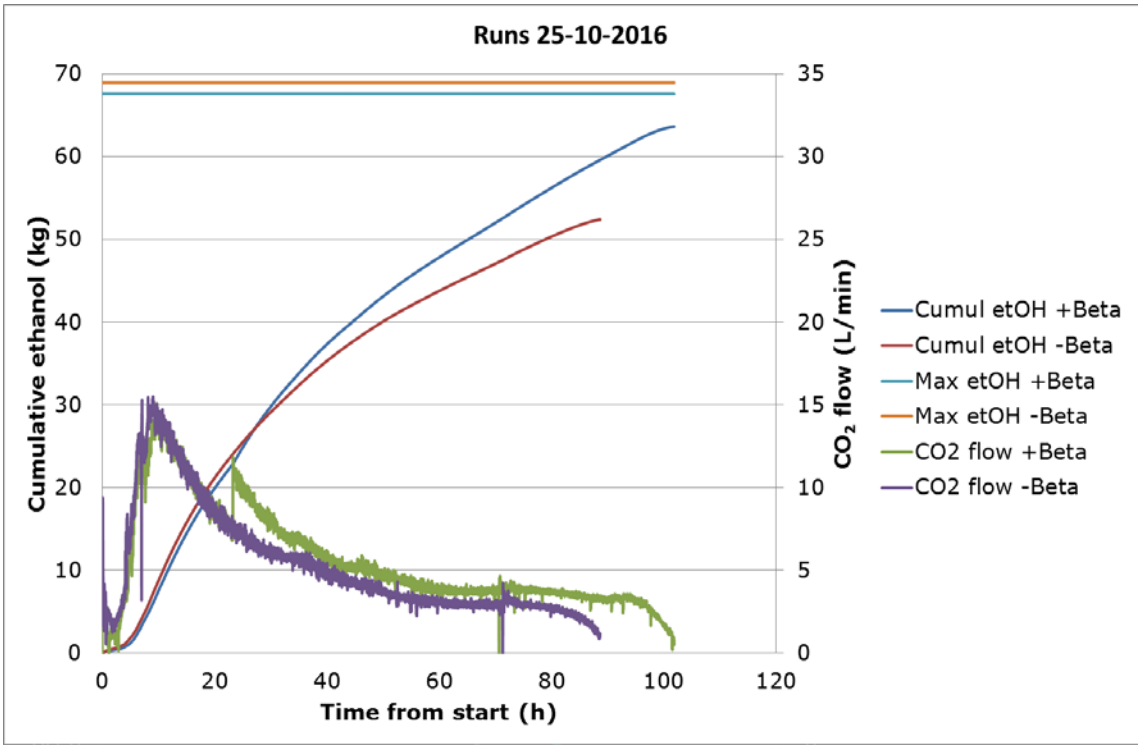

Figure 6 *CO_2 flow and ethanol formation, with and without Betaprocess; 25-10-2016 runs 18.1 wt% sugar in beet assumed for calculation of maximum ethanol production.*

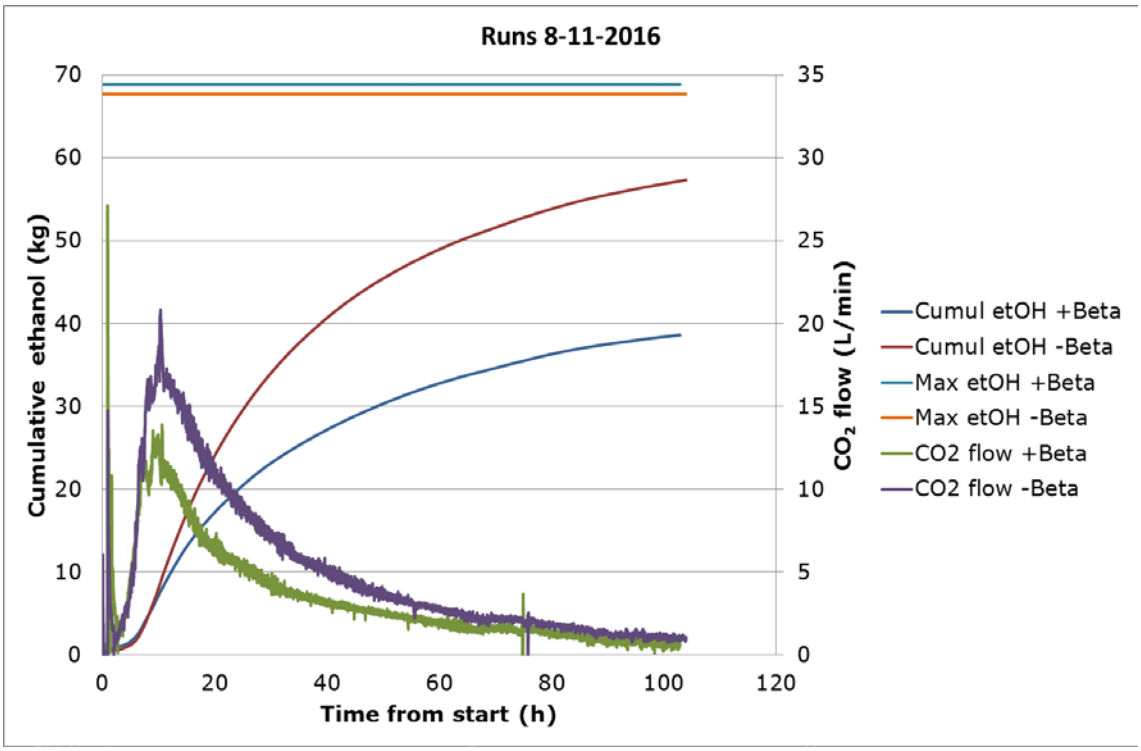

Figure 7 *CO_2 flow and ethanol formation, with and without Betaprocess; 8-11-2016 runs 18.1 wt% sugar in beet assumed for calculation of maximum ethanol production.*

4 Conclusions

Work package 1:

- With the adjustments realised, the ‘direct processing’ fermentation of sugar beets to ethanol was performed successfully.

- In future scale up, water addition should be minimised, which would necessitate adjustments to the heat exchanger and the fermenter’s mixing system.

- Either a different distillation setup is needed in order to be able to process fermented mash, or a solid/liquid separation of the mash.

Work package 2:

- In the first trial, final ethanol yields were 70 % to 82 %, according to chemical analysis. Gas flow measurements pointed towards a final ethanol yield of 83 % to 85 %. The combination of results leave about 5 % to 10 % room for yield improvement after optimisation.

- 80 % to 90 % of all ethanol was produced in the first 24 hours. Peak ethanol production was approximately 4 g/L per hour and took place after 8 h to 9 h from the start of the fermentation.

- No effect of Betaprocess was observed in the first trial.

- In the second trial, fermentation performance seems to have been seriously hampered due to the presence of fungi on the applied sugar beets. This possibility is an important matter to consider when designing direct sugar beet fermentation processes.

References

Deloitte, 2014. Opportunities for the fermentation-based chemical industry. An analysis of the market potential and competitiveness of North-West Europe.

Ingledew, W.M. 2009. The alcohol textbook. A reference for the beverage, fuel and industrial alcohol industries. Nottingham University Press.

Corresponding address for this report:
P.O. Box 430
8200 AK Lelystad
The Netherlands
T +31 (0)320 291111
www.wur.eu/plant-research
www.acrres.nl

Report WPR-744

www.acrres.nl