Report no: O3.7

TECHNICAL REPORT ON PILOT A TESTS IN SWEDEN

Henny Andersson, Eva Thorin, Johan Lindmark, Sebastian Schwede, Joakim Jansson, Anssi Suhonen, Ari Jääskeläinen, Tero Reijonen, Reino Laatikainen, Anneli Heitto, Elias Hakalehto

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1. Introduction

1.1 Basic background information

A biorefinery concept has been piloted at a chicken farm in Enköping, Sweden using mainly slaughterhouse waste and chicken manure from the farm as substrate. The process design has been outlined by the company Finnoflag Ltd in Finland and the basic idea is to provide a concept with an improved productivity and production of versatile products from various waste streams, for example waste from food industry and pulp industry, such as potato, whey and wastes from chemical pulp production. By improved productivity the products can be produced faster and the minimum facility size can be reduced. If end product concentration can be increased also downstream processing of products can be made more affordable.

The biorefinery process has previously been mentioned in the REMOWE State-of-art report (O4.1.1), and described in: Hakalehto et al. Production of energy and chemicals from biomasses by micro-organisms. In: Dahlquist: Biomass as energy source: resources, systems and applications, which will be published in 2012 by an international publisher (CRC Press, Taylor & Francis Group). REMOWE work has been utilized in part of this book.

The biorefinery process consists of pre-treatment of the substrate including dilution, pH adjustment and other physical-chemical steps, for example particle size reduction, necessary for the used substrate. The most essential biochemical routes utilized in the process are 2,3-butanediol-fermentation and acetone-butanol fermentation but also methane fermentation.

The outputs from the process are among others butanediol, butanol, ethanol, acetone, hydrogen which are valuble products that can be used as bulk chemicals, biomaterials and energy products. Butanediol can be further processed to butadiene, which is a raw material for synthetic rubber, plastic monomers, industrial fibers and anti-icing agent. Ethanol as well as butanol can be used for replacing petrol for fuelling cars or other internal combustion engines. Butanol and acetone are important industrial chemicals.

The bioprocess concept has been tested in laboratory in volumes of 1 L to 15 L and production rates 2-3 times higher than reported in other experiments have been experienced. In the pilot the bioprocess is scaled up to 200 L. The mobile pilot plant is constructed inside a freight container, including e.g. feed pre-processing, hydrolysis vessel, nitrogen gas bottles for enhancing the bioprocessing, two bioprocessing vessels, piping, heat exchanger, automation and control system and measurement equipment.

1.2 Location for pilot runs

During the pilot runs in Sweden the Pilot A was located at Hagby Gård, Tillinge 1, a chicken farm 6 kilometer west of Enköping (Figure 1). The population of the region is approx. 40000. The farm produces roughly 800 chickens a week for slaughter (figure for the year 2014). The farm produces chicken in small scale for direct delivery to customers in the County and for selling in their own store located in Västerås.

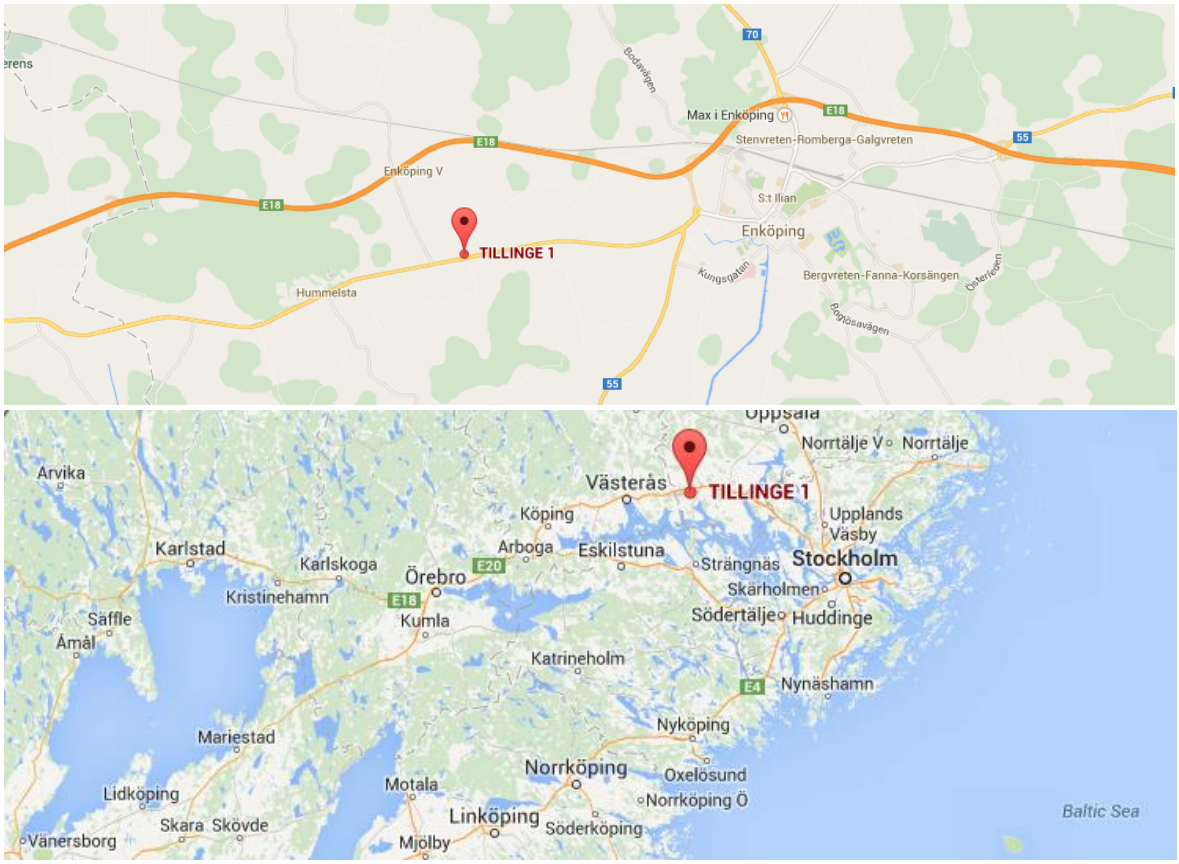

Figure 1: Location of Pilot A in Sweden. At Hagby Gård Enköping.

1.3 Transportation and installation

The Pilot was transported with a truck from Poland (see Figure 2). When unloaded from the truck stone slabs have been positioned under the corners in the front of the container in order to level it. The higher floor level in one side cause any leakage of fluid to pass on to the pumps located in the far end of the container (Figure 3). The plant was connected to the local electricity grid and to water supply from the chicken farm. After setting up the equipment, an inventory check has been performed to make sure everything (lab equipment, additional tools, etc.) was in its place.

Figure 2: Unloading of the container in Sweden at Hagby gård Enköping.

Figure 3: Levelling of the container with stone slabs.

2. On-site and additional testing

The substrate used during the Swedish operating period was chicken slaughterhouse waste, manure, straw (hey) and saw dust. In the following a description of the raw material and its characteristics will be given. The resulting consequences for on-site testing will be explained in the following description of the tests that have been performed. Besides the pilot runs the biogas potential of the different substrates used has been investigated in laboratory anaerobic digestion batch tests. Substrate and products content has also been analyzed at external laboratories.

2.1 Substrate

The substrate being used in Pilot A test runs in Sweden mostly comes from the chicken farm where the pilot was run. Both the manure and straw have been collected from the farm. Figure 4 shows the different kinds of substrates used, except the straw and saw dust. The saw dust being used is the same as used for the chickens to lie on at the farm.

Figure 4: From top left showing chicken leg, followed by feathers, intestines and manure.

Table 1 gives the dry matter and organic dry matter contents (volatile solids content, VS) of the different wastes measured at the laboratory at Mälardalen University in connection with biogas potential batch tests. Table 2 shows results from content analysis of the substrates carried out at an external laboratory (Eurofins Environment Sweden AB).

Table 1: Dry matter (TS) and organic dry matter (VS) contents of the different substrates. SD=standard deviation, FM= Fresh mass

	FEATHERS	INTESTINES	MANURE	HEY
TS [% FM]	35.83	30.78	47.05	89.30
SD	0.20	1.91	1.92	0.02
VS [% TS]	87.71	93.49	78.85	80.74
SD	2.52	1.42	0.75	1.61

Table 2: Content analysis of the substrate used in biorefinery test runs in Sweden. The runs are described further in Chapter 3. SD=standard deviation, FM= Fresh mass, SS= Swedish Standard, EN=European standard, NMKL= Nordisk Metodikkommitté för Livsmedel (Nordic committee of methods for food), SLVFS= Instructions according to the Swedish National Food agency

	Sub-strate mix Run1	Intes-tines Run2	Straw Run2	Straw Run3	Manure Run4-1	Manure Run4-2	Manure Run4-3	Measurement method
TS [% of FM]	56.4	26.9	87.2	88.1	30.2	49.8	67.7	SS EN 12880
VS [% of TS]	99.8	92.2	93.3	91.0	83.4	37.1	30.7	SS EN 12879
pH	6.6	6.2	6.4	5.9	7.2	8.8	9.0	EN ISO 15933:2012
Total- N [g/kg FM]	2.2	22	6.4	5.9	6.8	6.1	7.5	Kjeldahl, EN 13342
NH_4-N [g/kgFM]	0.46	6.5	0.55	0.47	1.3	2.5	3.4	Standard methods 1998, 4500 mod
Proteins [% of FM]	1.09	9.69	3.66	3.39	3.44	2.25	2.56	calculated
Fats [% of FM]	54.9	15.1	1.95	0.99	0.59	0.90	1.08	NMKL 131
Carbo-hydrates [% of FM]	0.20	0	76	76	21	15	17	SLVFS 1993:21 calculated
COD-Cr [g/l]	960	550	360	370	220	260	410	Spectroquant
Energy content [MJ/kg]	20	7.2	14	14	4.4	3.3	3.7	SLVFS 1993:21 calculated

Before feeding the substrate into the reactor it was crushed into smaller pieces and also hygienised.

2.2 Batch tests

Samples of substrates used in the pilot runs in Sweden were collected to determine the biogas potential in the laboratory at Mälardalen University in Västerås. The test was done following the VDI guideline 4630. Digestate from the Växtkraft biogas plant in Västerås was used as inoculum for the biogas potential test. Prior to the test the digestate was stored at 37°C and

sieved to remove particles >3 mm. Inoculum (~3 g volatile solids (VS)) and substrate samples (~3 g VS) were mixed with tap water to a total volume of 700 mL in 1 L sealed glass bottles. All samples were investigated in triplicate at mesophilic conditions (T = 34.5±0.5°C). The biogas volume produced was measured indirectly by determination of the pressure in the bottles. The gas volume was normalized to standard conditions (T = 273.15 K, p = 1013 hPa).

2.3 Pilot runs

The pilot was run following the pilot operating manuals. Figure 5 shows an overview of the plant. In short one run included the following steps:

- Substrate pretreatment by milling and slurrying: The substrate is shredded into smaller pieces by using an attached mixer to the pre-treatment tank. In the pretreatment tank the substrate is mixed with water and stirred into a homogenous mass.
- Hygienisation: The slurry is mowed into the hydrolyser tank where it's is heated up to about 80 degrees in one hour for hygienisation.
- Enzymatic hydrolysis- done using technology innovated by Finnoflag Ltd: After the mass in the hydrolyser tank has cooled to the desired level the pH is adjusted and enzymes added.
- Cultivation and incubation of microbes in PMEU (Portable Microbe Enrichment Unit, Samplion Ltd)– done using technology from Finnoflag Ltd
- Transferring cultivated microbes to the seed fermenters, and then to the reactor
- Bioprocess in reactor: Here it's possible to adjust pH and temperature. There is also possible to add gas and adjust its gas flow to optimize the mixture of gas to improve the bioprocess.
- Stabilization of process broth.

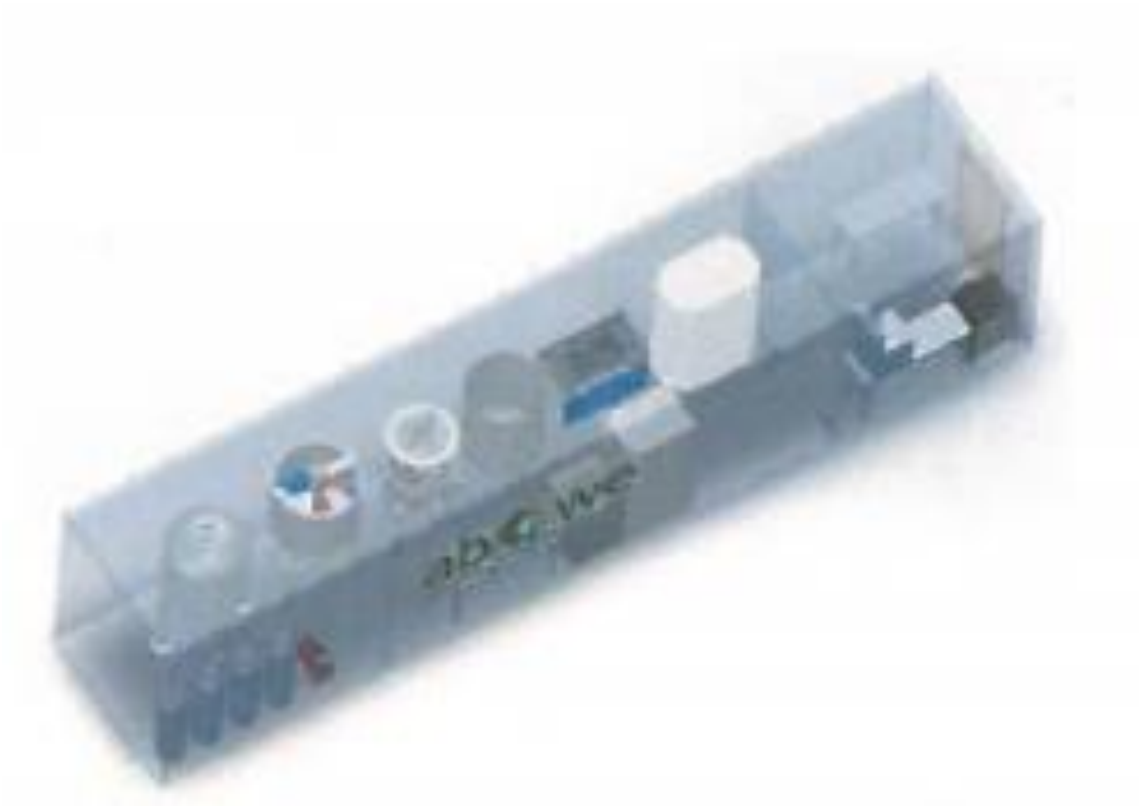

Figure 5. Overview of the biorefinery pilot plant situated in a container. To the left the different process reactors can be seen and to the right the on-site control and analysis equipment can be found.

Several samples were collected and some measurements done on-site during the runs. An overview of the measurements and sampling can be seen in Figure 6. During the runs the pilot was run all the time (24 h a day) for about one week. Personnel was at the plant to take samples, control the process and do measurements. All in all 4 runs were done during the piloting in Sweden. The runs are described more in detail in Chapter 3.

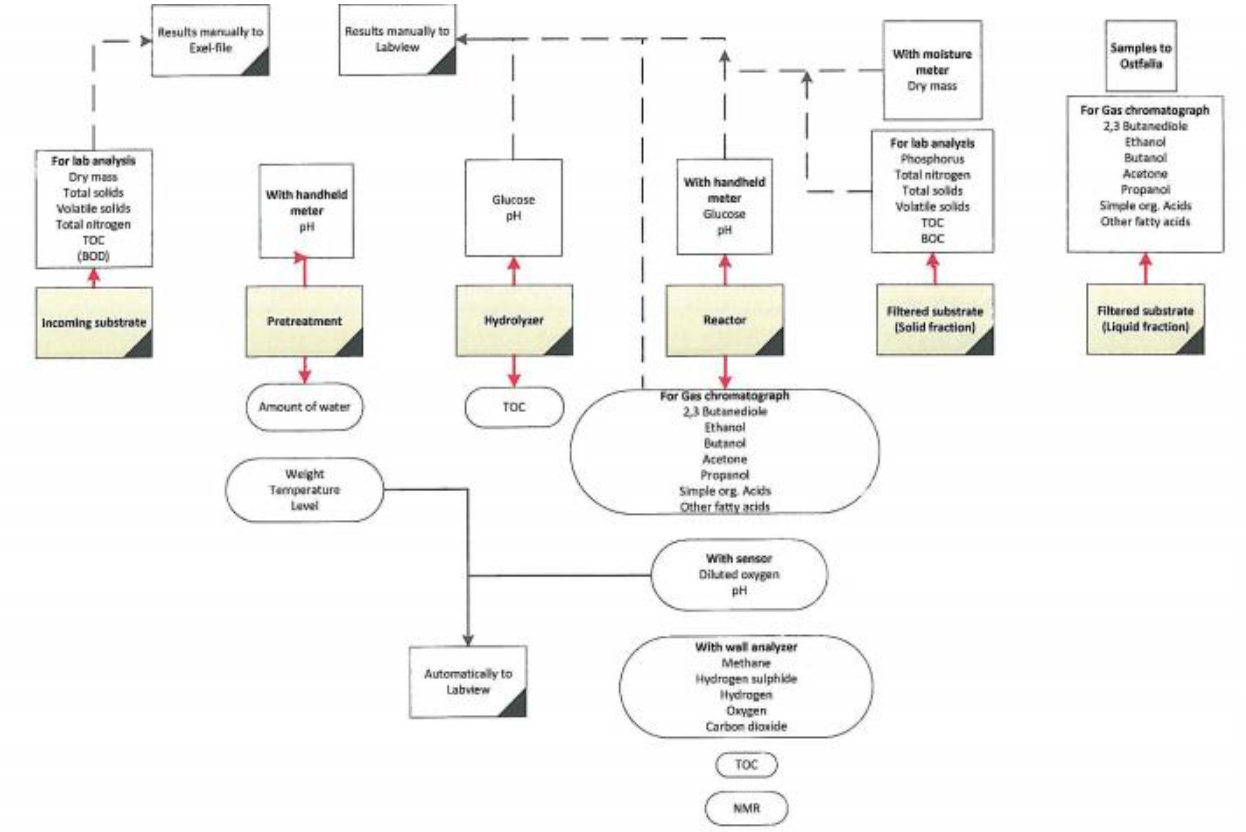

Figure 6. Overview of sampling and analysis during pilot plant operation.

2.4 Timeline of the Swedish operating period

Table gives an overview over mentionable events during the Swedish operating period. Major events will be described more in detail in Chapter 3.

Table 3: Timetable of mentionable events during the Swedish operating period.

Date	Event
04.08.2014	Plant arrival at Hagby farm, Sweden; Installation of the plant
11.08.2014	First Run, one week Monday to Friday
25.08.2014	Second Run, one week Monday to Friday
08.09.2014	Third Run, one week Monday to Friday
22.09.2014	Fourth Run, two weeks Monday to Wednesday
30.09.2014	Investor event
28.10.2014	Plant shipping back to Finland

3. Description of the pilot runs

In Table 4 an overview of the four different pilot runs done in Sweden is given. Besides the main substrates some carbohydrate sources were also added to the reactor when the measured glucose levels were decreasing. This was done with the aim to be able to continue the test run with the planned microorganisms and not risking that the preferred conversion path would stop due to lack of available carbohydrate source for the microbes.

Table 4 Overview of the different biorefinery runs done in Sweden.

	Added Carbohydrates	**Substrate**	**Condition**	**Microbes**
RUN1	sugar	straw, feathers, intestines (60kg)	Aerobic	*Klebsiella, E-coli*
RUN2	sugar, potato flour	straw, manure/wood, intestines (40kg), peptone, sawdust,	Anaerobic	*Clostridium butyricum, Clostridium acetobutylicum, Cellulomonas*
RUN3	apples	straw, intestines (100kg)	Anaerobic	*Clostridium butyricum, Clostridium acetobutylicum*
RUN4	blueberry soup (for trace elements mainly)	manure, saw dust, manure, intestines (34kg)	Anaerobic	*Clostridium butyricum, Clostridium acetobutylicum*

3.1 Run 1

3.1.1 Plan for Run1

The first idea of the run was to use the same ratio of feathers to intestines that is the result of the slaughter of the chickens, which is 60 % more intestines than feathers. Due to that the plant crusher did not manage to crush the feathers without getting stuck, it was not possible to pre-treat the feathers for the process. Therefore only a small amount was added in the first run and the feathers where excluded from the rest of the runs. The same problem occurred with the straw, but it could be crushed using a blender before they were feed to the process. Since this was a time consuming job only small amounts of straw were added. Therefore the main substrate used was the chicken intestines. The final mix of substrate for the first run can be seen in Table .

Table 5 Incoming substrate for run1 in Sweden.

Substrate	**Weight**	**Unit**
Straw 1:th addition	1.5	kg
Straw 2:nd addition	1	kg
Feathers	3.7	kg
Intestines	54.9	kg
Water	284	L
Chicken Liver 1:th addition	800	g
Chicken Liver 2:nd addition	400	g

3.1.2 Execution of Run1

The feeding to the pretreatment step and hydrolyser started at noon on Tuesday. The hydrolysis started in the evening on Tuesday. The aim was to reach 80 °C and keep that

temperature for one hour and then cool down to 55 °C. At 03:30 on Wednesday chicken liver and commercial enzymes where added to the hydrolyser (see Table 6), after the hydrolyzing step had been done. The temperature then measured 61 °C. Since both the heating and cooling system of the reactor where on at the same time the cooling system was not effective. After shutting the reactor heating down temporarily the temperature in the hydrolyser could reach 55 °C in the afternoon on Wednesday. Then another 400 g of chicken liver where added together with 1 kg of straw (Table)

Table 6 Enzymes added during Run1.

Enzymes added	**Amount**	**Unit**
viscamyl flow	0.,2	L
amylex,	0.2	L
alphalase NP	0.1	L
glucostar	0.1	L

On Wednesday at 18:30 the first microbes were added (7 Liters of *Klebisella* and 5 Liters of *E-coli*). On Thursday another 7 Liters of *Klebisella* were added together with 10 kg of sugar diluted in 15 Liters of water. (Table 7)

Table 7 Microbes added during Run1.

Microbes	**Amount**	**Unit**
Klebsiella 1st addition	7	L
E-coli	5	L
Klebsiella 2nd addition	7	L

Sampling was made every second hour from the reactor starting from 19:30 Wednesday the 13th of August and ending at 09:20 on Friday the 15th of August.

3.1.3 Problems encountered during Run1

Several practical and technical problems occurred during Run1 and they are summarized in the following list:

- Due to the lower limit of the scale for weighing the incoming substrate was to high the inaccuracy for the lower substrate weights might be high.
- Foaming in the rector: antifoam had to be used, 3 table spoons in total. When the level gets too high it is not possible to see through the sight glass.
- A lot of base had to be added to keep the pH at the recommended level. However, when no base was added, due to the run out of base solution, the pH stabilized at 5.3.
- Feathers could not be cut in the pilot crusher and it had to be excluded as a substrate in the runs. The feather that made it into the process in Run 1 ended up floating on top in the reactor causing a flouting layer to be formed together with the straw.
- Straw was not possible to get pre-treated in the pilot crusher but could be cut before feeding to the process, using a blender, instead.
- Crushing of intestines took a relatively long time.
- Straw and feathers that had not been cut properly got stuck in the pumps, therefore the lid had to be removed from the reactor for cleaning.
- The fat in the feed caused a thick layer in the tanks that was hard to remove when cleaning with only water.

- Centrifugation of the samples prior to analysis were problematic. The samples were not fully separated even after three times of centrifuging.
- The Glucose level was low and therefore sugar was added. However, the sugar was consumed fast.

3.2 Run 2

3.2.1 Plan for Run2

In the second run in Sweden the idea was to use mixed microbe cultures and also trying to utilize natural microbiological activities in the substrates. Previous Pilot A runs and Finnoflag experiments have shown that aerobic and anaerobic strains can get along in the same process and this was the idea to test. The aerobic flora exhausts the oxygen, and makes it possible to establish oxygen-free niches. The plan was to add *Cellulomonas* and *Klebsiella* together with a small amount of cellulose containing substrate to the reactor and leave them to adjust themselves for some time under aerobic gas flow. After that, to move the big portion of the wastes (including all slaughterhouse wastes) into the reactor, start making the content anaerobic with nitrogen flow. After reaching anaerobic conditions, inoculate with *Clostridium butyricum* and after letting the reactor content adjust for about three hours inoculate also with *Clostridium acetobutylicum*. During the continuation phase, after the clostridia seem to have adjusted, the bottom of the reactor can be carefully aerated with some air flow, whose oxygen would be consumed before it reaches the top layers. There the gas flow (to the upper ring) could and should remain strictly anaerobic.

In the second run the feathers had been excluded from the substrate mix used. Manure from the dunghill on the farm were added including wood (mainly saw dust). The substrates used are shown in Table .

Table 8 Incoming substrate for Run2 in Sweden.

Substrate	**Amount**	**Unit**
Intestines	36.9	kg
Water	213	Liters
Manure/wood	30	Liters
Straw (<5 mm pieces)	9	Liters

3.2.2 Execution of Run2

The blender used for cutting the straw broke down and part of the straw was therefore cut by hand instead. Since this took a longer time a smaller amount than first intended was added. The straw was added after the other substrates had been heated to 80 °C for an hour in the hydrolyzer step.

When the temperature in the hydrolyzer had cooled down to 50 °C pH was adjusted to 5.5 and the enzymes (see Table 9) were added. A mixture of peptone, sawdust and distilled water (see Table 10) was put in the reactor together with *Cellulomonas*. In a second substrate addition chicken liver was added to the substrate mixture in the hydrolyzer, the mixture was pumped into the reactor.

Table 9 Enzymes added during Run2.

Enzymes	Amount	Unit
Viscamyl	0.2	L
Amylex	0.2	L
Optimash	0.2	L
Alphalase	0.1	L
Glukostar	0.1	L

Table 10 Mixture first added to the reactor during the Run2 in Sweden.

Added	Amount	Unit
Peptone water	500 g peptone in 3 L distilled water	
Sawdust	5	Liters
Distilled water	40	Liters

Table 11 2nd substrate addition in Run2 Sweden.

Added	Amount	Unit
Chicken liver	900	g
Distilled water	10	Liters

The microbes used in Run 2 are shown in Table 2. At 18:15 on Tuesday, after the second substrate addition to the reactor took place and pH was adjusted, *Clostridium butyricum* was. Later the same evening *Clostridium acetobutylicum* was added. On Wednesday evening potato flour and some enzymes were added to the reactor (Table 3) and on Thursday afternoon a sugar solution was added with the aim to increase the glucose level in the reactor (Table 4).

Sampling was made every second hour from the reactor starting from 00:15 Wednesday the 27th of August and ending at 07:17 on Friday the 29th of August.

Table 2 Microbes added during Run2.

Microbes	Amount	Unit
Clostridium butyricum	7	L
Clostridium acetobutylicum	7	L
Cellulomonas	7	L

Table 3 Added potato flour and additional enzymes during Run 2 in Sweden.

Added	Amount	Unit	Time
Water	100	Liter	14:00
Potato flour	3	kg	14:00
Amylex	0,1	Liter	14:40
Potato flour	2	kg	15:40
Glucostar	0,1	Liter	16:30

Table 4 Added sugar during Run 2 in Sweden.

Added	Amount	Unit
Sugar	14	kg
Distilled water	12	Liters

3.2.3 Problems encountered during Run 2

Some practical and technical problems occurred also during Run 2 and they are summarized in the following list:

- Foaming
- Blender for cutting the straw broke down and part of the straw had to be cut by hand.
- Quite a lot of base and acid had to be used for pH adjustments.

3.3 Run 3

3.3.1 Plan for Run 3

The idea of the third run was to focus on using the microbe *Clostridium acetobutylicum,* and only use *Clostridium butyricum* in reserve. It was also decided to test using the intestines as the main substrate only adding a smaller amount of straw as carbohydrate source. The aim was to get a highly concentrated medium, and short time reactions due to the experience of the microbe's fast reaction time and the problem to get a carbon source being enough for the production in the previous runs. The substrates used are shown in Table 15.

In this run it was tested a method for improving the hydrolysis of the straw by adding it in a water solution at pH 4 together with Viscamyl TM enzyme, warm up to 50 °C and then leaving it in at room temperature for some days before adding it to the reactor.

The run was made mainly under anaerobic conditions.

Table 15 Incoming substrate for Run3 in Sweden.

Substrate	**Amount**	**Unit**
Intestines	100	kg
Water	200	Liters
Straw	4	kg
Water for straw	70	Liters

3.3.2 Execution of Run 3

The hydrolysis of the main substrate was performed at 80 °C during one hour. When the temperature reached 50 °C the first round of enzymes were added (see Table 5). A second hydrolysis step was done and after cooling the second round of enzymes was added. When the temperature in the hydrolyser reached 40 degrees 900 grams of chicken liver was added.

The straw was pre-treated in another container as described above (Chapter 3.3.1) and the straw mixture was added to the hydrolyser tank a few hours after the addition of chicken liver.

The microbes used in Run 3 are shown in Table 6. *Clostridium acebutylicum* was added at Wednesday afternoon and the *Colstridium butyricum* was added the day after in the evening.

Table 5 Enzymes added during Run 3 in Sweden.

Enzymes	**Amount**	**Unit**
First addition		
Alphalase	≈0.3	dl
Second addition		
Viscamyl	0.15	L
Amylex	0.2	L
Optimesh	0.2	L
Glucostar	0,3	dl

Third addition		
Viscamyl	0.12	L

Table 6 Microbes used in Run 3 in Sweden.

Microbes	**Amount**	**Unit**
Clostridium acetobutylicum	14	L
Clostridium butyricum	7	L

A third addition of enzymes was done after the first addition of microbes (see Table 16). Since the glucose level was low apples (15 kg peeled, boiled in 2 litres water and smashed) were added to the reactor as an additional carbohydrates sources on the evening after the first additions of microbes. Another 10 kg of apples in 1.5 litres of water was added the day after when adding the *Clostridium butyricum*.

3.3.3 Problems encountered during Run 3

Some practical and technical problems occurring during Run 3 were the following:

- A problem with the cooling occurred caused by low pressure in the pipe system causing slow cooling.
- The total pre-treated volume was too large for keeping the level in the reactor below the sight glass. Therefore about 143 liters of the pre-treated substrate mixture in the hydrolyser was removed. All of the 70 liters of straw mixture was added.
- It was not possible to add the straw mixture to the reactor with the circulation pump, because it was too thick. Instead it had to be poured into the hydrolyser tank and pumped inside the reactor.
- The pump under the hydrolyser tank broke and had to be changed. When opening the electrical part of the pump it was noticed that some part of it had been fixed with duct tape in an insufficient way. This pump was changed with the pump for the stabilizer that was not used in this run.
- Because of the breakdown of the pump, the cleaning of the tanks were delayed until the week after.
- Problem with seed fermenters occurred. When doing seed fermenter gas test, one gas distributor was missing and one was flouting in the fermenter. The gas flow was increased to 0.4 l/min to compensate for this and the flow was adjusted to produce mixing in all fermenters.

3.4 Run 4

3.4.1 Plan for Run 4

The experience from the previous runs indicated that the lack of simple hydrocarbons made it difficult to get higher levels of the products. Methods to elevating the levels are available but then more time would be necessary for a run. In the fourth run in Sweden the run was therefore prolonged to last from Monday the first week until Wednesday the week after starting up the test run and a fed-batch approach, to be able to better understand the speed of product formation (productivity), was tested.

The idea was to perform the hydrolysis in two steps with manure in one batch and intestines in another. The hydrolysis time was also extended and the manure was also treated with microbes in a separate step before adding the rest of the substrate.

The run was made mainly under anaerobic conditions. The substrates used are shown in Table 7.

Table 7 Incoming substrates during Run 4 in Sweden.

Substrate	**Amount**	**Unit**
First step		
Manure	34	kg
Water	407	Liters
Second step		
First addition		
Manure	70	Liters
Water	200	Liters
Intestines	34	kg
Saw dust/straw	10	Liters
Second addition		
Manure	14.7	kg
Water	100	Liters

3.4.2 Execution of Run 4

Step 1

The hydrolysis of the manure started at 80°C and was held steady for 60 minutes. The hydrolysed mixture was cooled down during 30 minutes to 50°C and enzymes (see Table 8) were added. No pH adjustment was made before adding the enzymes and therefore the hydrolysis and addition of enzymes (see Table 8) was repeated one more time this time with pH adjustment to 4.5. When the hydrolyzed mixture reached 37 °C, 250 liter of it was moved to the reactor and 900 grams of chicken liver was added to the mixture left in the hydrolyser tank. Another hydrolysis was started and enzymes added (see Table 8) when the temperature in the hydrolyser reached 40°C.

Table 8 Enzymes added during Step 1 of Run 4 in Sweden.

Enzymes	**Amount**	**Unit**
First addition		
Optimash	0.2	L
Viscamyl	0.15	L
Second addition		
Optimash	0.2	L
Viscamyl	0.15	L
Third addition		
Optimash	0.4	L

The microbes used in the first step of Run 4 are shown in Table 20. *Clostridium acetobutylicum* and *Clostridium butyricum*, were added to the hydrolysed manure mixture in the reactor. The day after more *Clostridium acetobutylicum* was added (Table 9).

pH was kept under 6.0 during the process in the reactor.

After about 12 hours 60 liters of the mixture in the reactor was removed and mixture from the hydrolyser was pumped into the reactor. Due to that the pump was stopped by a rock that got stuck only 30 liters from the hydrolyser was transferred to the reactor.

Table 9 Microbes added during Step 1 of Run 4 in Sweden.

Microbes	**Amount**	**Unit**
First addition		
Clostridium acetobutylicum	7	L
Clostridium butyricum	7	L
Second addition		
Costridium acetobutylicum	7	L

Some further pH adjustments were made for the rest of the run. Starting at pH 6.0 to 5.5 and 4.5. The first step run lasted from Wednesday midday until Monday evening. The process was left over the weekend with only checkup once a day. Due to problems with the heating system the temperature in the reactor varied between 30 to 50°C during the weekend.

Step 2

The first plan was to add 50 kg intestines in the second step but only 34 kg was possible to get from the slaughterhouse this week. Therefore more manure was also added in step 2. This time the manure was taken from a pile being more fresh manure than the one used in Step 1 (Table 7). After hydrolysis at 80°C for one hour and cooling, enzymes were added (Table 10).

Table 10 Enzymes added in Step2 of Run 4 in Sweden.

Enzymes	**Amount**	**Unit**
Optimesh	0.2	L
Glucostar	75	mL
Viscamyl	1	table spoon

When the temperature had been kept at 65°C for about 6 hours the temperature was set to 37°C and when reaching 45°C 900 grams of chicken liver was added. The mixture in the hydrolyser was added to the reactor with the circulation pump. Microbes (see Table 13) were also added to reactor. pH in the reactor was kept between 5.5 and 6.0 during the run.

Table 11 Added microbes during Step 2 of Run 4 in Sweden.

Microbes	**Amount**	**Unit**
First addtion		
Clostridium acetobutylicum	7	L
Clostridium butyricum	7	L
Second addition		
Clostridium acetobutylicum	7	L

Another batch of manure and water was added to hydrolyser that was heated to 37°C. pH was adjusted down to 4.3 (aim was pH 4.5). After removing 60 Litres of the mixture in the reactor 60 litres was added from the hydrolyse tank. Also more of the microbe *Clostridium acetobutylicum* was added (Table 12).

pH was adjusted in the reactor with the aim to reach down to 4.5 in the reactor but there was not enough acid available at the pilot plant. Before new acid arrived to the pilot an attempt to

get the pH down using citric acid was made with slow progress. When new acid arrived the pH was adjusted to 4.5.

Later in the evening carbon source in the form of blueberry soup (Table 13) was added.

Table 13 Added blueberry soup

Addition	Amount	Unit
Blueberry soup	6	L

3.4.3 Problems encountered during Run 4

Some practical and technical problems which occurred during Run 3 were the following:

- There were some problems with the computer system, causing problems with the control of gas flow in the PMEU. The PMEU syringes where bubbled for some time and then sealed over the weekend. When coming back on Sunday pressure had been built up in the syringes. One of the syringes was broken and some fluid was lost from the others. Therefore in the end, less microbes where used and some contamination could maybe not be excluded.
- The pH was not adjusted after the first hydrolyze and therefore it had to be done again.
- The power supply was down a few times during this run causing shutdown of the system and restart was needed. There were some complications with components because of this.
- After one of the power breaks the heating system was turned off and it did not get turned on when the system was restarted. This led to a decrease in reactor temperature.
- The heating system was very unstable, oscillating between 60 to 32°C. This could have been caused by the sedimentation in the reactor leading to inaccurate measurements in the reactor for the control system, when set to reading in the tank. By changing the control to “heating water temp” the problem seemed to be fixed.
- Sedimentation in the reactor caused vacuum to build up in the circulation pump.
- The valve used for sampling in the circulation pipe was leaking and samples had to be taken from the left side and the leaking side was shut off.
- The manure contained sand and rocks that got stuck in the pumps.
- The range of the pH meter in the hydrolyser was not enough to be able to measure the aimed pH of 4.5 in this run. A handheld meter needed to be used instead.

4. Results

4.1 Results from batch tests

Figure 7 shows the results of the anaerobic digestion batch tests done on the different substrates used in the biorefinery pilot runs in Sweden and the residues/digestate after the first pilot run. The residues/digestate is here the liquid phase in the reactor after the first run. There was also a solid phase that was not possible to sample. The liquid sample was centrifuged and the biogas potential test was made on the solid phase after centrifugation. Additionally, a solid layer (probably a fat layer) was formed at the top of the vials. That phase was also recovered and included into the biogas batch test.

The intestines show the largest biogas potential and it can also be seen that there is a large biogas potential in the residues after the short period for the pilot runs. The latter reveals the potential and the demand to further use the residues in an anaerobic digestion process for biogas production. To get a value of the full potential of all the residues from the process also the solid phase in the reactor should be investigated. This phase might lower the biogas potential (if this material is not that fast degradable as the one recovered from the liquid phase), but increase the biogas productivity since it includes the potential of all available material.

The dry matter content and volatile solid content of the residues is shown in Table 24.

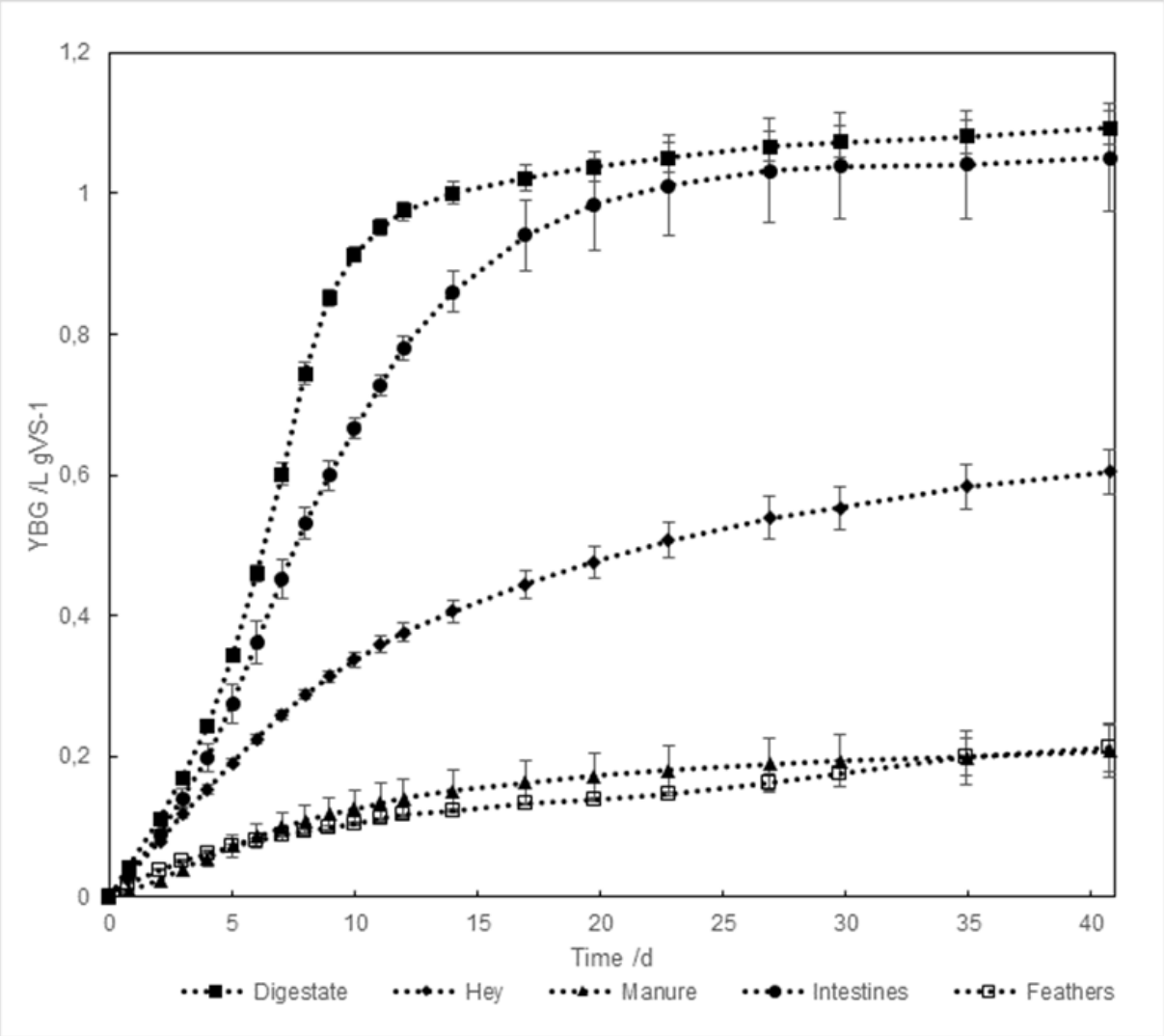

Figure 7. Biogas yield of the different substrates used in the biorefinery pilot runs in Sweden and the residues/digestate after the first pilot run. The y-axis shows the yield in liter biogas per grams of volatile solids of the substrate.

Table 24. Dry matter (TS) and organic dry matter (VS) contents of the residues (digestate) from the first pilot run. SD=standard deviation, FM= Fresh mass

	DIGESTATE_SEPARATED
TS [% FM]	15.09
SD	0.055
VS [% TS]	96.72
SD	0.050

4.2 Results from pilot plant operation with slaughterhouse waste in Sweden

The results of the different measurement on-site as well as in external laboratories are shown for the different runs in Sweden below.

The production of the gas products H_2S and CH_4 are given as the concentration in the gas flow from the reactor at different times of the runs.

The production of the different products in the reactor liquid have been measured with the GC in the pilot plant. For Run 2 the products has also been measured with NMR.

Besides the products also the results from measurements of TOC (Total Organic Carbon), BOD_7 (Biological Oxygen Demand), total nitrogen and phosphorous in the form of PO_4-P (unfiltered) are shown for samples from the reactor. For some samples also the content of fructose, glucose, lactose, maltose and sucrose are shown.

4.2.1 Run 1

Figure 8 and Figure 9 show the production of H_2S and CH_4, respectively. The GC results for the different products in the reactor liquid are shown in Figure 10-15 and Table 25 shows the TOC, BOD7, total nitrogen and phosphorous in the reactor during Run 1.

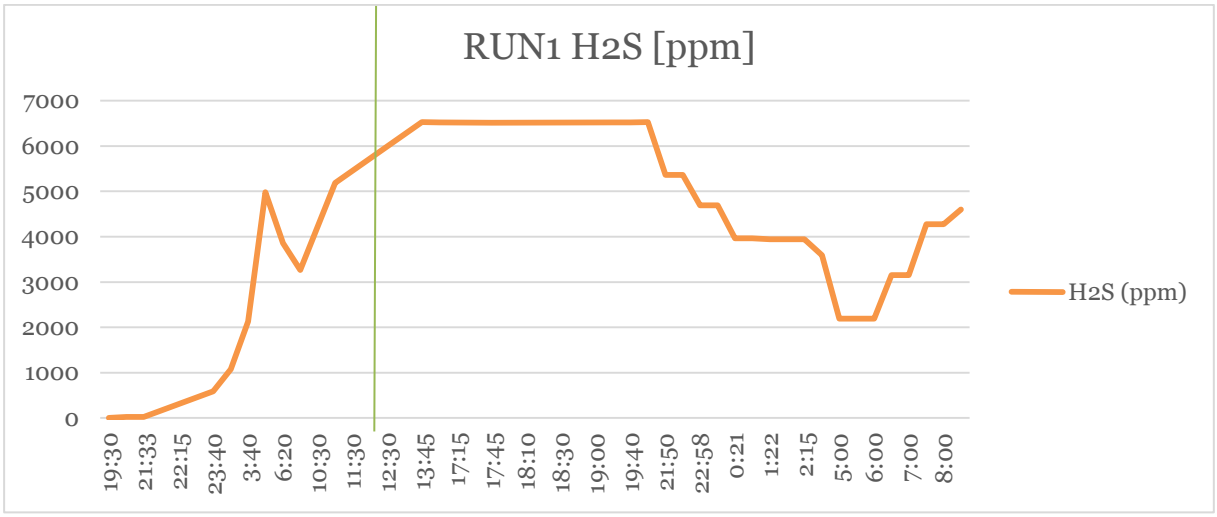

Figure 8 Hydrogen sulfur produced in parts per million. The green line shows the time for addition of sugar and the microbe *Klebsiella*-bacterial strain.

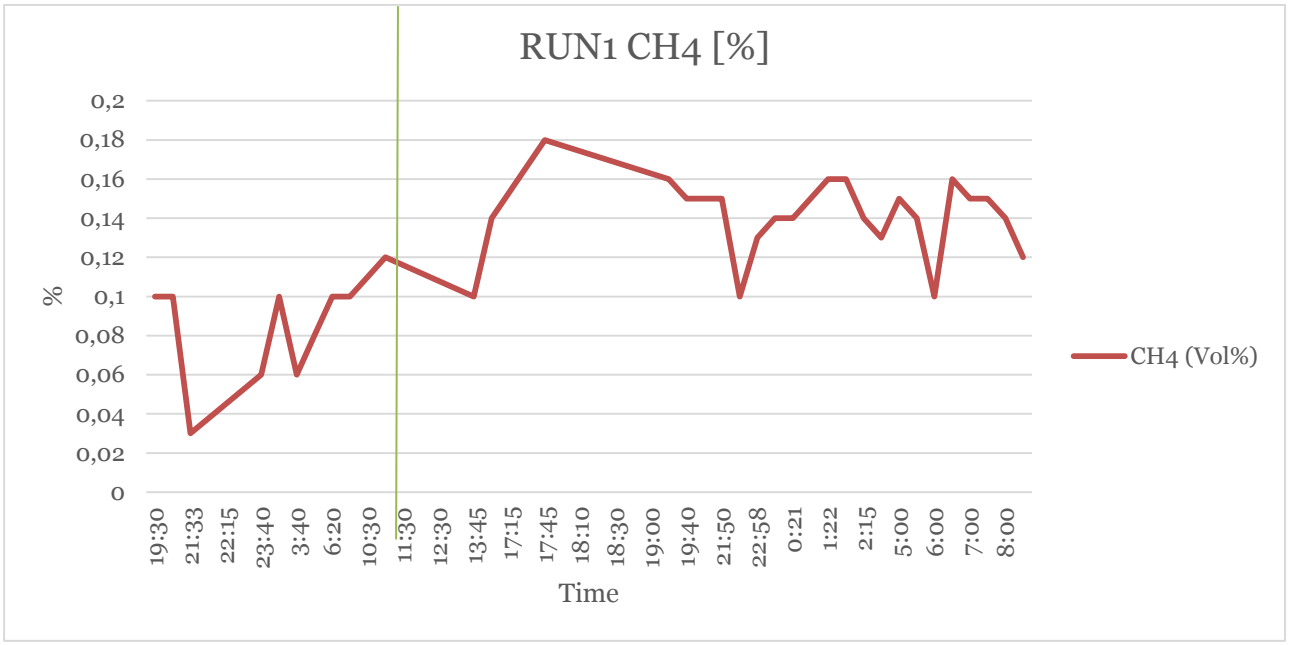

Figure 9 Methane level in percent of total volume gas. The green line shows the time for addition of sugar and the microbe *Klebsiella*.

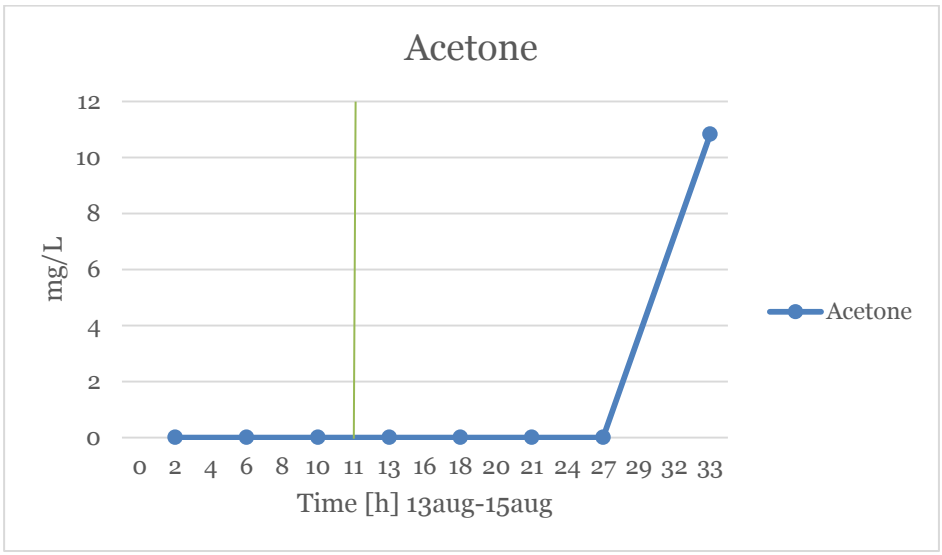

Figure 5 Acetone produced during the first run. The green line shows the time for addition of sugar and the microbe *Klebsiella*.

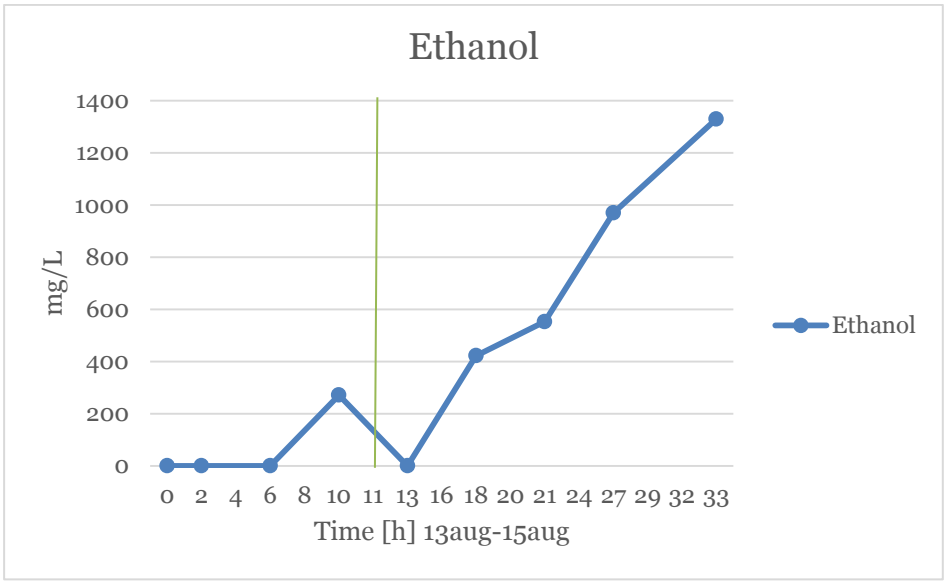

Figure 11 Ethanol produced during the first run. The green line shows the time for addition of sugar and the microbe *Klebsiella*.

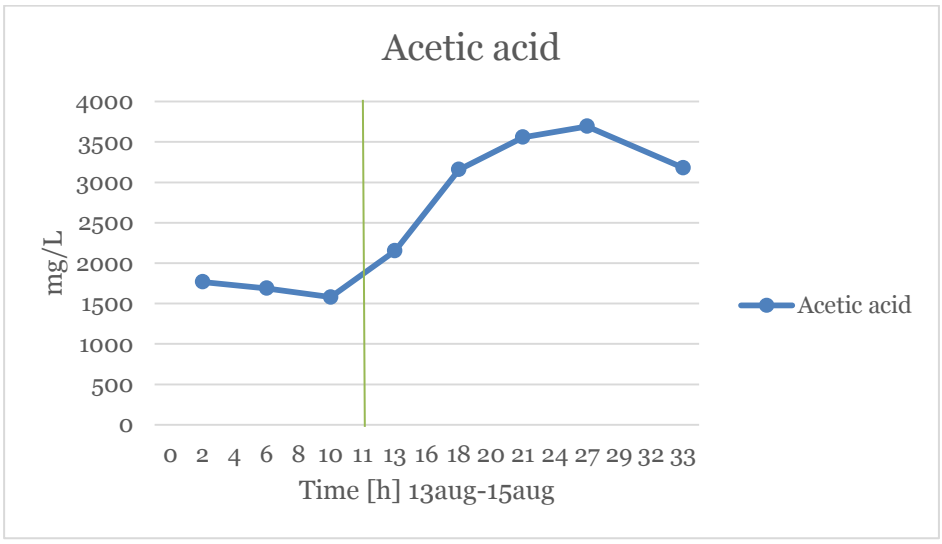

Figure 6 Acetic acid produced during the first run. The green line shows the time for addition of sugar and the microbe *Klebsiella*.

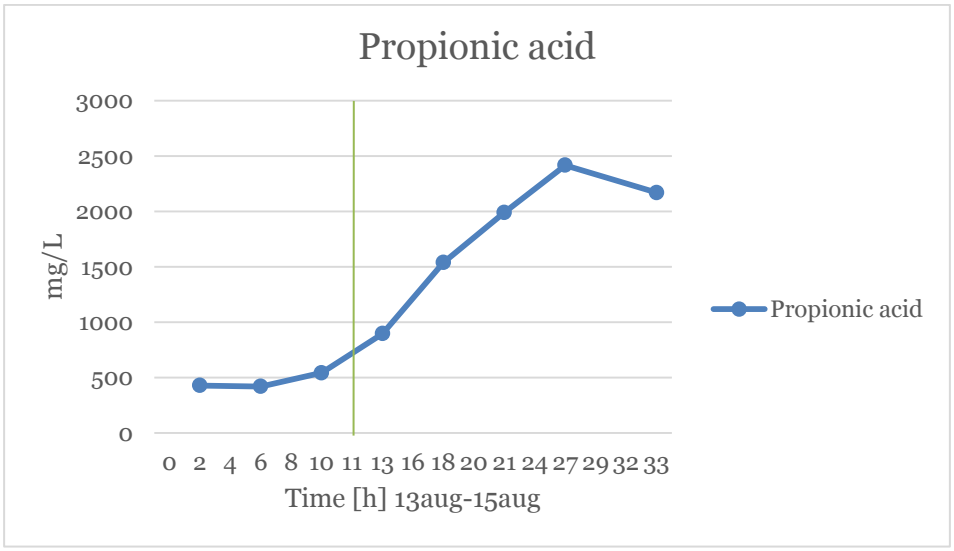

Figure 7 propionic acid produced during the first run. The green line shows the time for addition of sugar and the microbe *Klebsiella*.

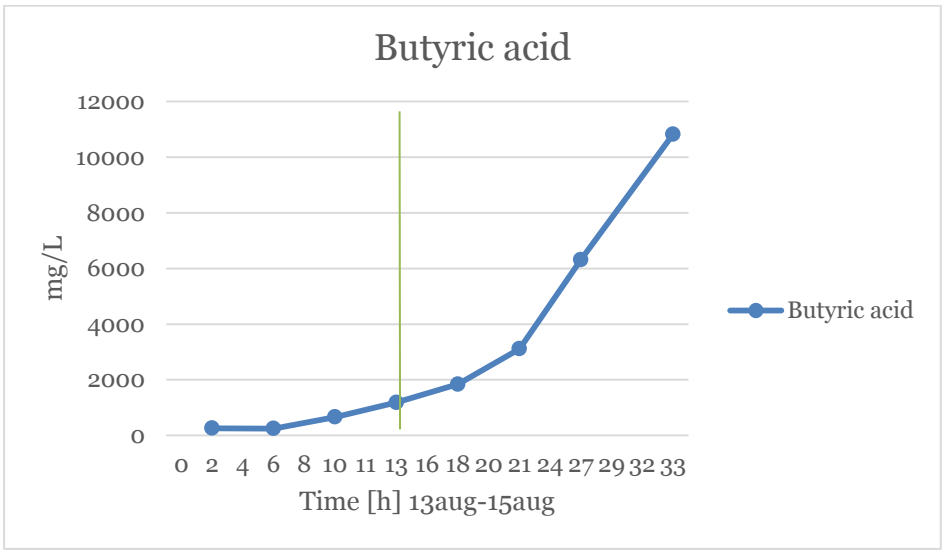

Figure 8 Butyric acid produced during the first run. The green line shows the time for addition of sugar and the microbe *Klebsiella*.

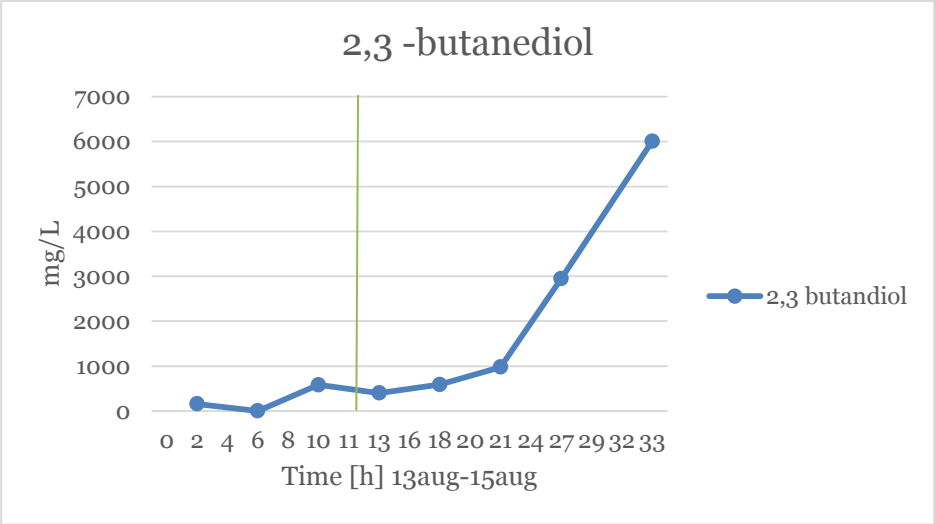

Figure 9 2,3 butanediol produced during the first run. The green line shows the time for addition of sugar and the microbe *Klebsiella*.

Table 25 TOC, BOD_7, total nitrogen and phosphorous in the reactor during Run 1.

Date	Time	TOC [g/l]	BOD7 [g/l]	Total N [g/l]	PO4-P, unfiltered [g/l]
2014-08-14	06:20	11	23	2.4	0.19
2014-08-15	09:30	19	83	2.2	0.16

4.2.2 Run 2

Figure 16 and Figure 17 show the production of H_2S and CH_4, respectively. The GC results for the different products in the reactor liquid are shown in Figure 18-23.

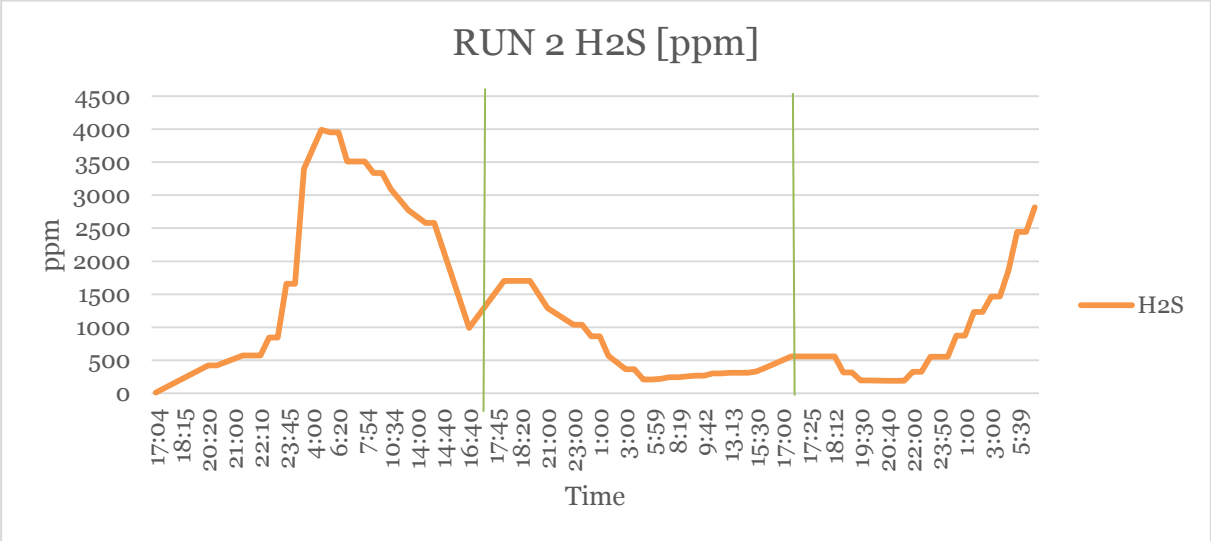

Figure 16 Hydrogen sulfur in parts per million produced in Run 2. The first line shows the time for addition of potato flour and the second line shows the addition of sugar.

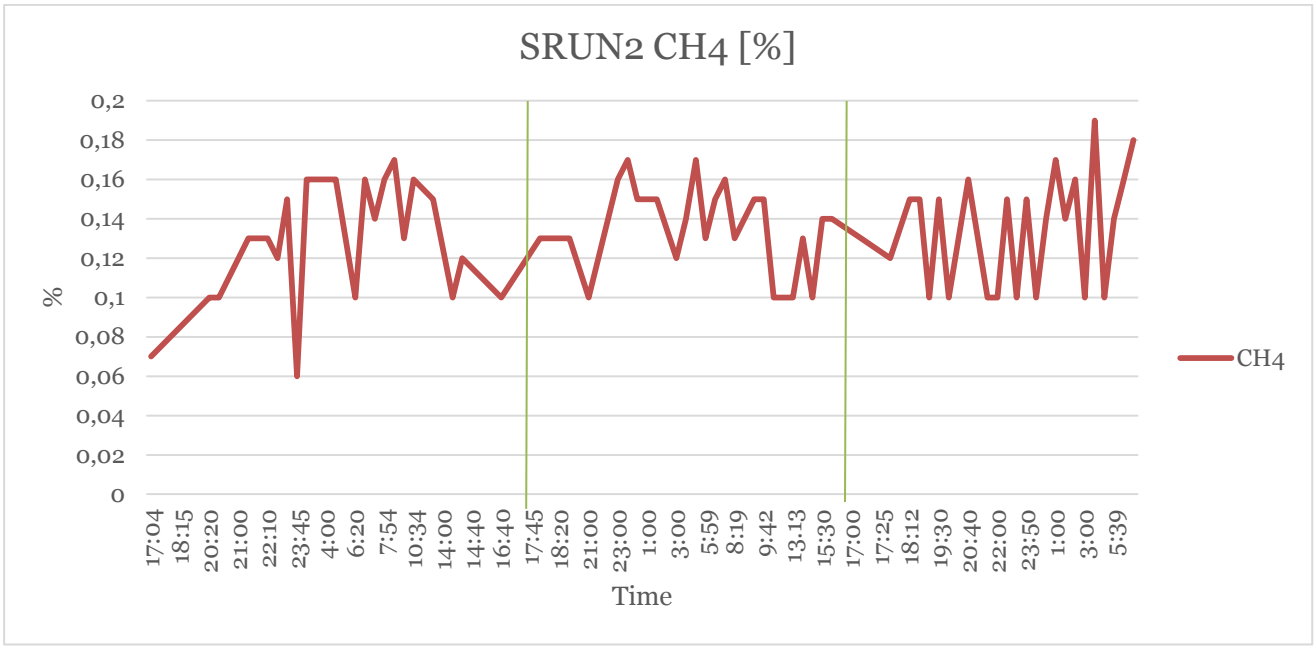

Figure 17. Methane level in percent of total volume gas. The first line shows the time for addition of potato flour and the second line shows the addition of sugar.

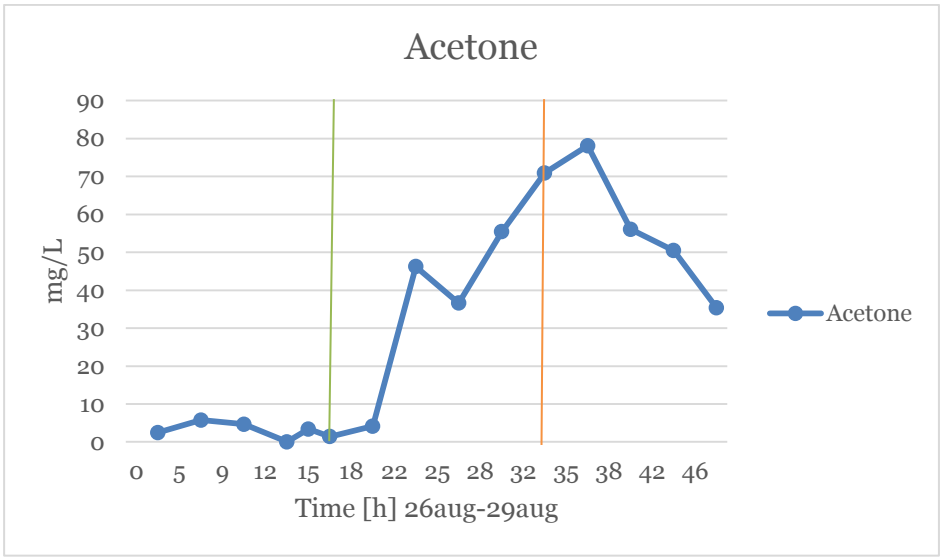

Figure 18 Acetone produced during the second run. The first line shows the time for addition of potato flour and the second line shows the addition of sugar.

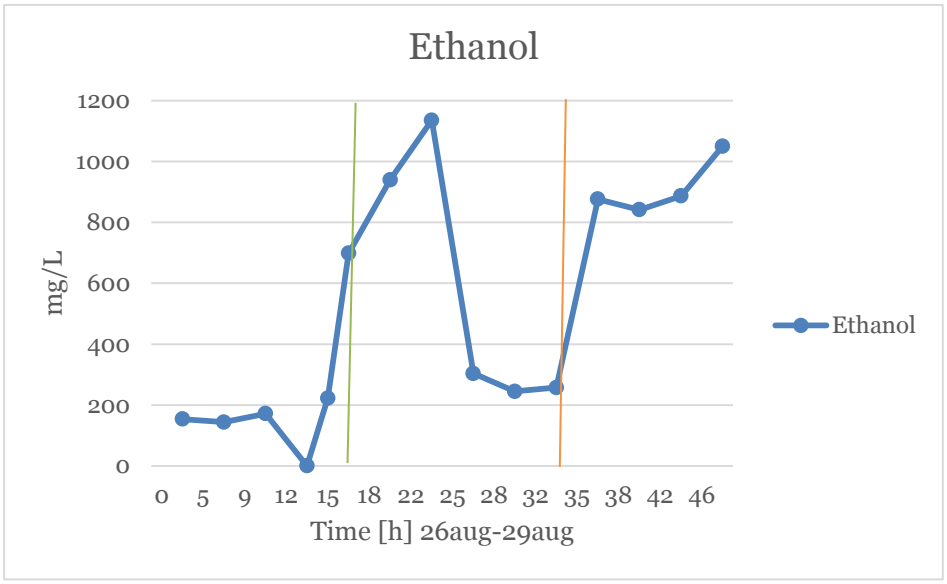

Figure 10 Ethanol produced during the second run. The first line shows the time for addition of potato flour and the second line shows the addition of sugar.

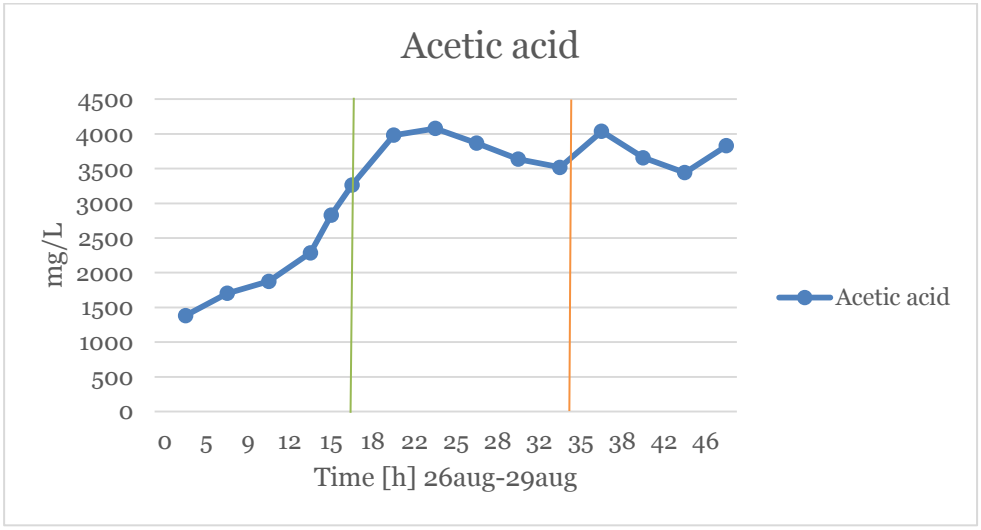

Figure 11 Acetic acid produced during the second run. The first line shows the time for addition of potato flour and the second line shows the addition of sugar.

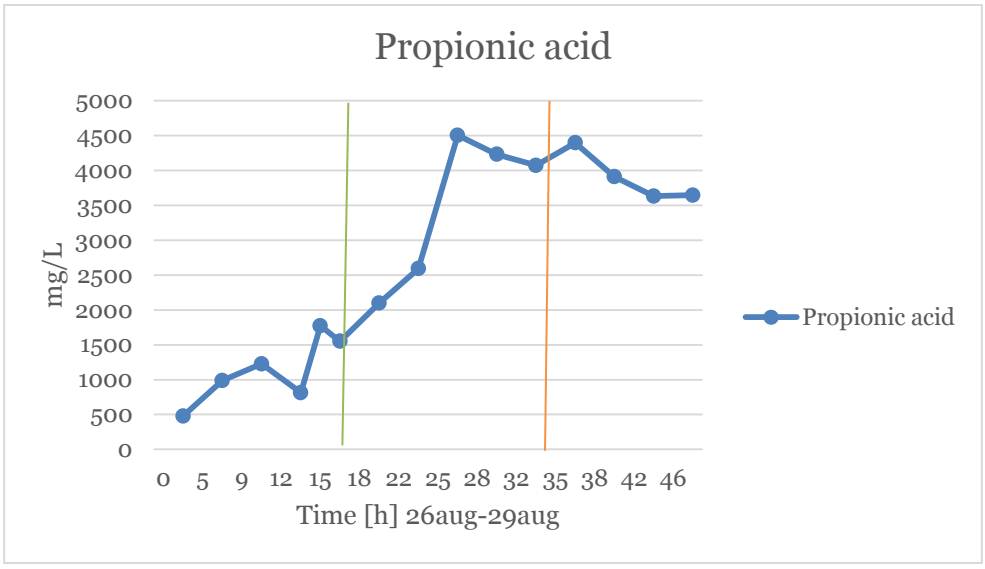

Figure 21 Propionic acid produced during the second run. The first line shows the time for addition of potato flour and the second line shows the addition of sugar.

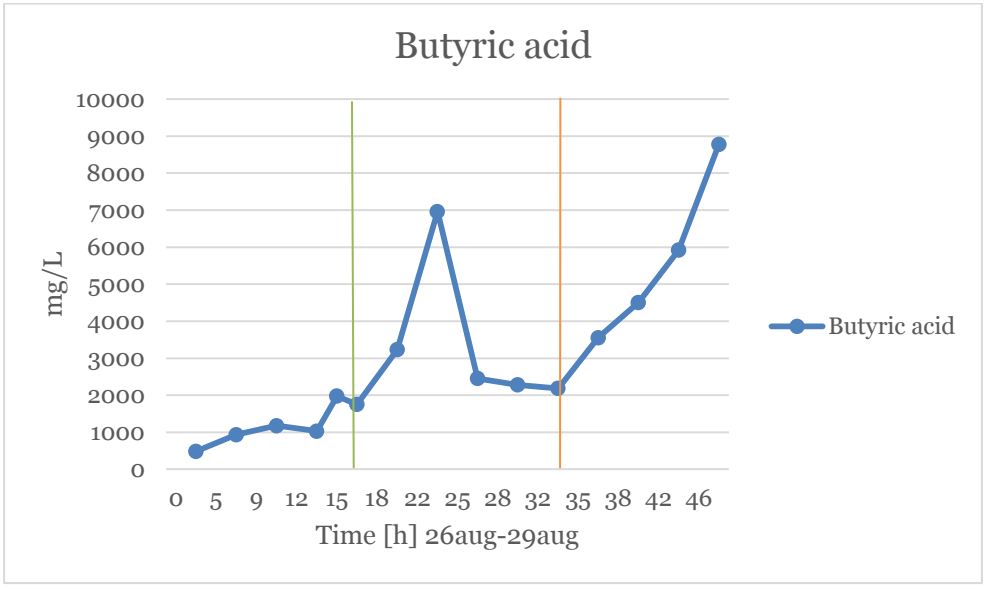

Figure 22 Butyric acid produced during the second run. The first line shows the time for addition of potato flour and the second line shows the addition of sugar.

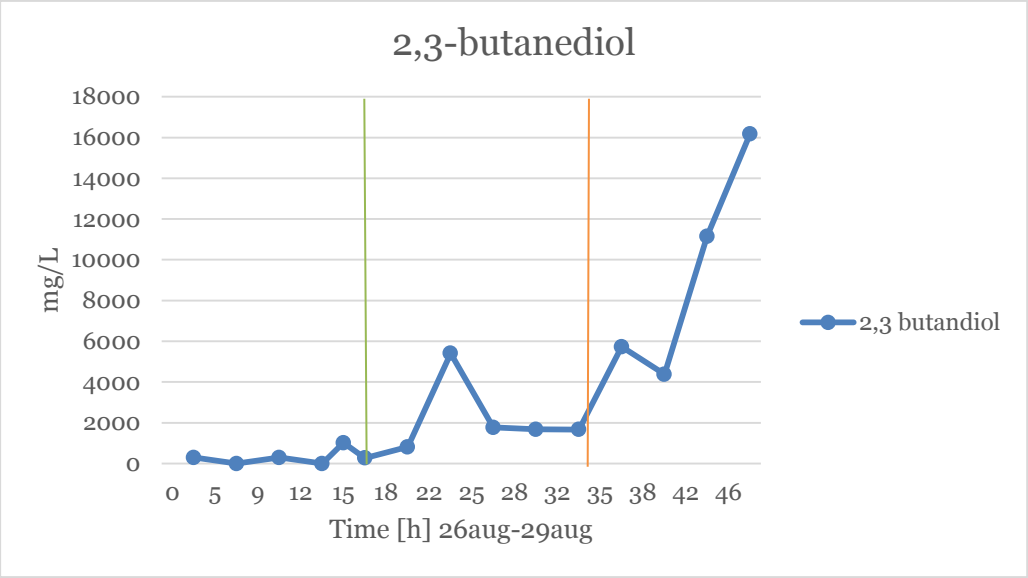

Figure 23 2,3-butanediol produced during the second run. The first line shows the time for addition of potato flour and the second line shows the addition of sugar.

4.2.3 Run 3

Figure 24 and Figure 25 show the production of H_2S and CH_4, respectively. The GC results for the different products in the reactor liquid are shown in Figure 26-31 and Table 27 shows the TOC, BOD7, total nitrogen and phosphorus in the reactor during Run 3. Table 28 shows the content of BOD7, total nitrogen, phosphorus and different sugars during the hydrolysis in Run 3.

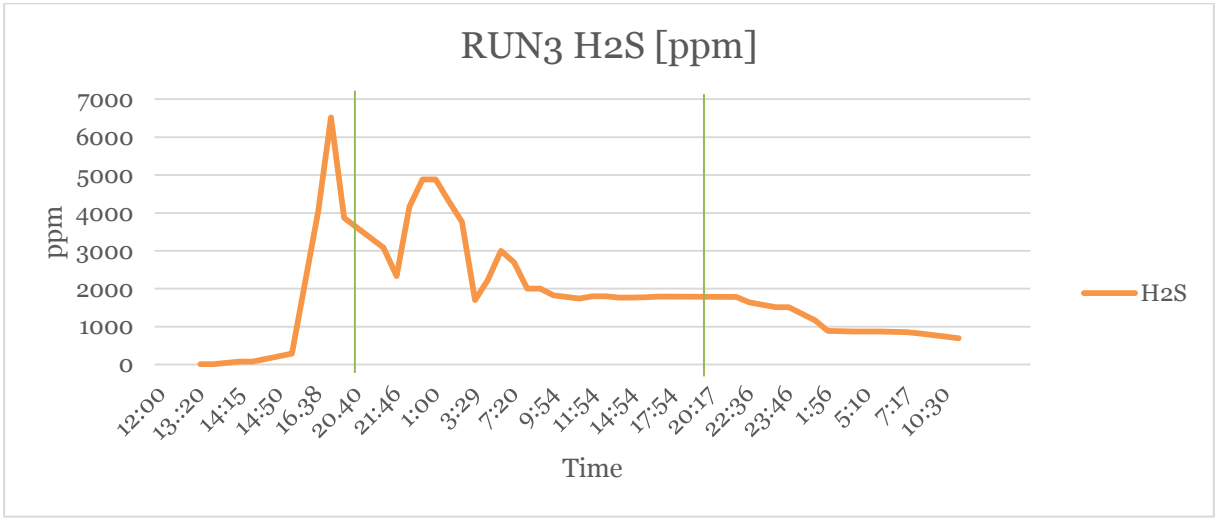

Figure 24 Hydrogen sulfur production in parts per million produced. The lines shows the times for addition of apples to the reactor during Run 3.

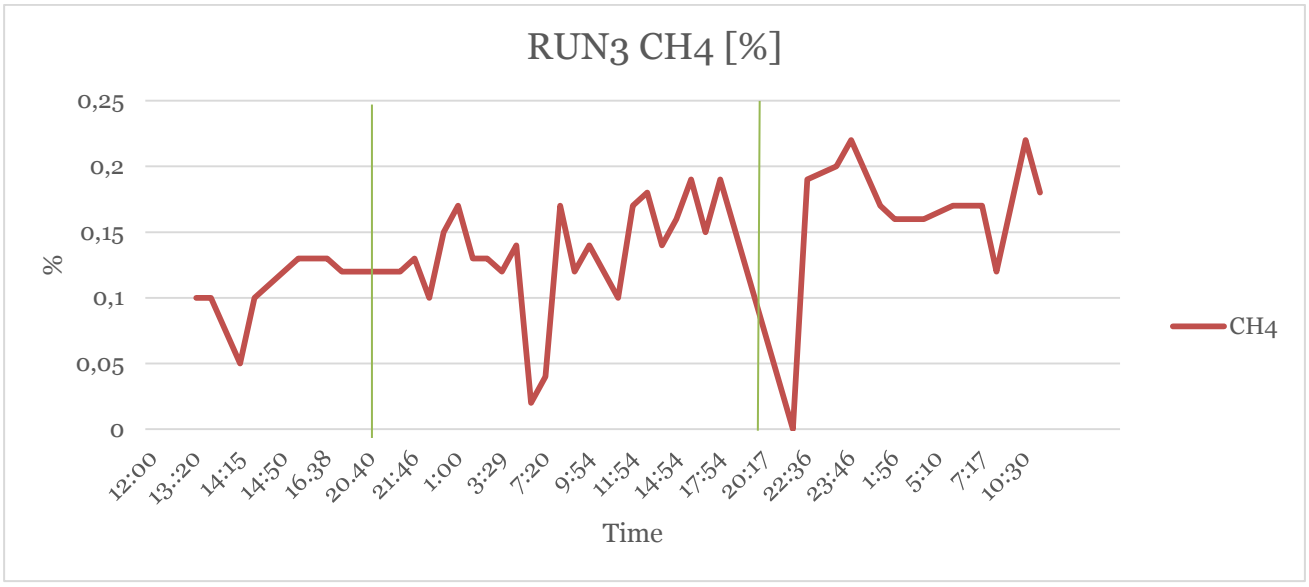

Figure 12 Methane level in percent of total volume gas. The lines shows the times for addition of apples to the reactor during Run 3.

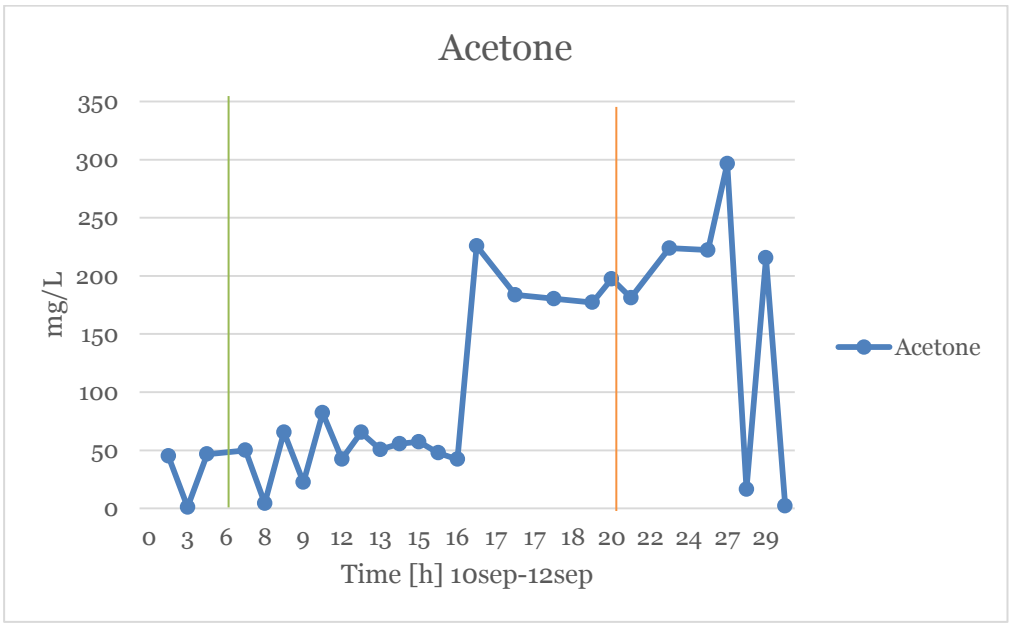

Figure 13 Acetone produced during the third run. The lines shows the times for addition of apples to the reactor during Run 3.

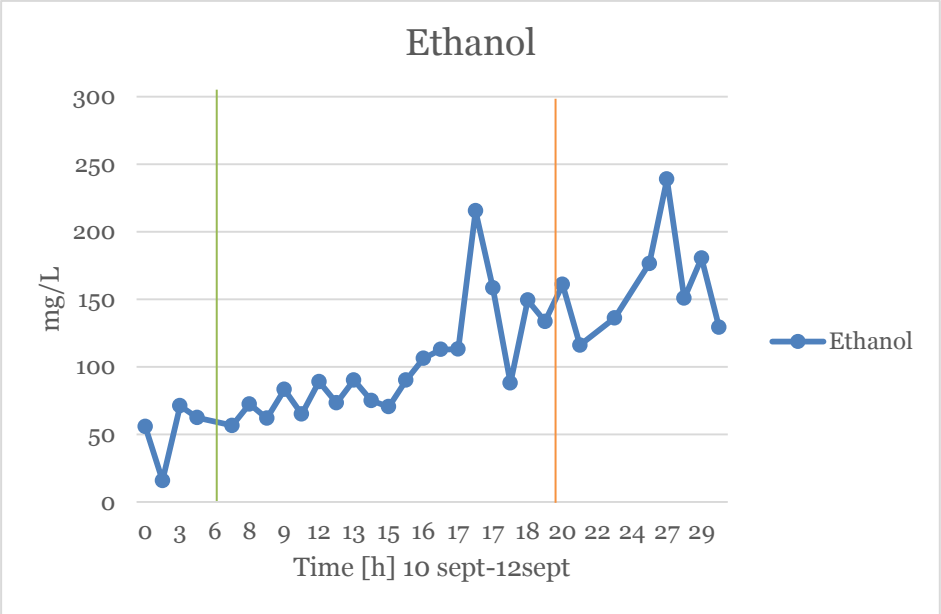

Figure 14 Ethanol produced during the third run. The lines shows the times for addition of apples to the reactor during Run 3.

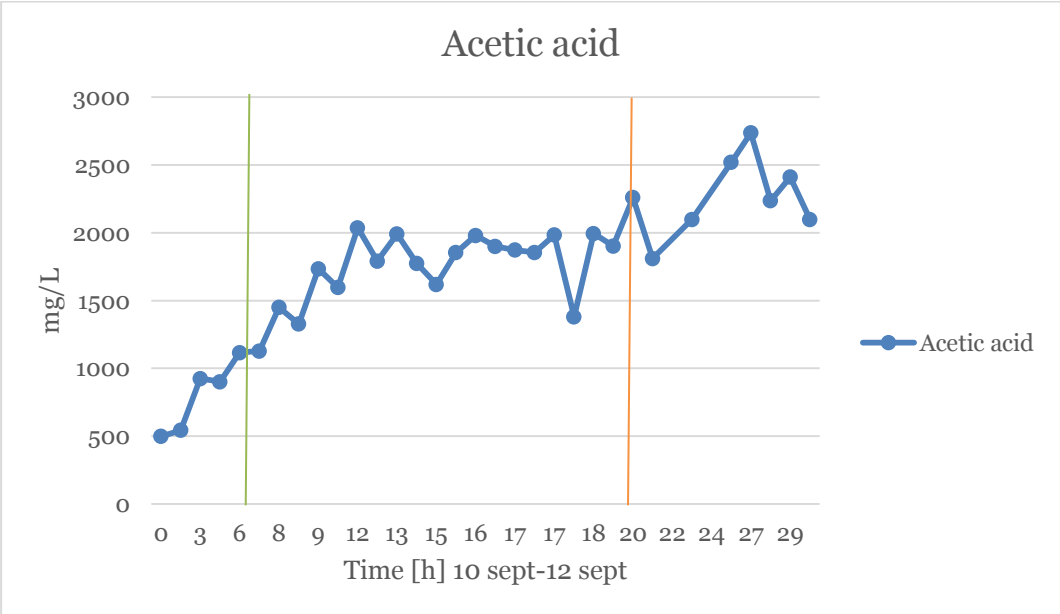

Figure 15 Acetic acid produced during the third run. The lines shows the times for addition of apples to the reactor during Run 3.

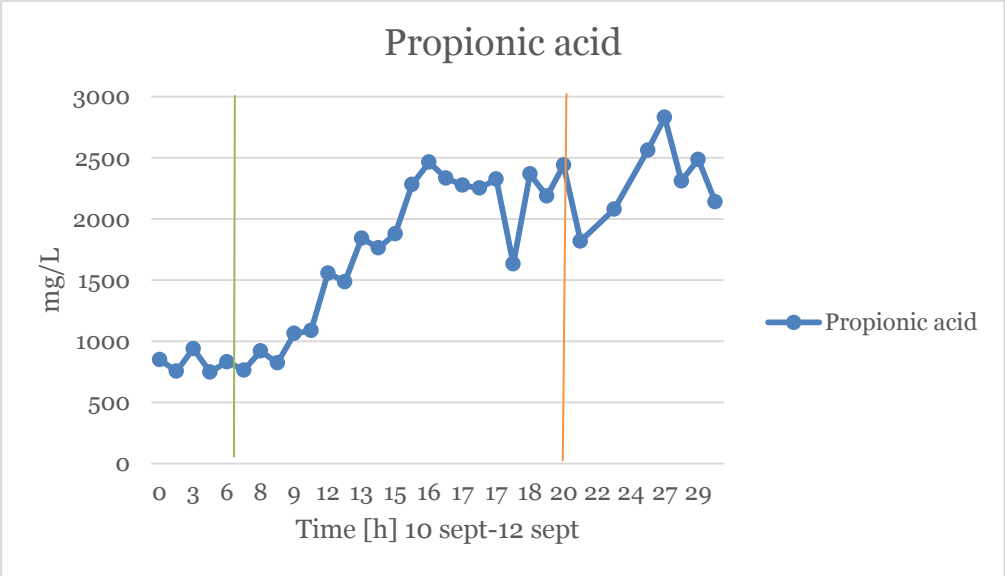

Figure 16 Propionic acid produced during the third run. The lines shows the times for addition of apples to the reactor during Run 3.

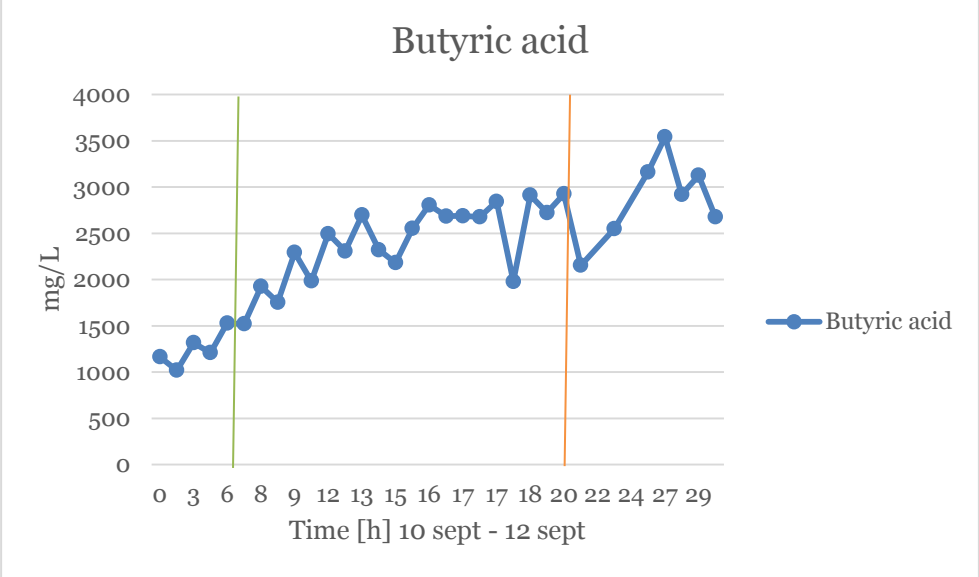

Figure 17 Butyric acid produced during the third run. The lines shows the times for addition of apples to the reactor during Run 3.

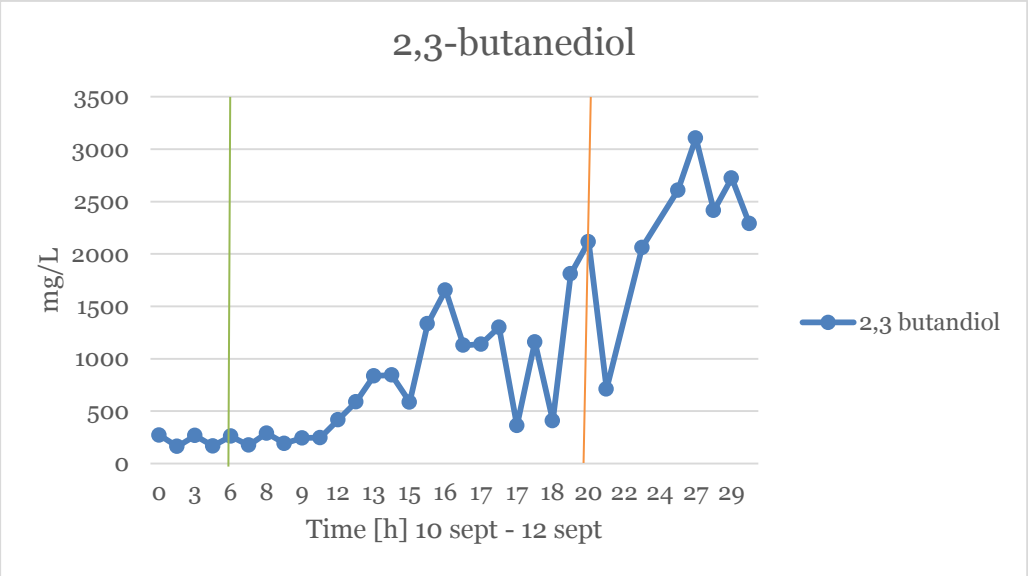

Figure 18 2,3 Butandiol produced during the third run. The lines shows the times for addition of apples to the reactor during Run 3.

Table 27 TOC, BOD_7, total nitrogen and phosphorous in the reactor during Run 3.

Date	Time	TOC [g/l]	BOD7 [g/l]	Total N [g/l]	PO4-P, unfiltered [g/l]
2014-09-10	12:45	9.4	25	2.1	0.16

Table 28 BOD7, total nitrogen, phosphorous and different sugars during the hydrolysis in Run 3.

Sample	BOD7 [g/l]	Total N [g/l]	PO4-P, unfiltered [g/l]	Fructose %	Glucose %	Lactose %	Maltose %	Saccharose %
Intestines before hydrolysis	320	10	0.72	<0.04	<0.04	<0.04	<0.04	<0.04
Straw in hydrolyser	9.9	0.27	0.092	0.33	0.25	<0.04	<0.04	<0.04
Hydrolyser before adding liver	73	5.9	0.41	<0.04	<0.04	<0.04	<0.04	<0.04

4.2.4 Run 4

Figure 32 and Figure 33 show the production of H_2S and CH_4, respectively. The GC results for the different products in the reactor liquid are shown in Figure 34-39 and Table 29 shows the TOC, BOD7, total nitrogen and phosphorus in the reactor during Run 4. Table 30 shows the content of BOD7, total nitrogen, phosphorus and different sugars during the hydrolysis in Run 4.

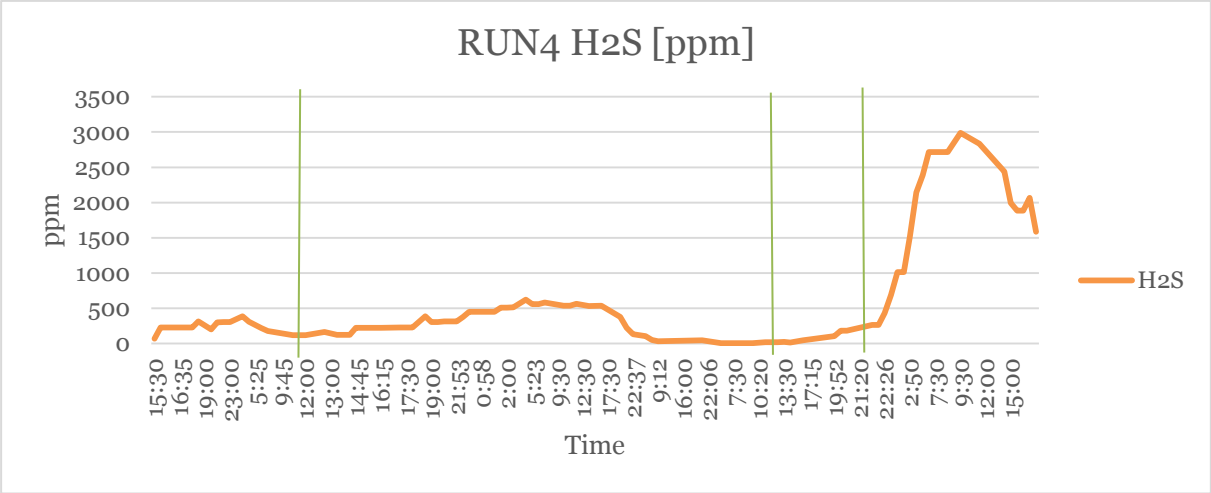

Figure 32 Hydrogen sulfur in parts per million produced. First line shows the time for adding *Clostridium acetobutylicum*, the second line the time for adding *Clostridium acetobutylicum* and *Clostridium butyricum*, and the third line shows the time for adding Blueberry soup to the reactor.

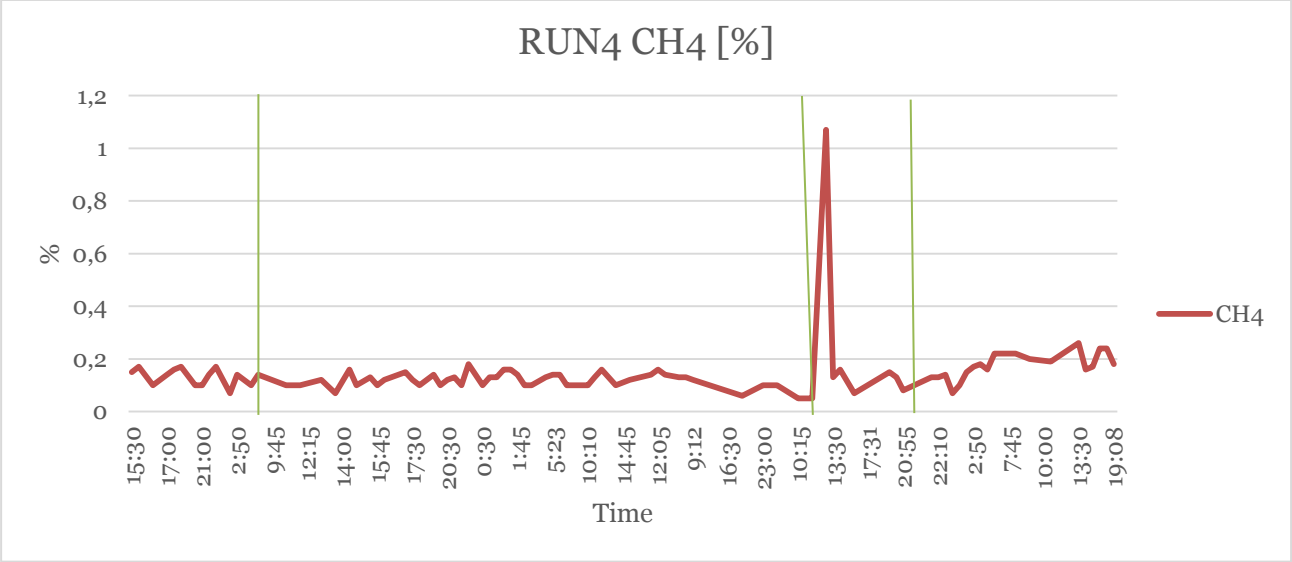

Figure 19 Methane level in percent of total volume gas. First line shows the time for adding *Clostridium acetobutylicum*, the second line the time for adding *Clostridium acetobutylicum* and *Clostridium butyricum*, and the third line shows the time for adding Blueberry soup to the reactor.

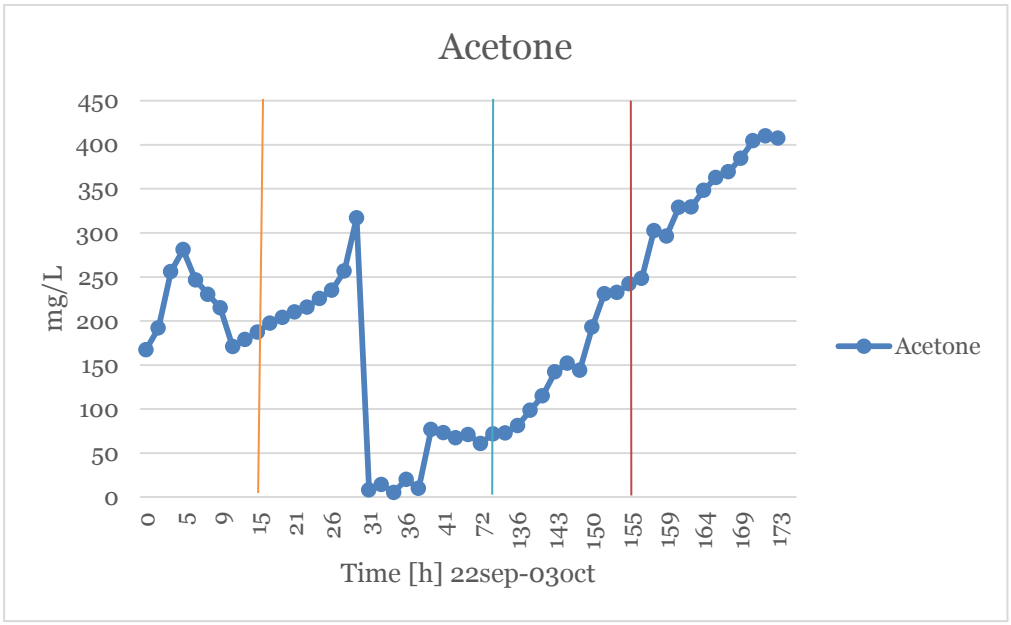

Figure 20 Acetone produced during the fourth run. First line shows the time for adding *Clostridium acetobutylicum*, the second line the time for adding *Clostridium acetobutylicum* and *Clostridium butyricum*, and the third line shows the time for adding Blueberry soup to the reactor.

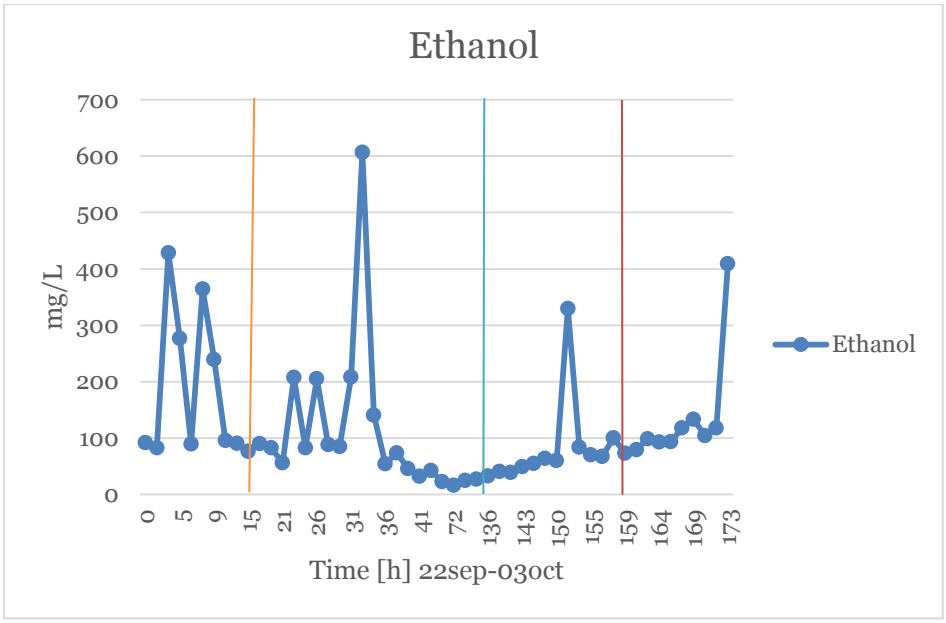

Figure 35 Ethanol produced during the fourth run. First line shows the time for adding *Clostridium acetobutylicum*, the second line the time for adding *Clostridium actoebutylicum* and *Clostridium butyricum*, and the third line shows the time for adding Blueberry soup to the reactor.

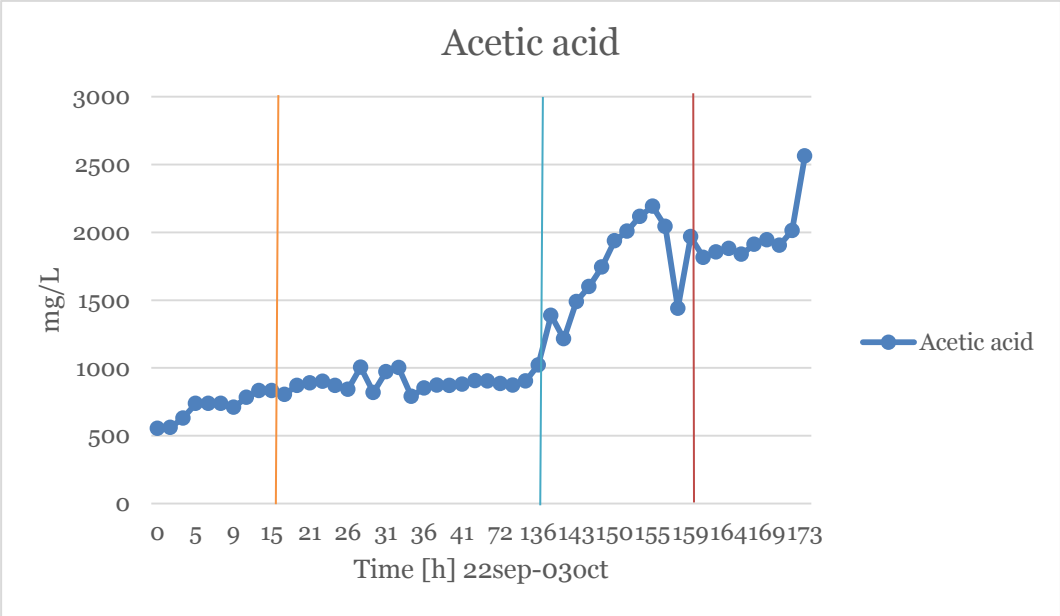

Figure 21 Acetic acid produced during the fourth run. First line shows the time for adding *Clostridium acetobutylicum,* the second line the time for adding *Clostridium acetobutylicum* and *Clostridium butyricum*, and the third line shows the time for adding Blueberry soup to the reactor.

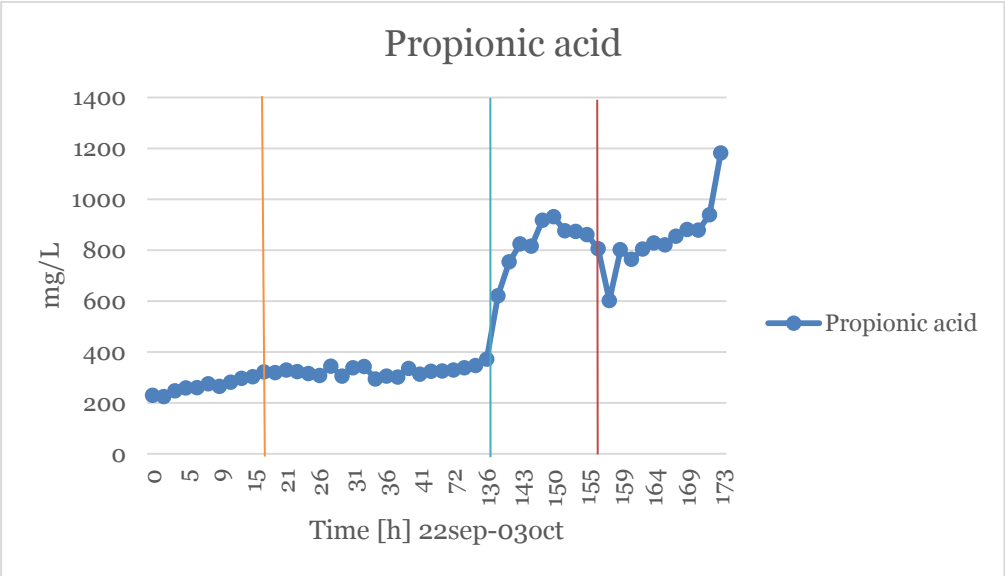

Figure 22 Propionic acid produced during the fourth run. First line shows the time for adding *Clostridium acetobutylicum*, the second line the time for adding *Clostridium acetobutylicum* and *Clostridium butyricum*, and the third line shows the time for adding Blueberry soup to the reactor.

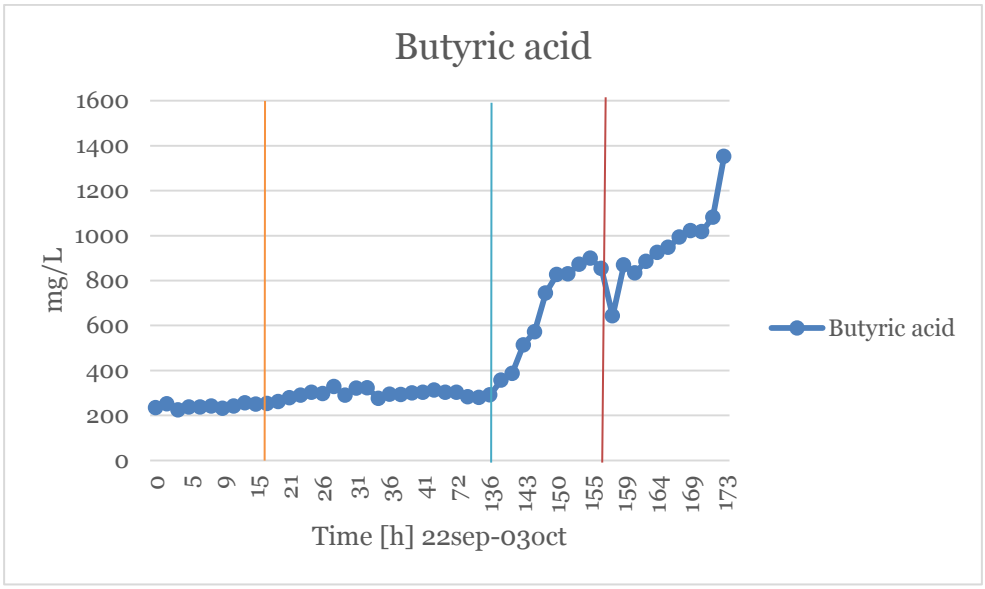

Figure 23 Butyric acid produced during the fourth run. First line shows the time for adding *Clostridium acetobutylicum*, the second line the time for adding *Clostridium acetobutylicum* and *Clostridium butyricum*, and the third line shows the time for adding Blueberry soup to the reactor.

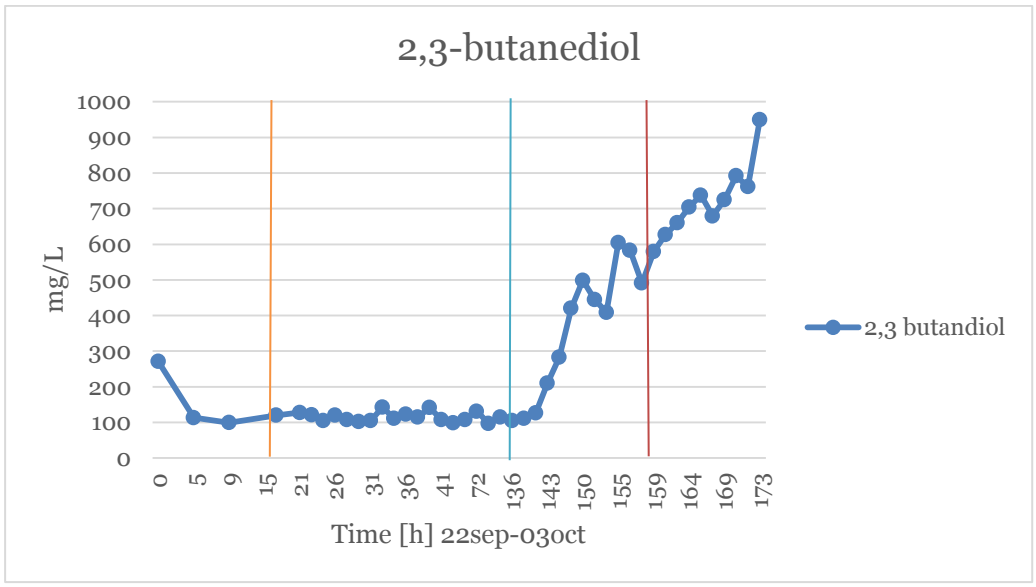

Figure 24 2,3 Butanediol produced during the fourth run. First line shows the time for adding *Clostridium acetobutylicum*, the second line the time for adding *Clostridium acetobutylicum* and *Clostridium butyricum*, and the third line shows the time for adding Blueberry soup to the reactor.

Table 29 TOC, BOD_7, total nitrogen and phosphorous in the reactor during Run 4.

Date	Time [h]	TOC [g/l]	BOD7 [g/l]	Total N [g/l]	PO4-P, unfiltered [g/l]
2014-09-24	13.17	1.4	1.5	0.49	0.28
2014-09-24	23:00	1.2	1.4	0.51	0.25
2014-09-25	00:50	1.3	1.6	0.38	0.26
2014-09-25	02:40	1.4	1.9	0.44	0.25
2014-09-25	07:37	0.47	0.65	0.32	0.27
2014-09-25	10:00	1100	1600	400	270
2014-09-25	16:15	1400	1900	450	240
2014-09-25	19:00	1500	1800	460	240
2014-09-26	03:23	1300	1800	370	290
2014-09-26	07:01	1400	1700	420	280
2014-09-26	15:30	1.1	1.4	0.39	0.31
2014-09-30	12:14	6100	15000	2000	340
2014-10-01	17:00	4800	11000	1700	280
2014-10-01	23:00	4900	5400	1700	280
2014-10-02	14:55	4600	10000	1700	280

Table 30 BOD7, total nitrogen, phosphorous and different sugars during the hydrolysis in Run 4.

Sample	BOD7 [g/l]	Total N [g/l]	PO4-P, unfiltered [g/l]	Fructose %	Glucose %	Lactose %	Maltose %	Saccharose %
9.5 hours after addition of enzymes in 1st step hydrolyse	0.82	0.29	0.19	<0,04	<0,04	<0,04	<0,04	<0,04
	1.5	0.36	0.004	<0,04	<0,04	<0,04	<0,04	<0,04
Start of 2nd step hydrolyse	27		0.4		-	-	-	-
Before adding chicken liver 2nd step hydrolyse	49	3.7	0.66	<0,04	<0,04	<0,04	<0,04	<0,04
30 minutes after adding chicken liver 2nd step hydrolyse	34	0.098	0.38	-	-	-	-	-

4.2.5 Comparison

In Figure 25 the production of the different products are compared for the 4 runs.

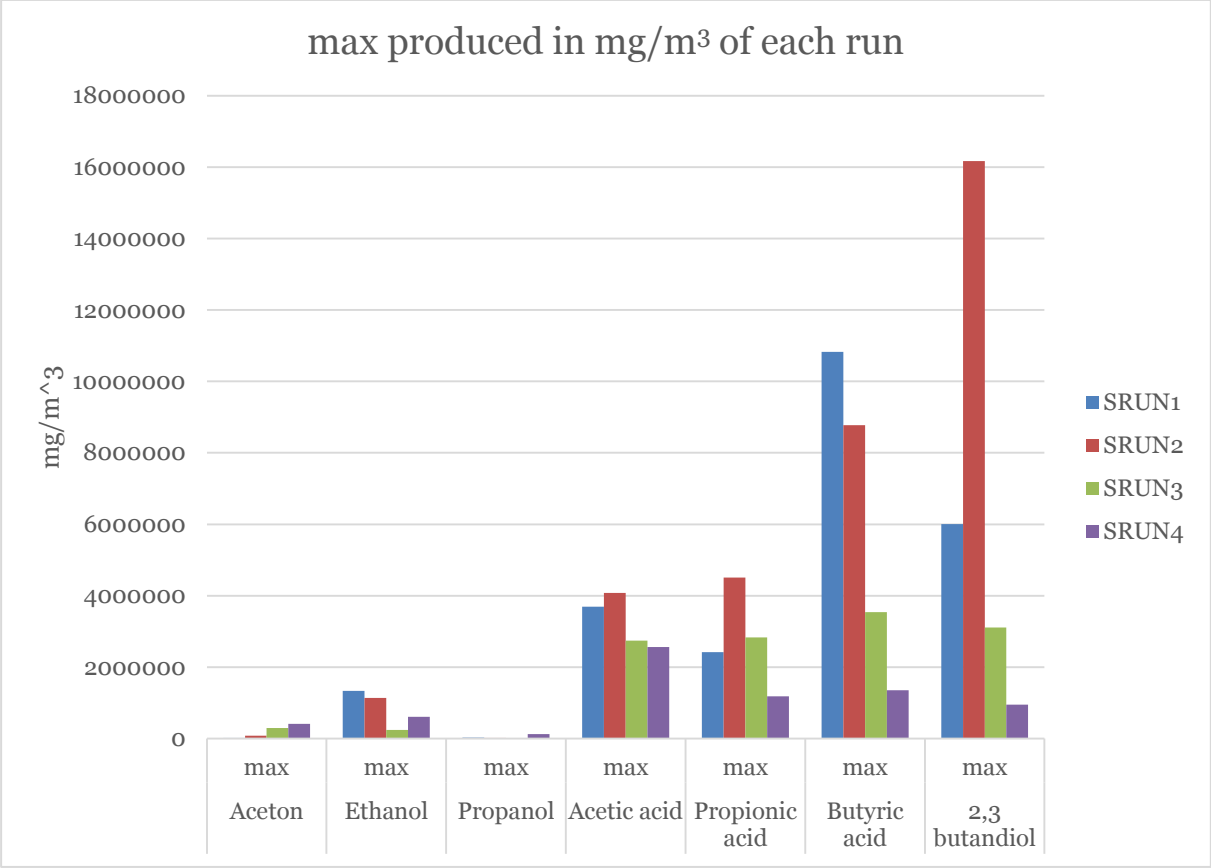

Figure 25. Max production from GC result in mg/m3 between the different runs.

4.3 Discussion of the results

The results show that products have been produced both in the runs with aerobic as well as anaerobic conditions. A higher production is observed when easily accessed carbohydrates and sugars are available.

The second run gave the highest levels of ethanol, acetic acid, propionic acid and 2,3-butanediol. This might be a result of the easy accessed carbohydrates in the added sugars and potato flour. Also the first run where sugar also was added shows higher levels than the later runs.

Clostridium acetobutylicum and *Clostridium butyricum* produced organic acids like acetate, propionate and butyrate. Comparing the GC results with NMR tests, done at the University of Eastern Finland on samples from the process, shows that the GC results for 2,3-butanediol probably is to a big part due to the presence of valeric acid in the samples. Acetate and propionate derived by bacteriological activity can react with each other to form valeric acid, which also is a valuable product. Its price is 2-3 times that of 2,3-butanediol. Valeric acid can be used as raw material to similar chemical products as 2,3-butanediol.

The results show that during the short time process that the runs represented proteins and fats could be used by the *Clostridia* to produce acids. Acids that later on could have been reduced to alcoholic substances and aliphatic substances if there had been more time for the experiment.

Klebsiella was not effective for 2,3-butanediol production due to the glucose limitation of the raw material. Further improvements of the pumps and the mass transfer could facilitate higher glucose levels, and thus make it possible to gain industrial levels. At present, several organic acids were produced in high quantities. Also in their production improved pretreatments and elevated small carbon molecules would increase the yield.

Hydrogen production was rather high during intensive bacteriological activity periods, which could give leads to the biohydrogen production from the organic wastes, such as the animal or plant residues from the agriculture.

No lactate was produced. Waste hygienization probably eliminated the lactic acid bacteria, such as *Lactobacillus sp*.

As a conclusion it can be stated that the ABOWE Pilot A provided a tool to quickly convert tedious waste mixtures into useful substrates. During the two month testing period a good starting point for later optimization of the process and the equipment could be obtained.

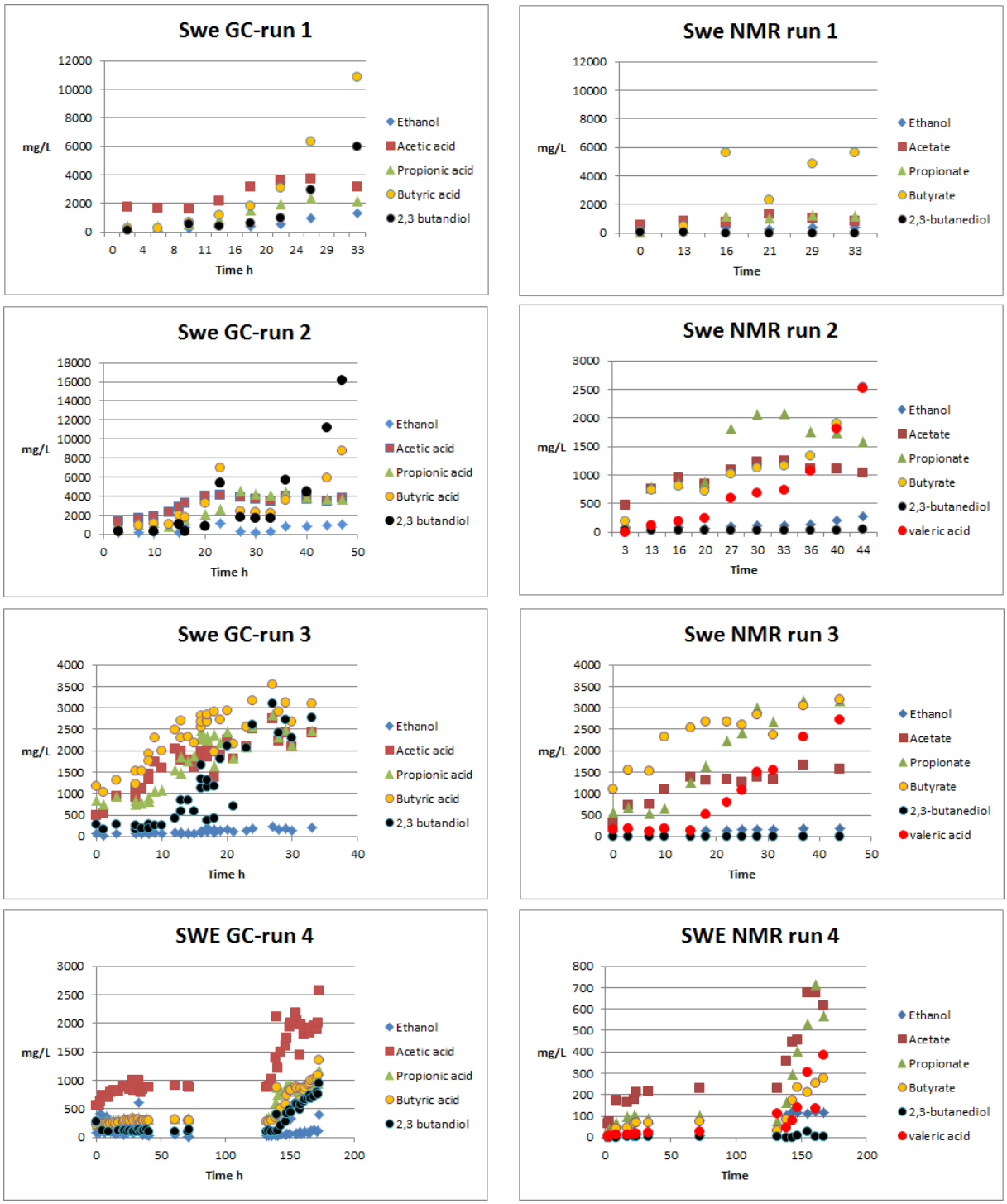

Figure 26. GC and NMR results of the Swedish test runs. In some of the tests the latter gave clearly lower results probably due to the longer preservation times and transportation of the samples.