abowe
Implementing Advanced Concepts for Biological Utilization of Waste

Report no: O3.5

REPORT ON START-UP OF PILOT A

Savon Sellu tests in Kuopio February-March 2014

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1. General estimation of the Pilot A process concept

From the bioprocess view, all the test runs were preliminary ones. Their main purpose was to get tested and fixed the various technical details and features of the Pilot A experimental station which was ready for use 1-2 month later than was originally scheduled. Consequently, the focus was not in optimizing the bioprocess but, besides the technical runs, different characteristics of biomass waste as raw material and the use of mixed microbial cultures were experimented. Also, basics of the biochemical processes were charted. These first experimentations were thus producing:

1. Technical validation, reparation data and further technical preparation of the engineered pilot plant
2. Introduction of the process principles
3. Education of the users
4. Preliminary testing with the cellulosic waste material with proof of concept on using the Pilot A type of solution in the treatment of forest industry wastes

This evaluation period could have continued as actual optimization and improvement of the process, but the pilot was to be delivered to Poland due to ABOWE schedules. However, some encouraging first results were obtained. For example, the constant hydrogen containing gas flow observed earlier in the Finnoflag Oy´s laboratory was detected also during the ABOWE piloting. Unfortunately, in the Pilot A it was not possible to measure the hydrogen levels exactly due to the upper limit of the gas detector system for hydrogen concentration. The generation of molecular hydrogen was combined with the production of liquid chemicals, which production levels were subjected to improvement due to the preliminary nature of the testing. The diminishment in the environmental load was already comparable to the biogas process, and actually the biorefining for chemical commodities could be combined with subsequent biogas production for the highest decrease in the climatological effects in the waste treatment.

During the testing it was possible to conceive the actual purpose of the Pilot A as a device for producing the proof of concept for various biomass waste treatments. In all planning, the sustainability principles were valued high in the process design. For example, reused parts were applied for the construction of the Pilot A.

2. Introduction

This report is one output of ABOWE project (Implementing Advanced Concepts for Biological Utilization of Waste) which belongs to EU Baltic Sea Region Programme 2007-2013. The purpose of this report is to gather together essential information from Finnoflag biorefinery technology test runs with Pilot A at Savon Sellu cartonboard factory's waste water treatment plant in Kuopio during February-March 2014. The main purpose has been to test the equipment functions of the pilot plant which was designed and realized in the project and then transferred to the factory waste treatment site on 18.1. 2014, which was perhaps the coldest day of that winter (Figure 1).

Figure 1. The first transfer of Pilot A from its construction site to the first testing location.

Because of the harsh climate conditions with temperatures between -25°C and -30 °C the functions of the Pilot A were put into a real "climate test". Fluids tend to get iced in the tubes during their pumping into the unit. Also the raw material for the experiments, the dried sludge from the waste treatment plant was cooling down rapidly in the piles where it was collected from (Figures 2 and 3).

Figure 2. Pilot A in its first testing site at Savon Sellu Oy.

Figure 3. Dried waste water treatment sludge in pile at Savon Sellu Oy.

The waste waters of this factory are treated with a sophisticated purification plant including at the time of construction first forest industry active sludge process in Finland. The treatment process is controlled from the same central operation control room as the pretreatment of the raw material wood into woodchips when it arrives to the factory site either by trucks, or trains, or via waterways in summertime (Figures 4 and 5). On the basis of previous experiments in the Savonia biogas pilot, some 40-50% of the total solids of the sludge were biodegradable in the biogas process (Huopana et.al. 2014).

Figure 4. Wood is transported e.g. by trucks and also via waterways.

Figure 5. Wood waiting for pretreatment before manufacturing process at Savon Sellu Oy.

The previous experience of Savonia UAS with the biogas pilot plant was partially at the usage of the Pilot A team. Also the background know-how previously obtained in the Finnoflag Oy laboratory for treating the cellulosic wastes was available in the process design. Some general outlines of the Finnoflag biorefinery strategies have been presented earlier (Hakalehto et al. 2008; Hakalehto et al. 2013).

3. Description of Finnoflag biorefinery technology

The main tanks in the Pilot A biorefinery process are:

1. HOMOGENIZER is the first of the four main tanks of the Pilot A. It is equipped with a biomass crushing unit and effective mixing function. It is also one of the three recycled and modified pieces of the main equipment in the Pilot A used for the upstream bioprocessing sequence. In the homogenizer various biomasses are being mechanically broken in micro- and macroscale. Their dry weight and total masses of solid and liquid raw materials are measured with a weighing sensor installed in the support frame of this tank. The design and functions of the homogenizer, as well as all other parts of the Pilot A are resulting from cooperation between Savonia University of Applied Sciences and Finnoflag Oy during 2013. The joint team has been made operational by the project manager Ari Jääskeläinen of Savonia UAS. The engineering and construction processes of Pilot A were under the responsibility of Senior Lecturer Anssi Suhonen of Savonia UAS.

2. HYDROLYZER is a thermostatic and pH controlled reactor for producing, maintaining and adjusting the optimal conditions for chemical and/or enzymatic hydrolysis of the macromolecules in the raw material biomasses. Main parameters are the water content (adjusted partially in the homogenizer), fill in level, temperature (can be lifted up to 90 degrees Celsius), pH of the biomass, viscosity and the hydrolysis time. This reactor tank is also an ecologically sustainable product of the Savonia Engineering Works, originating from the Finlayson Oy cotton factory in Tampere, which is the city in Southern Finland where our metal engineering and other industrialization began almost 200 years ago. There the tank was used for staining textiles before it was modified in Kuopio into a crucial part of the chain in recycling waste biomasses in the Pilot A experimental station. During this era of modern reindustrialization.

3. BIOREACTOR is the sole entirely novel big tank in the Pilot A. It has been manufactured by Brandente Oy in Kuopio according to the instructions of the innovator Dr. Elias Hakalehto of Finnoflag Oy and Senior Lecturer Anssi Suhonen of Savonia UAS. The patented design is based on numerous bioprocess runs in Finnoflag Oy's laboratory projects preceding the ABOWE project. During ABOWE a joint team of about 50 experts have been participating in the planning and construction of the Pilot A. Different homogenized and hydrolyzed biomasses are processed in adjustable gas conditions in the bioreactor in order to produce biofuels, gases and chemicals by the metabolic activities of bacteria and other micro-organisms. During the process runs pH, dissolved oxygen, temperature, total volume (biomass input and process fluid outflow), as well as the gas mixing and measurement are adjusted by the central computer control together with real time operating activity by the personnel on site and connected via 3G network to the Pilot A.

Major fields of responsibility during the buildup of the Pilot A have been:

- Process, mechanical and lay-out engineering (M.Sc Anssi Suhonen)
- Mechanical installations (Tech. Juhani Mikkonen)
- Automation and thermal control (M.Sc Risto Rissanen),
- Gas flow system (Eng. Tero Kuhmonen),
- Control and monitoring system (M.Sc Asmo Jakorinne),
- Electrical installations (Eng. Toni Hirvonen),
- Procurement (M.Sc Osmo Miinalainen).

The microbiological inocula are produced first in the PMEU equipment (Portable Microbe Enrichment Unit) (Samplion Oy, Siilinjärvi, Finland), and then in the seed fermenters connected to the main bioreactor. In PMEU it is possible to get homogenous cultures in same active growth phase in a few hours of cultivation (Figure. 6). The entire microbiological and biotechnical process control practices are designed by Finnoflag Oy.

Figure 6. PMEU – Portable Microbe Enrichment Unit. See also Hakalehto and Heitto (2012).

4. STABILIZER is modified from a food industry boiling tank into a cooled collection unit of the bioprocess fluid containing liquid (and possibly solid) products of the Pilot A. There the temperature is lowered to 15-18 degrees Celsius from the usually much higher production temperatures in order to avoid losses in the product concentrations after the process. The gaseous products are recorded from the volatile outflow of the bioreactor prior to the stabilization. Modification of this unit, mechanical assembly work and the routings of piping of the Pilot A are hand made by Juhani Mikkonen and other professionals of the construction teams of Savonia UAS, Savo Vocational College and subcontracting companies.

The process fluid is further analyzed at the University of Eastern Finland in Kuopio, and in Ostfalia University of Applied Sciences in Germany (under the supervision of Prof. Thorsten Ahrens), where the downstream processing of some of the bioprocess products are being provisionally experimented.

The leading principle in the Finnoflag Oy's biorefinery technology is the implementation of degradative and recycling function of the Nature's microbiota into industrial applications. This requires understanding on the interactions between the biomass (whose composition is subjected to variations), its natural flora, and the added strains and enzymes.

The original idea of the piloting experiments is to study the combination of gaseous, liquid and solid phases in the reactor in order to produce bioenergy, chemicals and fertilizers, or their raw materials. Breaking the biochemical process into bits and pieces could form this basis for any experimentation in the future (Hakalehto *et al.* 2013; Hakalehto 2015).

Pilot A is described in more detail in the Pilot A User's Manual (ABOWE report O3.4).

4. Practical experiments with Pilot A

4.1 Test organization and testing team

Tests were carried out under the supervision of Dr. Elias Hakalehto. In the testing team there were two participants from Finnoflag Oy; Laboratory manager Anneli Heitto and System engineer Kevin King. From Savonia Environmental Engineering unit there were Project engineer Tero Reijonen, Laboratory worker Henna Huopainen and Bachelor student Petteri Rautvuori. Project engineer Tero Kuhmonen was taking care of technical arrangements at the site. Two process operator students from Savo Vocational College participated in the test runs; Mikko Auriola and Samuli Räsänen. As contact person at Savon Sellu Oy was the environmental manager Kari Koistinen, and personnel of the waste water treatment plant was providing necessary information every now and then.

4.2 Analyses in various laboratories

Various samples from Pilot A test runs were sent to:

- Savonia's water laboratory for TS/VS (Total Solids/Volatile Solids tests)
- Finnoflag laboratory for microbiological samples
- University of Eastern Finland for NMR (Nuclear Magnetic Resonance) analyzer
- Ostfalia University of Applied Sciences, Germany for Downstream Processing experiments received the raw sludge from the testing site for simulations on the 2,3-butanediol purification
- Commercial laboratory of Savo-Karjalan Ympäristötutkimus Oy

4.3 The six test runs

In testing period six runs were made to test the Pilot A in different circumstances and with different bacterial strains. The waste materials used in these test were the solid sludge and waste water going to active sludge plant. In some occasions raw woody cellulose broth and smaller amounts of pulp from the factory was used also for increasing the substrate concentration. During the hydrolyzation of sludge Viscamyl Flow™-enzyme were used.

Test run 1 took place between 27th and 30th of January. Microbe used during this first test run was *Probionibacterium acidipropionici*. It was chosen for test organism eg. because of its safety (EFSA, European Food Safety Authority 2012). Process was anaerobic. For the adjusting pH $NaHCO_3$ and HCl were used. The main purpose of run 1 was to make sure that all the components in the Pilot A facility were working properly, so no major results were expected. Due to some extreme foaming happening in the reactor phase, it was decided that

the run would be cancelled on 30th of January. Notes were taken and improvements made for next run.

Test run 2 was performed during 10.-12.2. On this run microbes used in bioreactor process were *Klebsiella mobilis* and *Echerichia coli*. Process was containing oxygen in this case. Small amount of pulp from Savon Sellu's factory and sugar was added to process for boosting the fermentation. pH was adjusted with NaOH, $NaHCO_3$ and HCl. Much like the first run, run 2 was meant to be a testing run. Foaming didn't occure anymore, partially due to adding small amounts of antifoaming agent to the process. Due to problems in bioreactor phase, regarding the high H_2S levels in the outgoing gas, run 2 was short, since we wanted to avoid the growth of sulphate reducing bacteria.

Test run 3 run was carried out 17.-20.2.2014. Microbes used were again *Klebsiella mobilis* and *E.coli*. In this case raw woody cellulose broth was used as one of the source materials besides sludge, waste water and small amount of pulp. Adjusting pH NaOH, $NaHCO_3$ and HCl were used. Run 3 was the first run where the bioreactor phase could be completed without any issues regarding H_2S formation. Besides the oxygen in the ingoing gas, this was due to the nutrient bed application, where the initial inoculum was cultivated in the reactor, within raw pulp or woody liquor.

Test run 4 started 4th of March and ended on the 6th. Microbes used were again *Klebsiella mobilis* and *E.coli*. Source materials were raw woody cellulose broth, sludge and wastewater. Also end product of previous test was added to reactor as well as small amounts of pulp and sugar. Later also small amount of sour milk was added in order to stabilize the microflora and to compensate the effects of the relatively robust natural flora. Adjusting pH NaOH, $NaHCO_3$ and HCl were used.

Test run 5 took place between 17-21st of March. Microbial strains were *Clostridium butyricum* and *Clostridium acetobutylicum*, and the process was strictly anaerobic. In this case filtered lake water was used instead of waste water for dilutions. Due to some encouraging preliminary results, pulp was used again on this run as a feed material alongside the sludge. On this run there were some problems with the heating process in the hydrolyzer vessel.

Test run 6 started on 26th of March and ended 3rd of April. Microbes were again *Clostridium butyricum* and *Clostridium acetobutylicum*. This time pulp and bacteria was added to reactor first to give them good start as an inoculated nutrient bed. Sludge/wastewater mass was added three hours later. This final test run lasted longer than others in order to observe the duration of the active bioreactor phase. New round of hydrolyzed mass was added 68 hours after the first batch. Also small amounts of sugar were added to process in order to boost the microflora.

Summary of all six test runs is presented in Table 1.

	substrate	additions	atmosphere	microbes
RUN1	100 kg dry sludge 275 waste water	0,3 l chalk, 3 kg sugar in 15 l water	anaerobic	*Propionibacterium acidipropionici*
RUN2	103 kg dry sludge 275 l waste water	0,2 l chalk, 20 l hydrolyzed pulp, 10 l saturated sugar solution	aerobic	*Klebsiella mobilis*
RUN3	120 l raw wood liquor, 37 kg dry sludge 120 l waste water	10 l pulp 30 l hydrolyzed pulp	aerobic	*Klebsiella mobilis* *Echerichia coli*
RUN4	140l raw wood liquor, 30 kg dry sludge 120l endproduct from previous run	30 l hydrolyzed pulp 3 kg sugar in 2 l water (20 l of sour milk + 3kg sugar in 2l water)	aerobic	*Klebsiella mobilis* *Echerichia coli*
RUN 5	80 kg dry sludge 200 l technical water	30 l hydrolyzed pulp 6 kg sugar in 20 l water	anaerobic	*Clostridium butyricum* *Clostridium acetobutylicum* ("cellubed")
RUN 6	97 kg dry sludge in waste water	60 l hydrolyzed pulp 0,2 l chalk 15 kg sugar in 16l water	anaerobic	*Clostridium butyricum* *Clostridium acetobutylicum* ("cellubed")

In none of the experiments the fed-batch principle was used due to the risk of the formation of the toxic H_2S gas which prevented the gradual collection of the process fluids into the stabilizer. The H_2S emission was partially avoided in the latter test runs by careful preparation of the inocula.

In overall the testing period in Savon Sellu went without any major problems. Only actual drawback was the failure of the heating stage in the hydrolysis process. The filter at the end of the process also proved to be problematic, and could not be used to filter any samples in large extent. Otherwise all parts of the facility worked rather well for the first runs, and improvements were made to the equipment on the basis of practical experience.

5. Results and their analysis

5.1 Results from the six test runs

Test run 1

The production organism intended for used during this first run was *Propionibacterium acidipropionici*, which was accepted as food grade by EFSA (European Food Safety Authority 2012). However, the natural flora in the biowaste sludge was containing natural microflora, which was not completely eliminated in the hydrolysis temperatures. Consequently, some thermofilic sulfate reducing bacteria were conquering the space in the reactor. They produced excessive amounts of H2S. Because of these rapidly growing natural bacteria propionibacteria could not get propagated well enough for overcoming the background flora. In the literature the maximal concentration of propionic acid should be achieved within 70-80 hours, and even with the enhanced Finnoflag method this takes about 30 hours.

Due to the surprisingly high H2S concentration we tested the methods for restricting the bacteria producing this volatile substrate. This was mainly carried out by temporal elevation of oxygen levels in the ingoing gas flow. This was effective against the sulphate reducing thermophiles, but the original idea of anaerobic conversion of lactate into propionic acid was not functionable. Therefore, the natural production of organic acids was dominating in this run.

During this experiment the homogenization and pH adjustment of the waste suspension was examined. These adjustments were working out satisfactorily. Some difficulties were met in termostating the hydrolysis tank and bioreactor, and some changes were made to heat exchange system later on. Levels above 30 mmol/l of glucose were achieved in the hydrolysis. The arctic outside temperatures – 25-30 ^{o}C were disturbing the pumping of technical water to the biomass, and some other additions or functions. The first runs of the computer control system was carried out simultaneously with the bioprocess experiments.

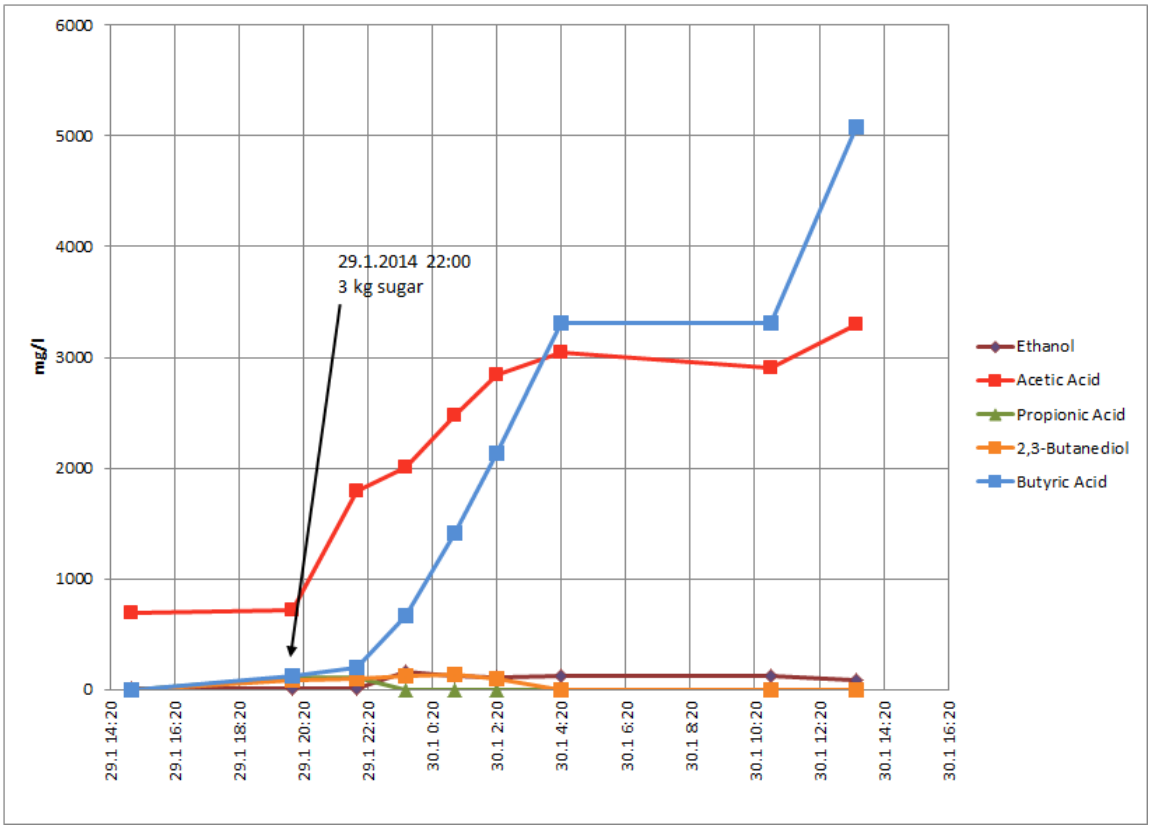

Figure 7. Amounts of ethanol, actic acid, propionic acid, butyric acid and 2,3- butanediol during run 1, GC-measurements.

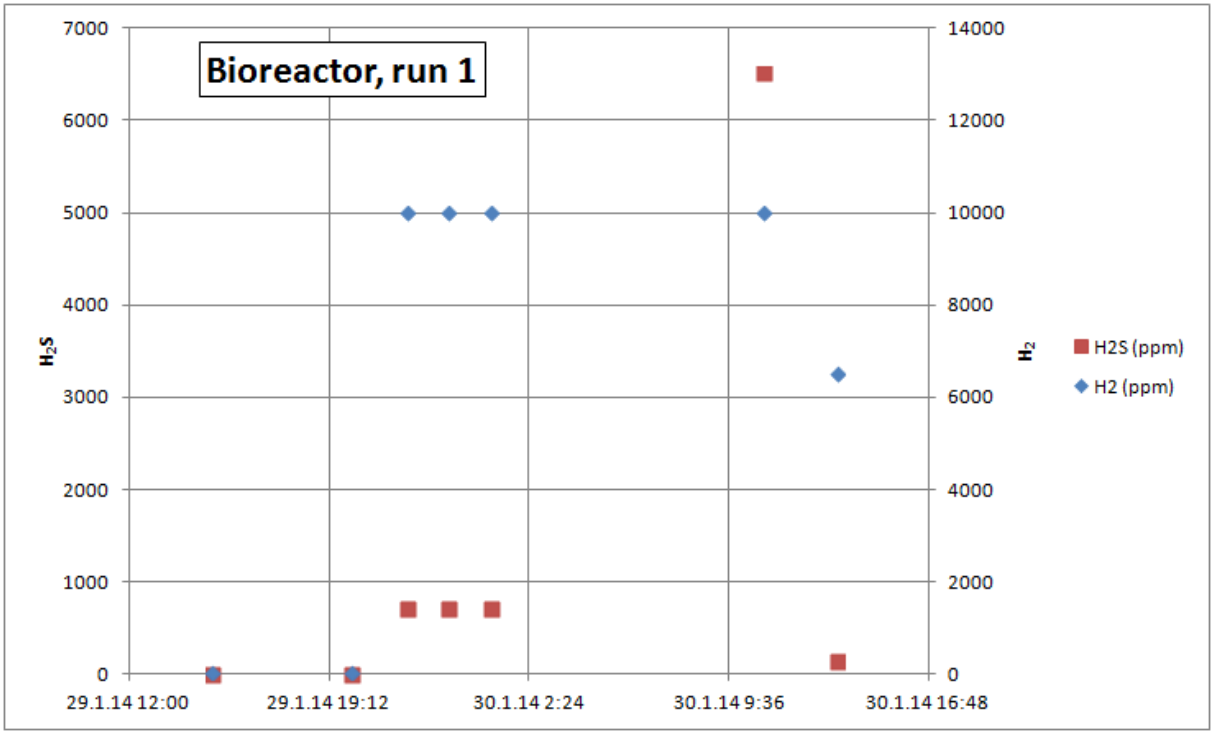

Figure 8. Amounts of hydrogen and hydrogen sulfide during run 1.

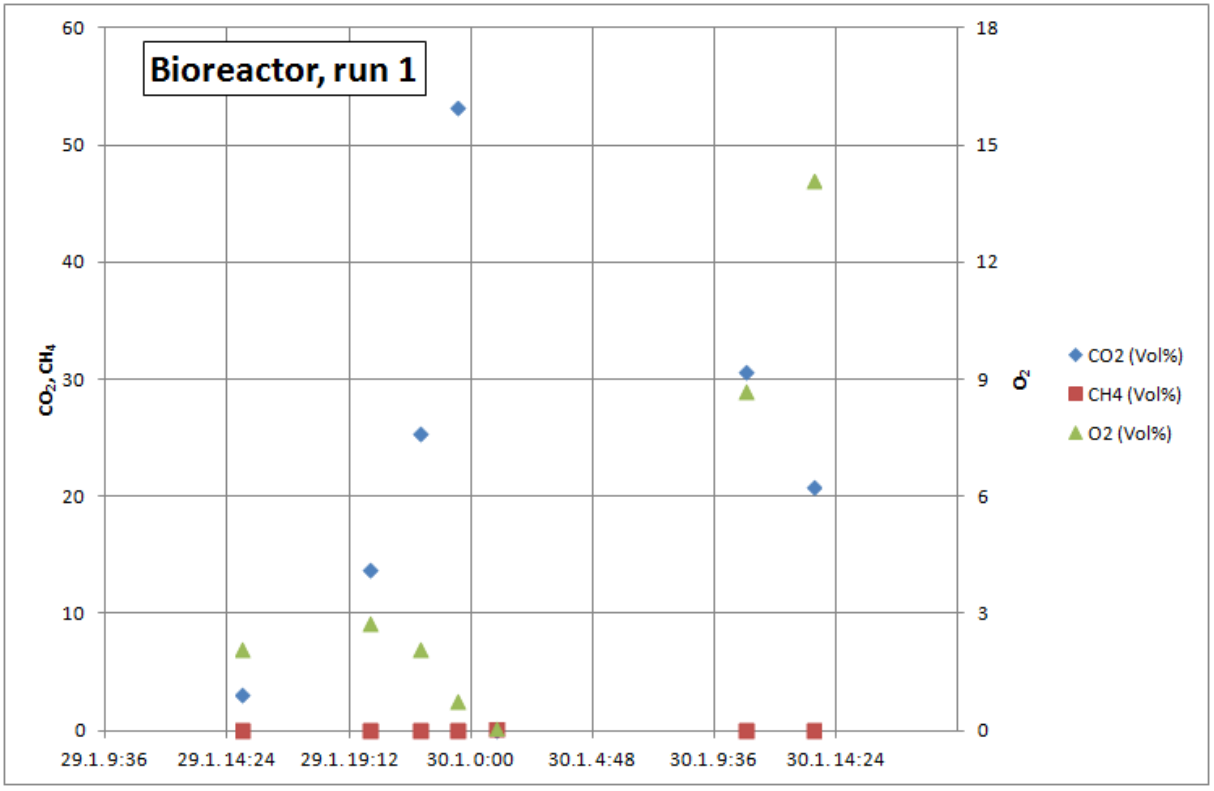

Figure 9. Amounts of carbon dioxide, methane and oxygen during run 1.

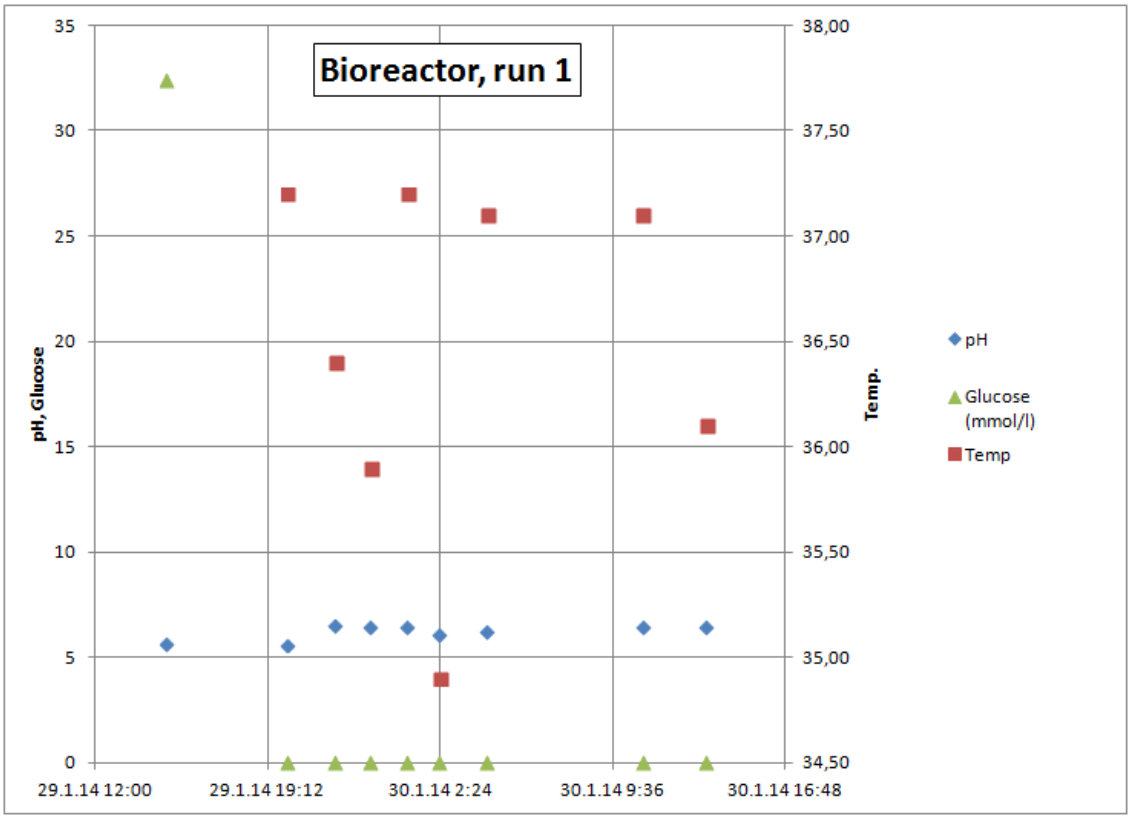

Figure 10. Temperature, pH and the amount of glucose during run 1.

Test run 2

In order to make possible the control and restriction of hydrogen sulfide emissions during the waste treatment facultative anaerobic *Klebsiella mobilis* and *E.coli* were used as production organisms. The original amount of biomass was about 100 kg which was then diluted with 275 l of wastewater, resulting in the dry matter content of 10.3%.Two desiliters of chalk was added for additional buffering of the solution. In the hydrolysis cellulolytic enzymes produced relatively high levels of glucose (55 mmol/l) from the raw cellulose but the product formation was disturbed by the background microflora. Consequently, the glucose level was inadequate. This run was interrupted because of the interference cause by the natural bacteria.

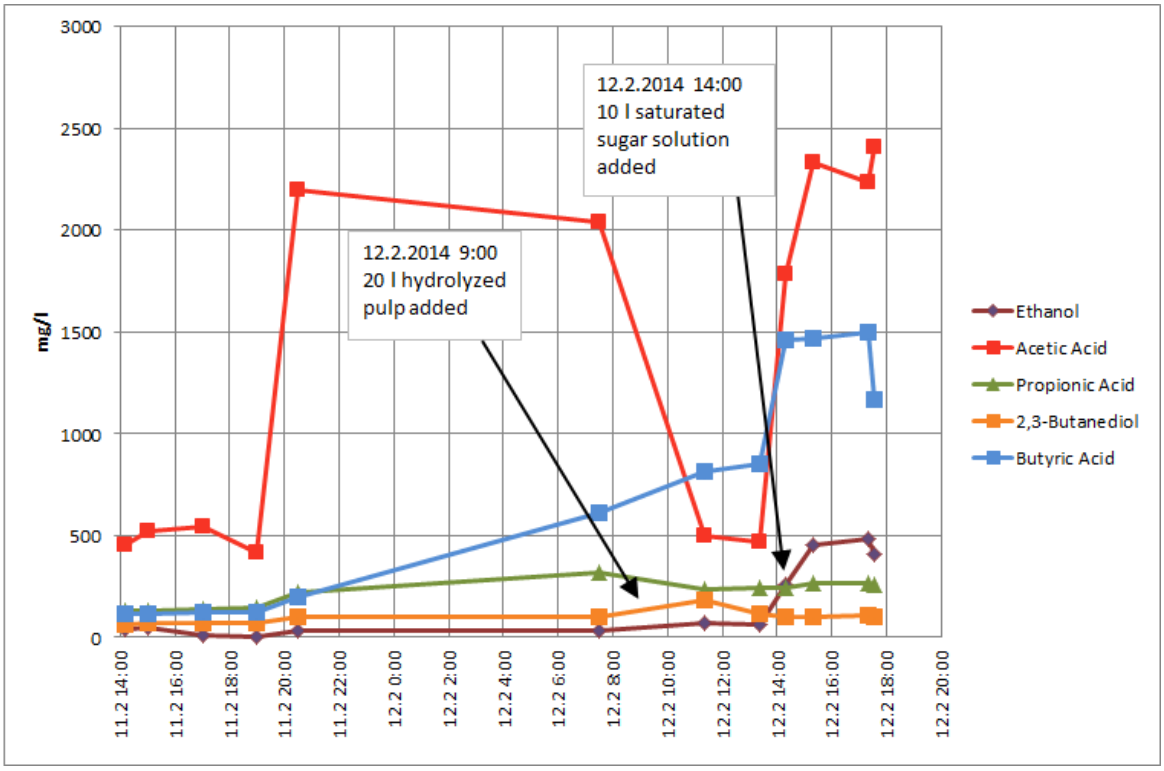

Figure 11. Amounts of ethanol, actic acid, propionic acid, butyric acid and 2,3- butanediol during run 2, GC-measurements.

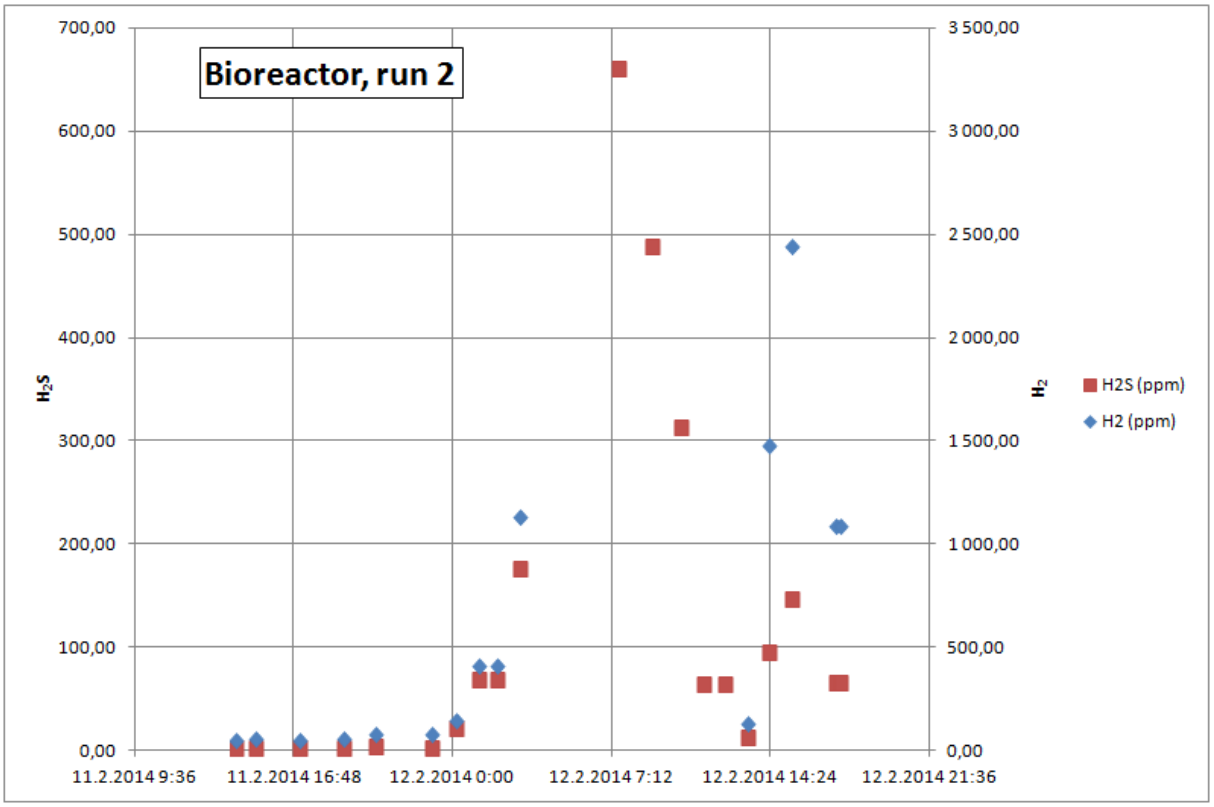

Figure 12. Amounts of hydrogen and hydrogen sulfide during run 2.

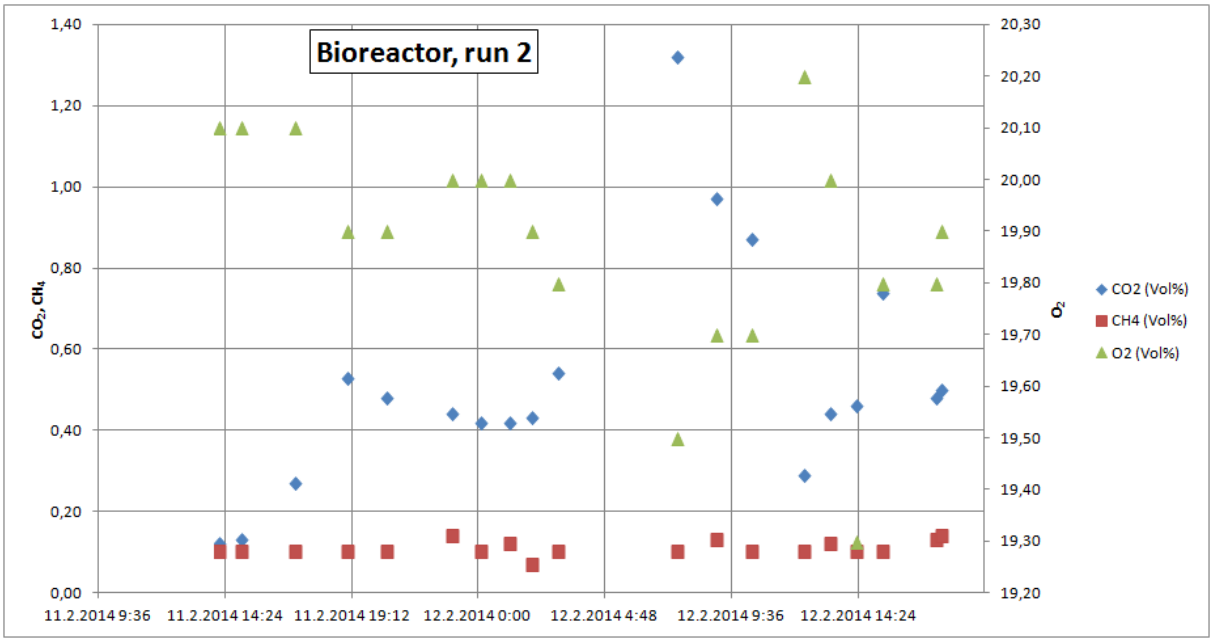

Figure 13. Amounts of carbon dioxide, methane and oxygen during run 2.

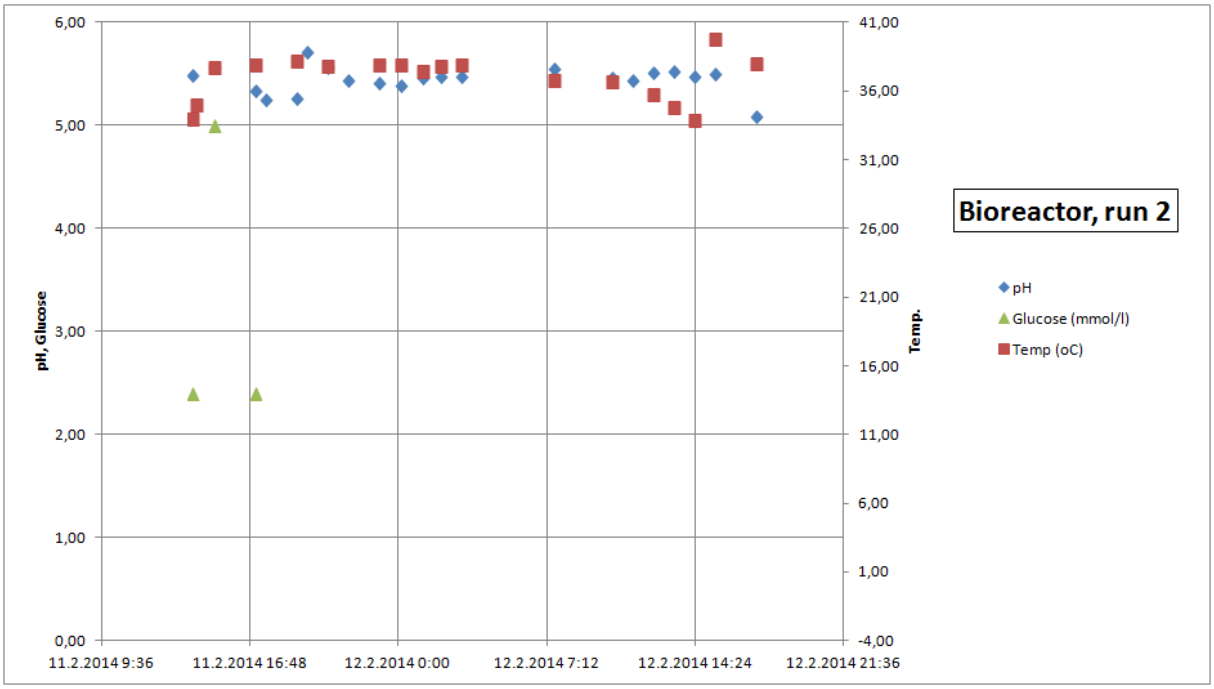

Figure 14. Temperature, pH and the amount of glucose during run 2.

Test run 3

Some 2,3-butanediol was formed, and the levels of small organic acids was higher than in the previous runs. Traces of ethanol and hydrogen were also detected. The product formation was still not remarkable quantitatively, but at least the H2S formation was prevented in this run. However, the O2 content was nearly 20 % in the outgoing gas. Main purpose of this run was to test the nutrient bed method of inoculation where the production organisms were readily waiting for the actual biomass in the reactor. Glucose was exhausted in 6 hours from the biological reaction environment, partially due to low initial concentration. It was likely that some components of the natural microflora were interfering the process. The elevated initial levels of acetic acid and 2,3-butanediol were caused by the activities of the microbes in the nutrient bed.

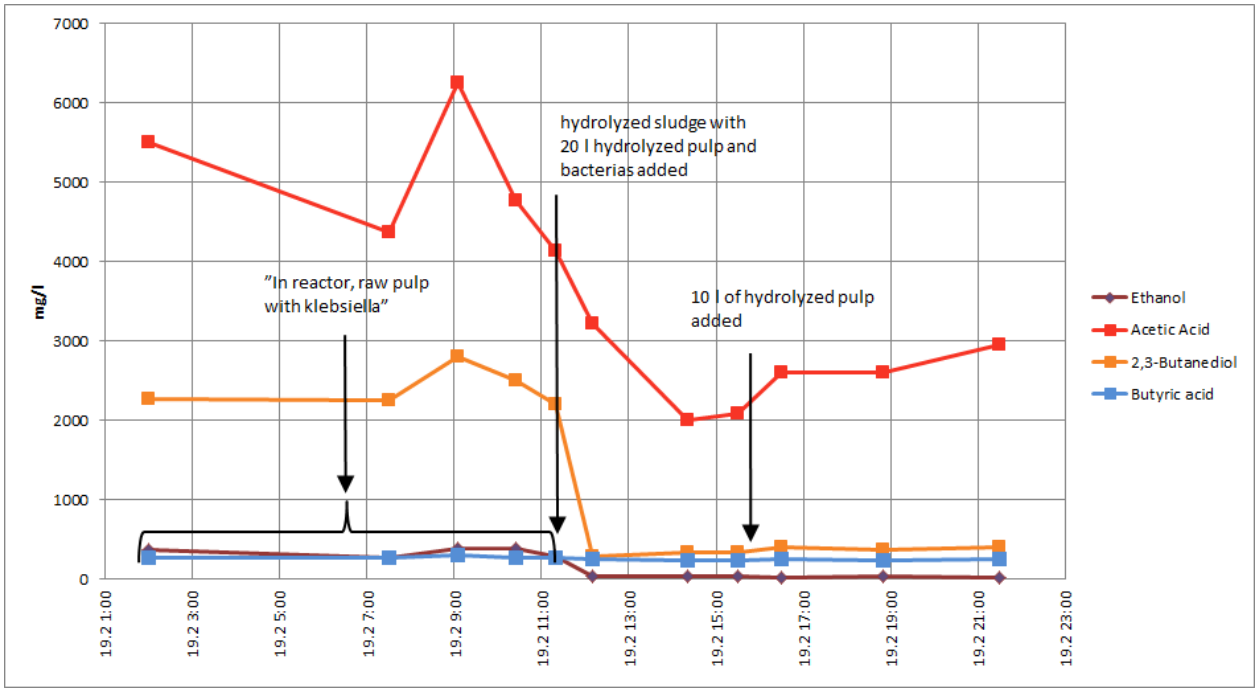

Figure 15. Amounts of ethanol, actic acid, butyric acid and 2,3- butanediol during run 3, GC-measurements.

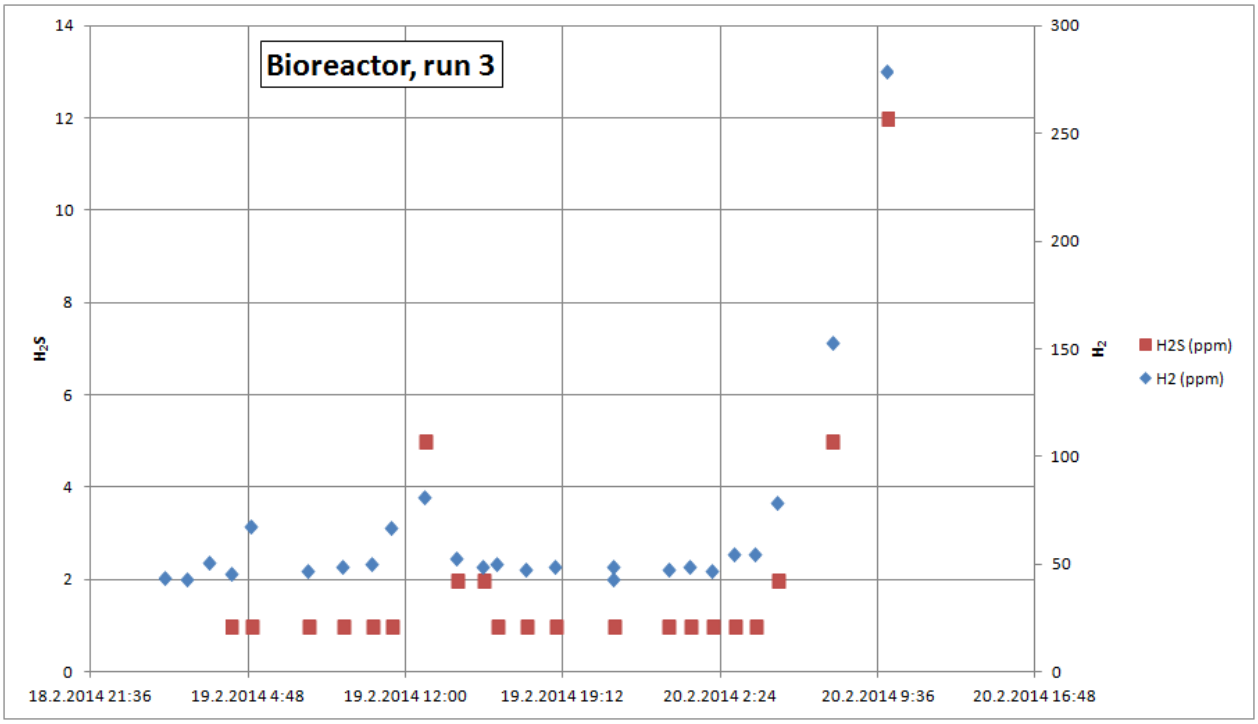

Figure 16. Amounts of hydrogen and hydrogen sulfide during run 3.

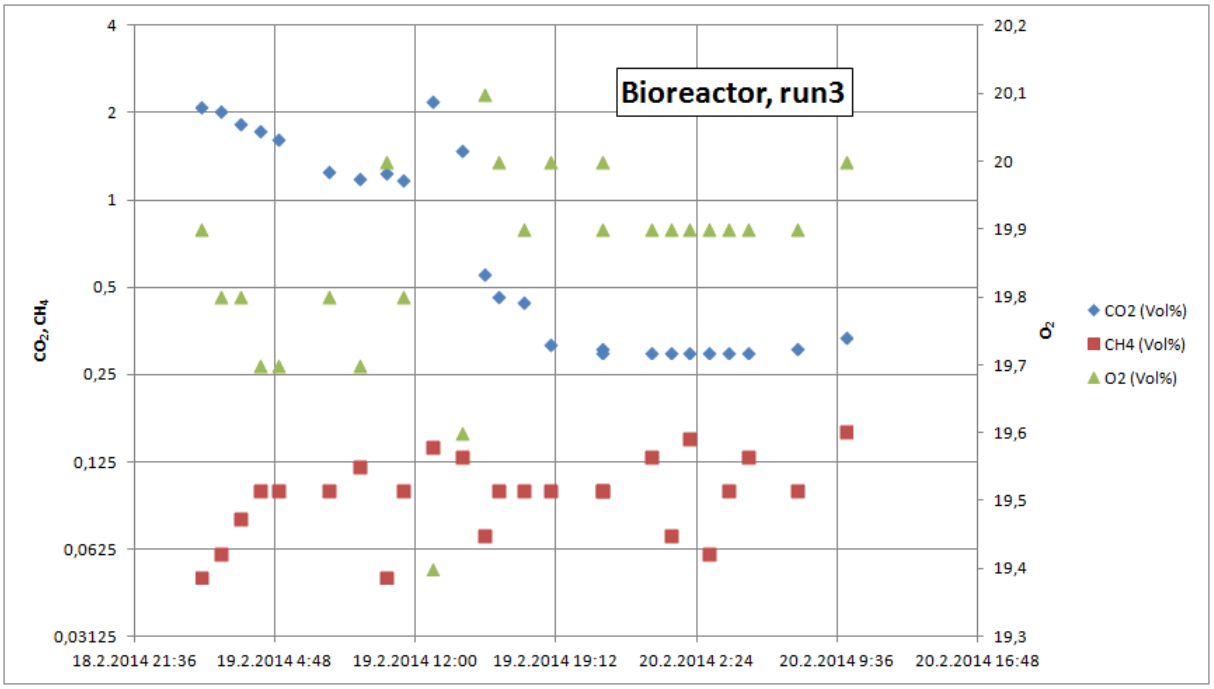

Figure 17. Amounts of carbon dioxide, methan and oxygen during run 3.

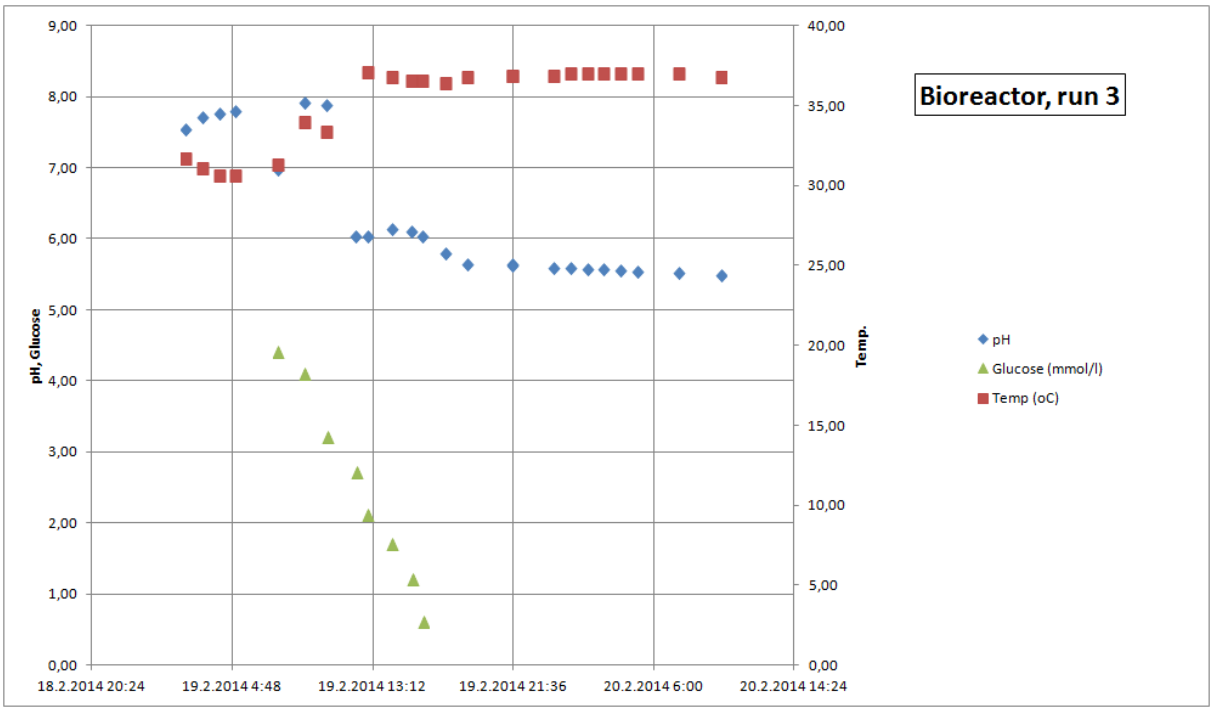

Figure 18. Temperature, pH and the amount of glucose during run 3.

Test run 4

In this run O2 content was still too high but the H2 level reached the upper limit of the measurement (10 000 ppm). As before the product formation was otherwise quite low due to the aerobic conditions. In this trial and in subsequent experiments the glucose levels after

hydrolysis were not adequate for full production of metabolites. This was probably due to the partial takeover of hydrolyzed suspension by the thermophilic bacteria during its cooling down phase in the hydrolyzer. Therefore, more experience, knowledge and learning of the parameters is needed for optimal performance of the non-aseptic process runs with partially undefined microbial cultures.

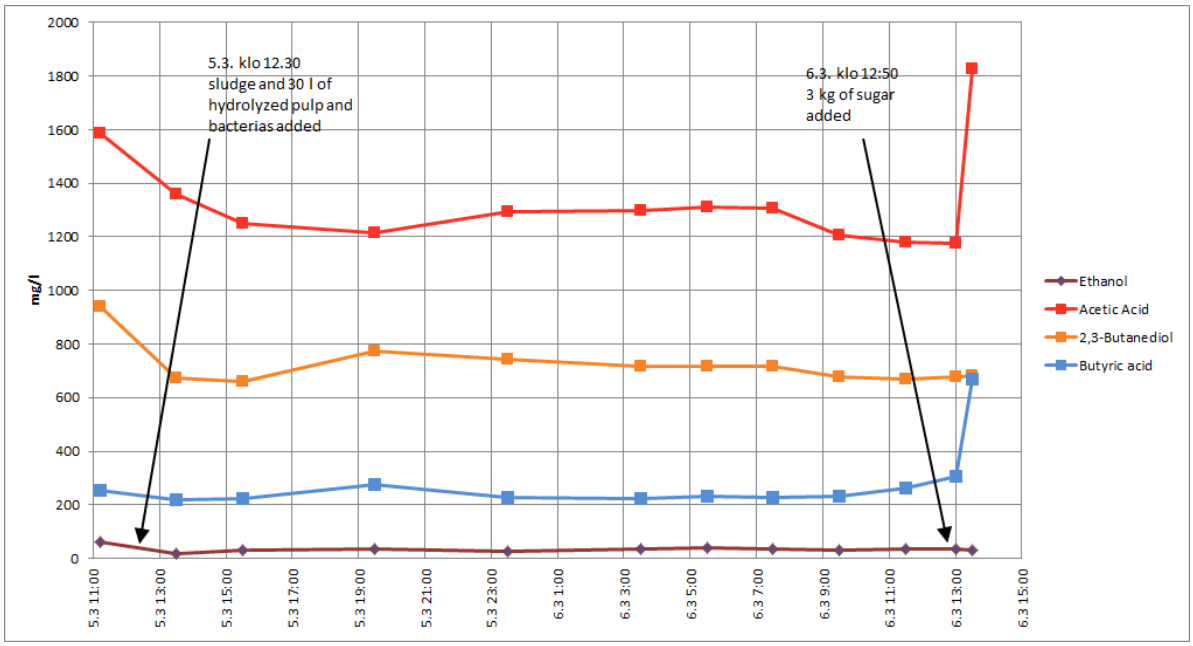

Figure 19. Amounts of ethanol, actic acid, butyric acid and 2,3- butanediol during run 4, GC-measurements.

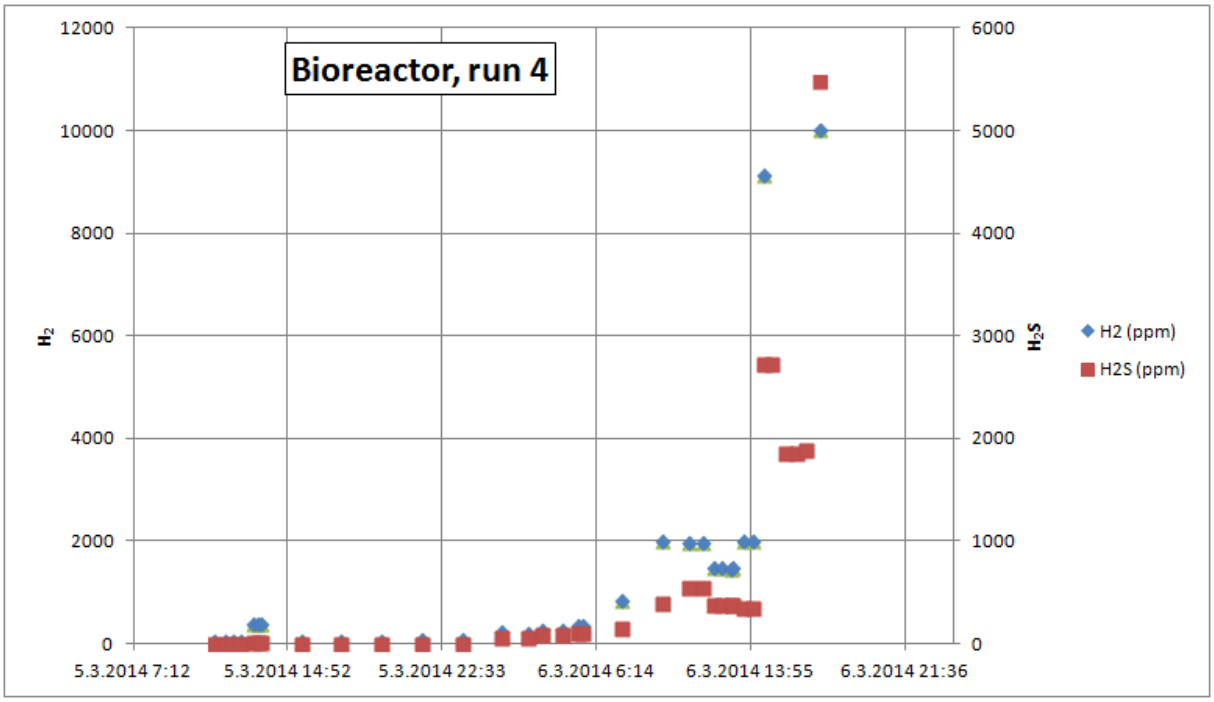

Figure 20. Amounts of hydrogen and hydrogen sulfide during run 4.

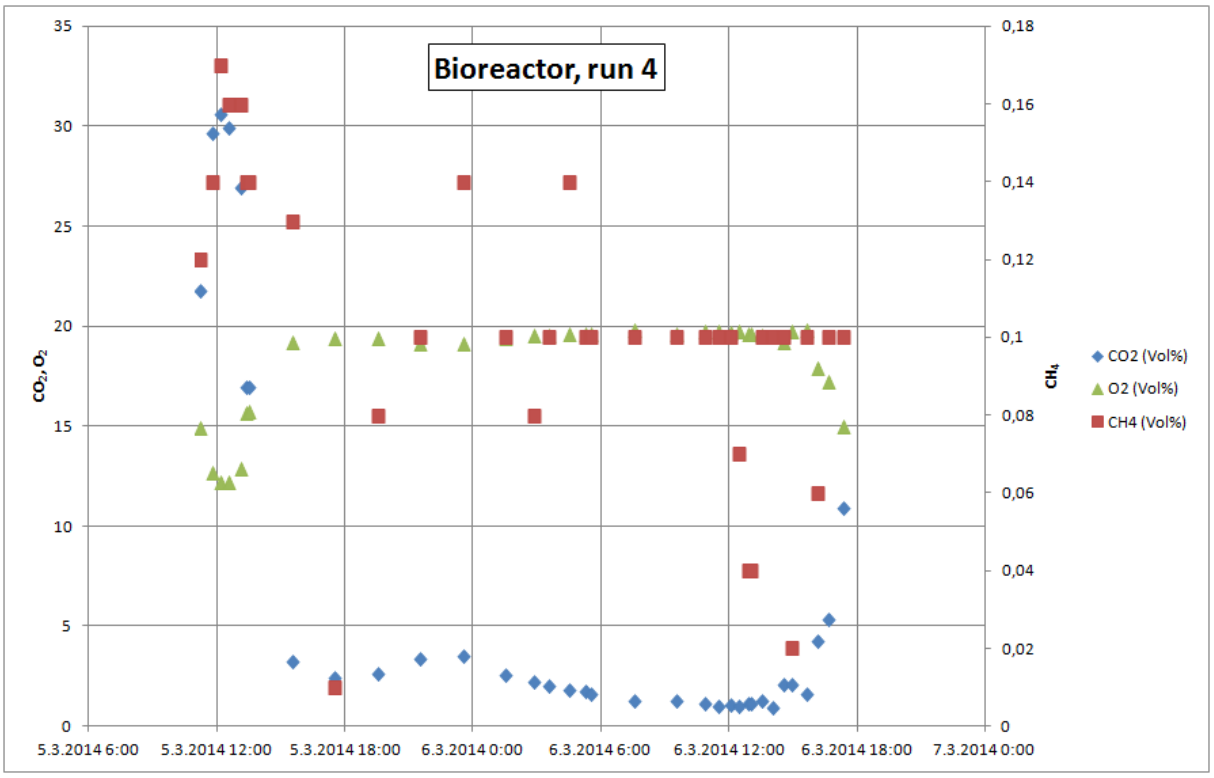

Figure 21. Amounts of carbon dioxide, methane and oxygen during run 4.

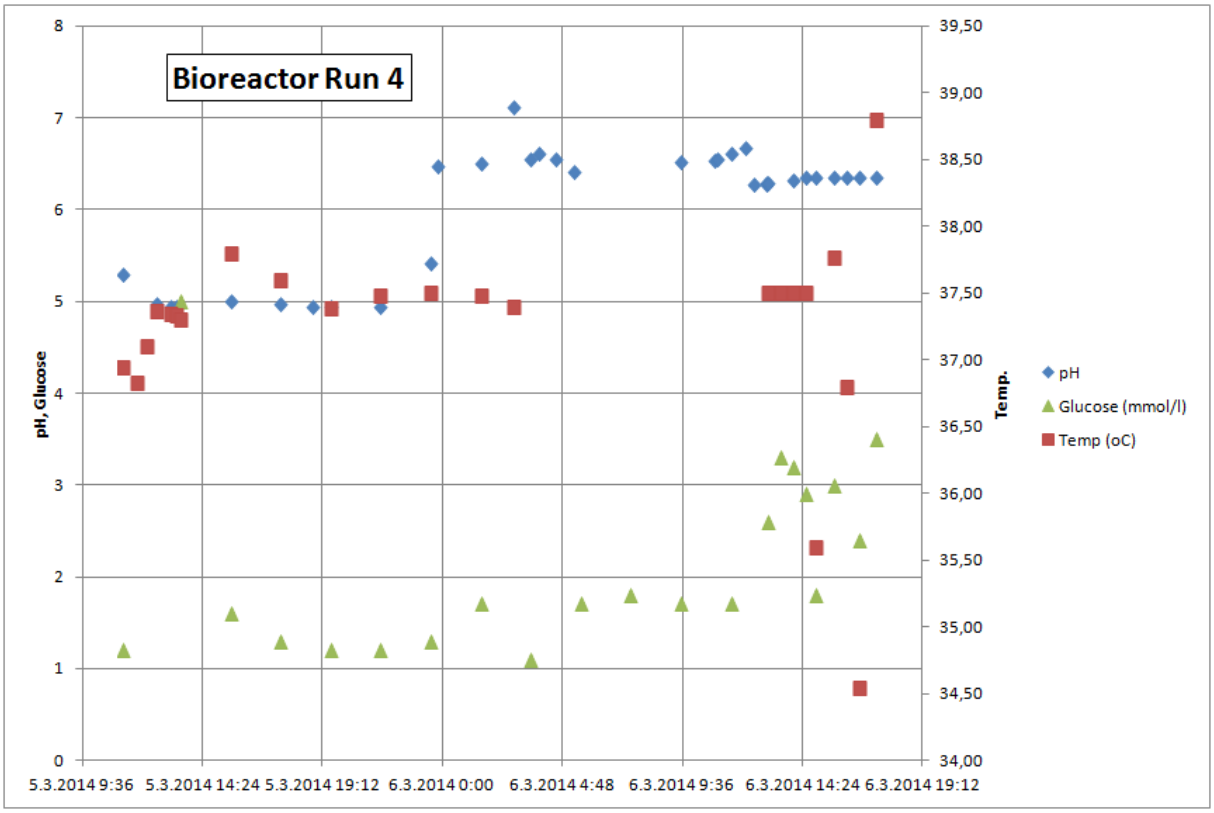

Figure 22. Temperature, pH and the amount of glucose during run 4.

Test run 5

In order to exercise anaerobic conditions in the rest of the experiments, *Clostidium butyricum* and *Clostridium acetobutylicum* were chosen for production organisms. Traces of acetone, butanol and ethanol were detected together with somewhat higher amounts of acetate and butyrate. Also in this run the nutrient bed system was in use. The H2 production exceeded 10 000 ppm remaining high during the rest of the 2.5 days of bioprocessing. H2S level remained mostly below 2000 ppm. Some difficulties were met in running the hydrolysis and in the sampling from bioreactor because of the blocked valves. Initial glucose level was not high enough for full production. In the cellulose-water mixture butanol level of 0,2% was achieved in 20 hours.

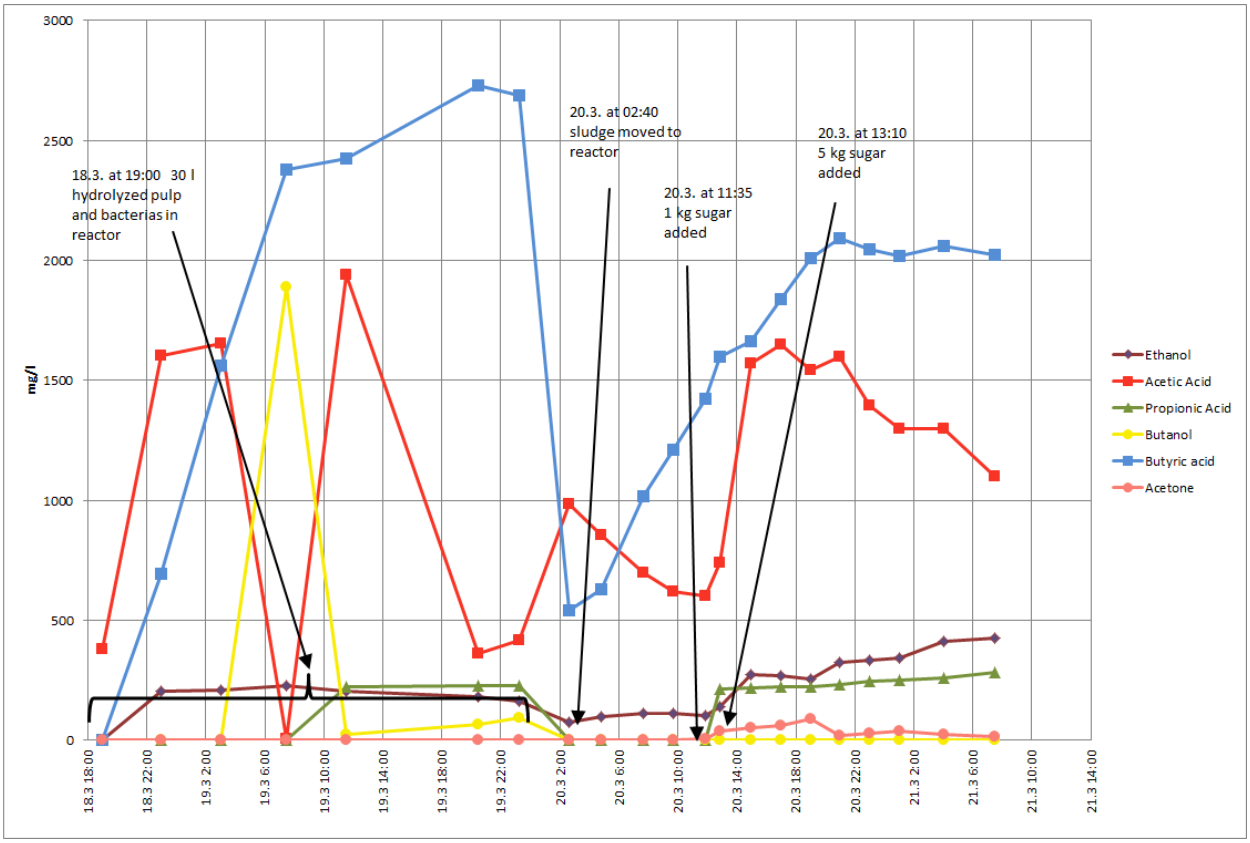

Figure 23. Amounts of ethanol, actic acid, butyric acid, propionic acid, butanol and 2,3-butanediol during run 5, GC-measurements.

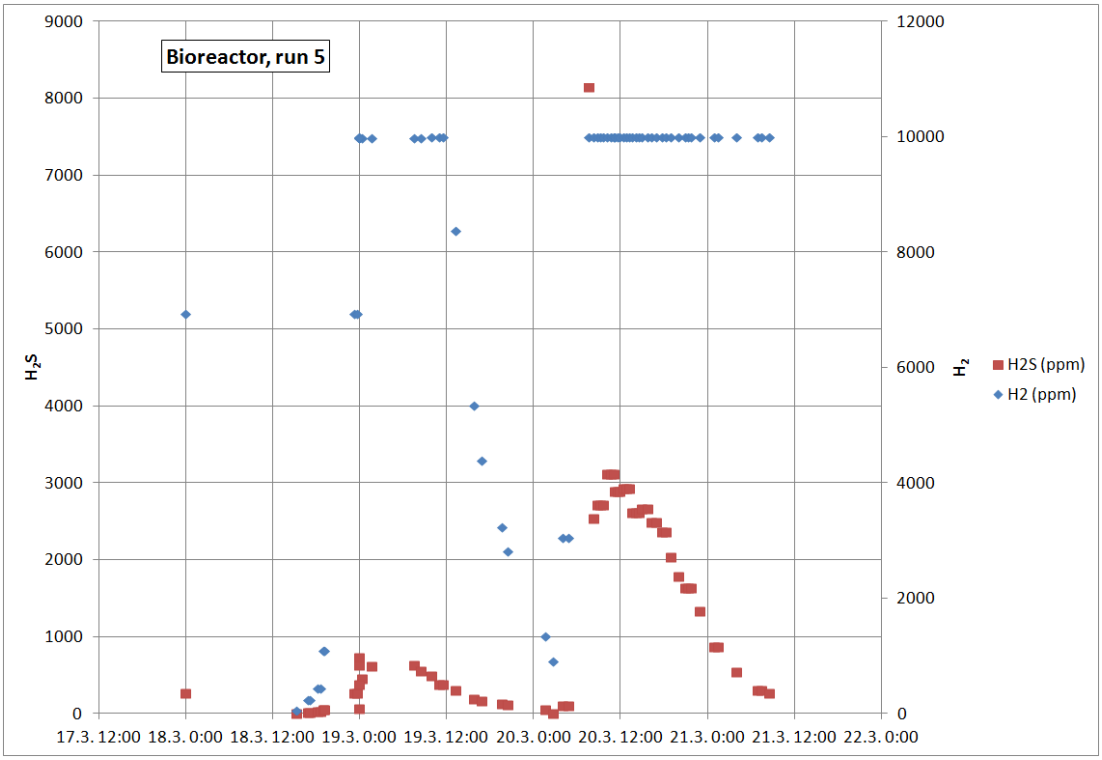

Figure 24. Amounts of hydrogen and hydrogen sulfide during run 5.

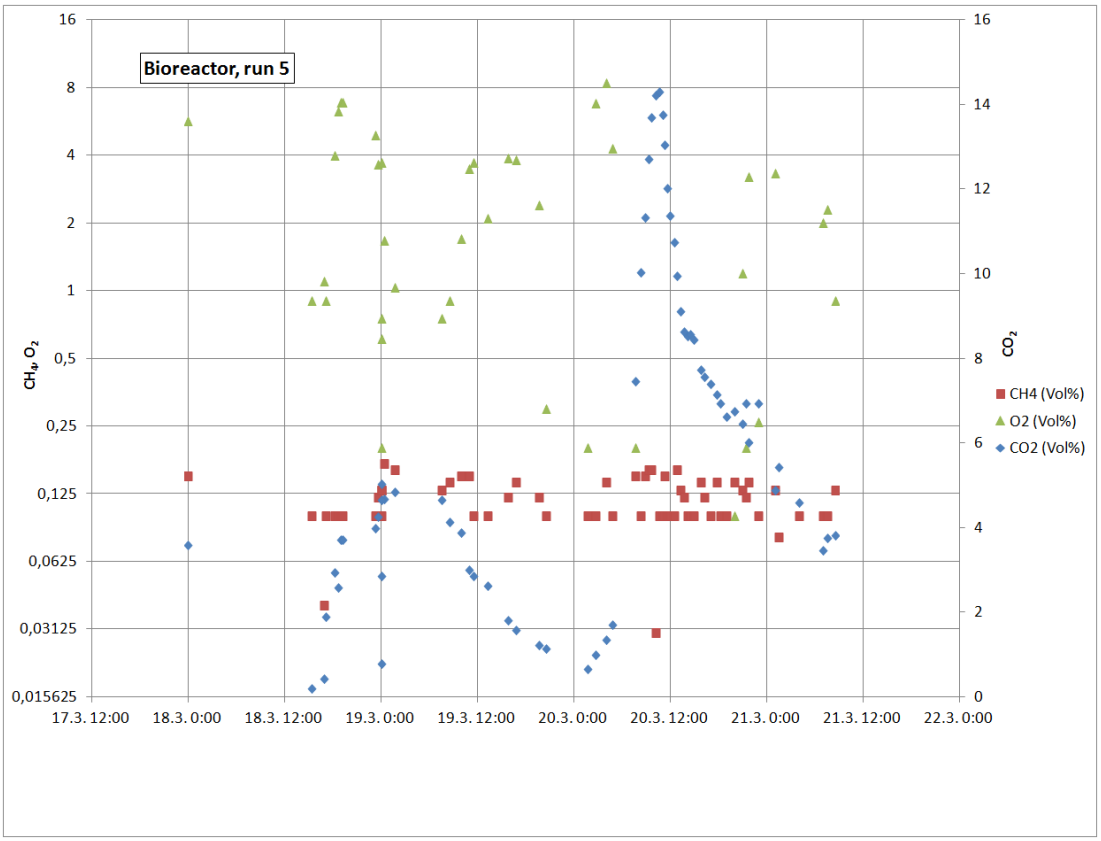

Figure 25. Amounts of carbon dioxide, methan and oxygen during run 5.

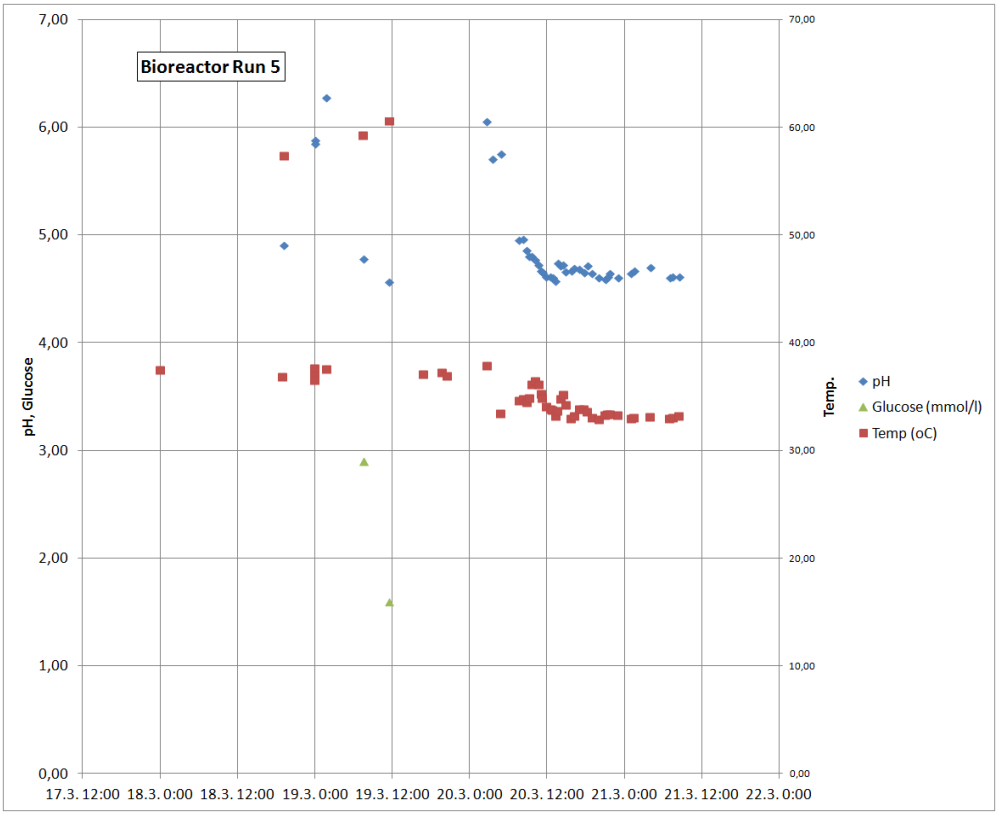

Figure 26. Temperature, pH and the amount of glucose during run 5

Test run 6

In the last 6th run we wanted to exploit the experience acquired in the previous runs with respect to the setting up of the rector and its control system. In the start of this experiment the glucose level was about 17 mmol/l at maximum which was better than before but should still be elevated. The anaerobic clostridia were used as like in the previous run. Inoculation was carried out again as seeded nutrient bed. This time the ethanol level was nearly 0,2% . Some butanol and 2,3-butanediol as well as acetate, propionate and butyrate were detected. Hydrogen level exceeded 10 000 ppm for almost one week. H_2S was remaining mostly below 3000 ppm.

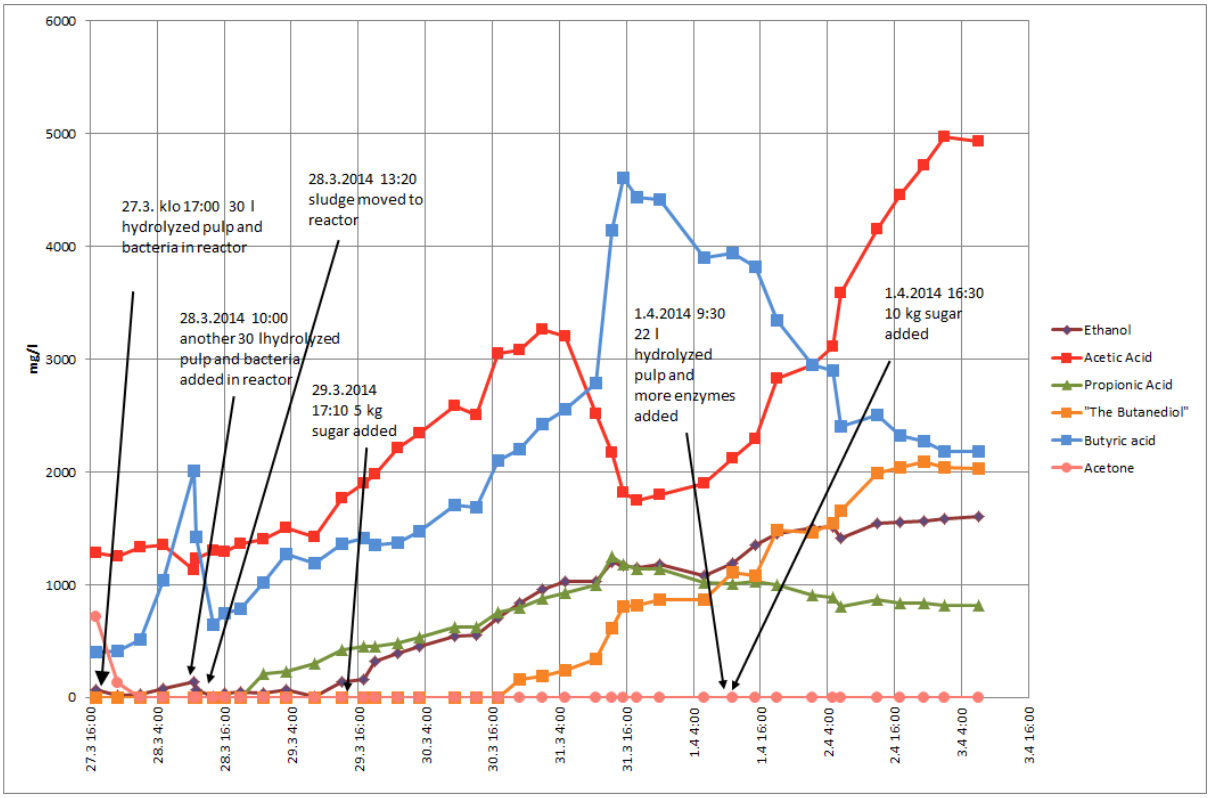

Figure 27. Amounts of acetone, ethanol, acetic acid, propionic acid, butyric acid and 2,3-butanediol during run 6, GC-measurements.

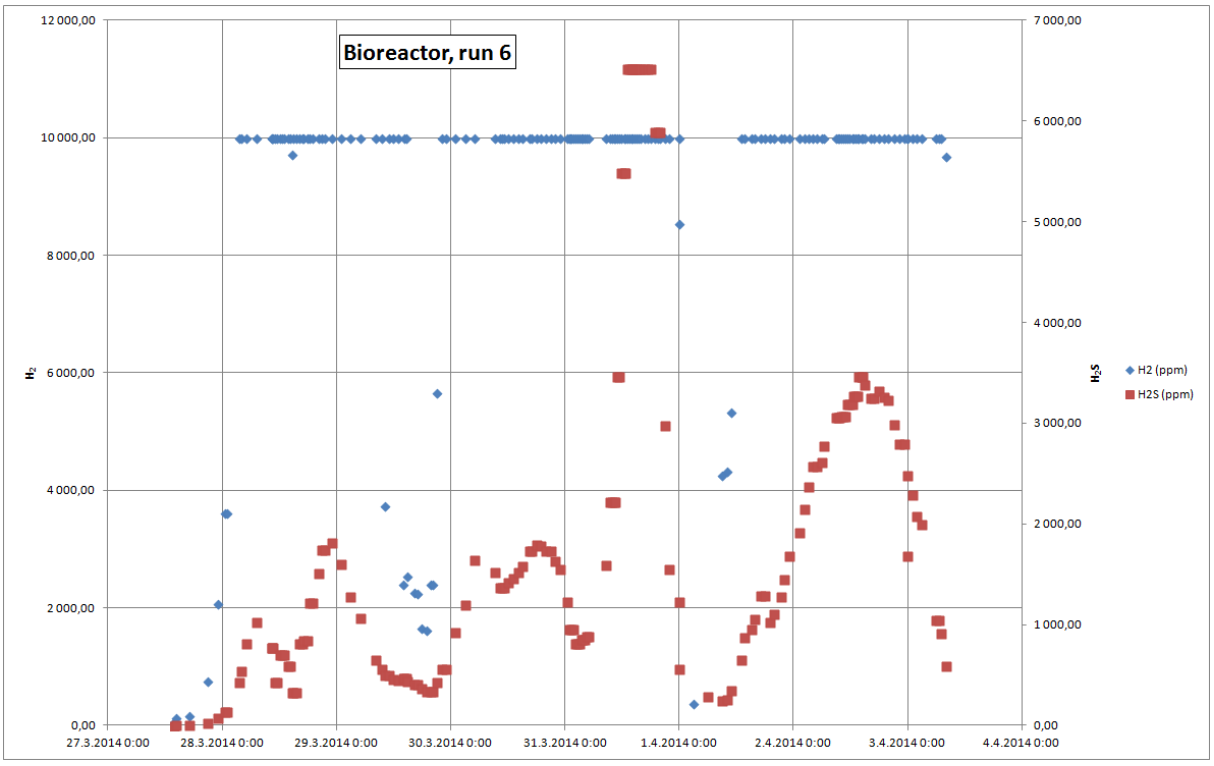

Figure 28. Amounts of hydrogen and hydrogen sulfide during run 6.

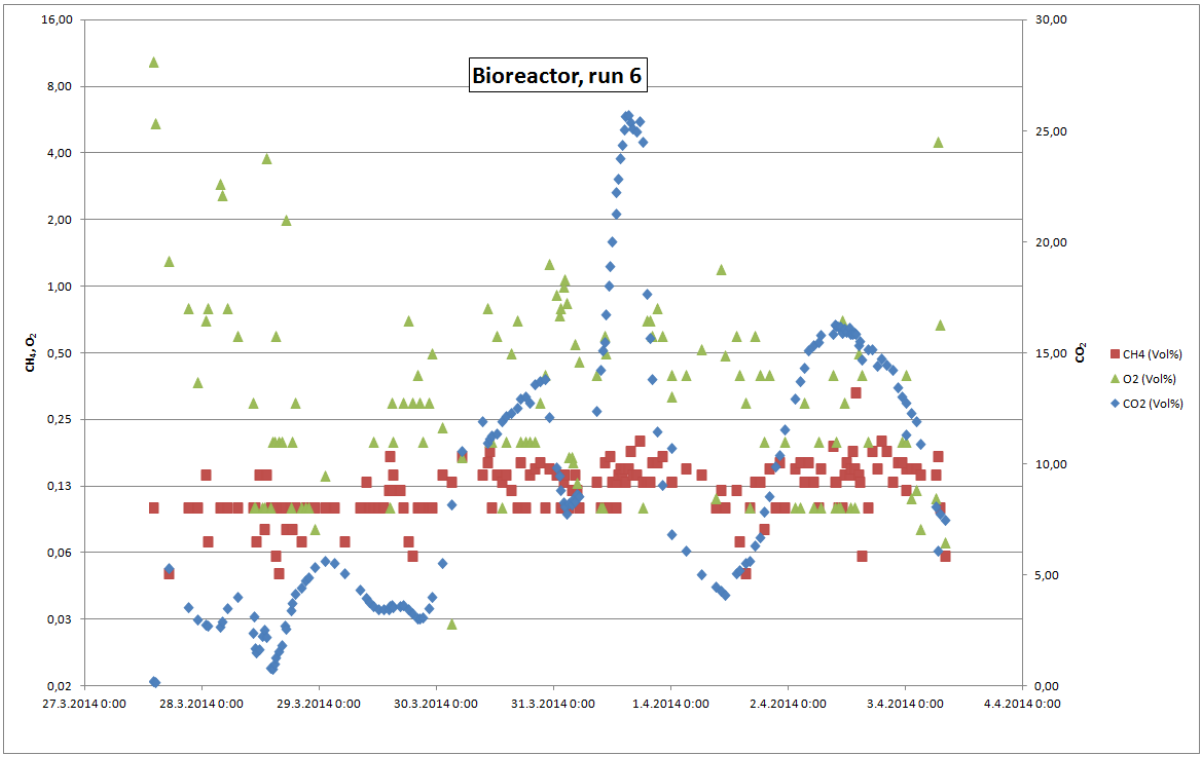

Figure 29. Amounts of carbon dioxide, methan and oxygen during run 6.

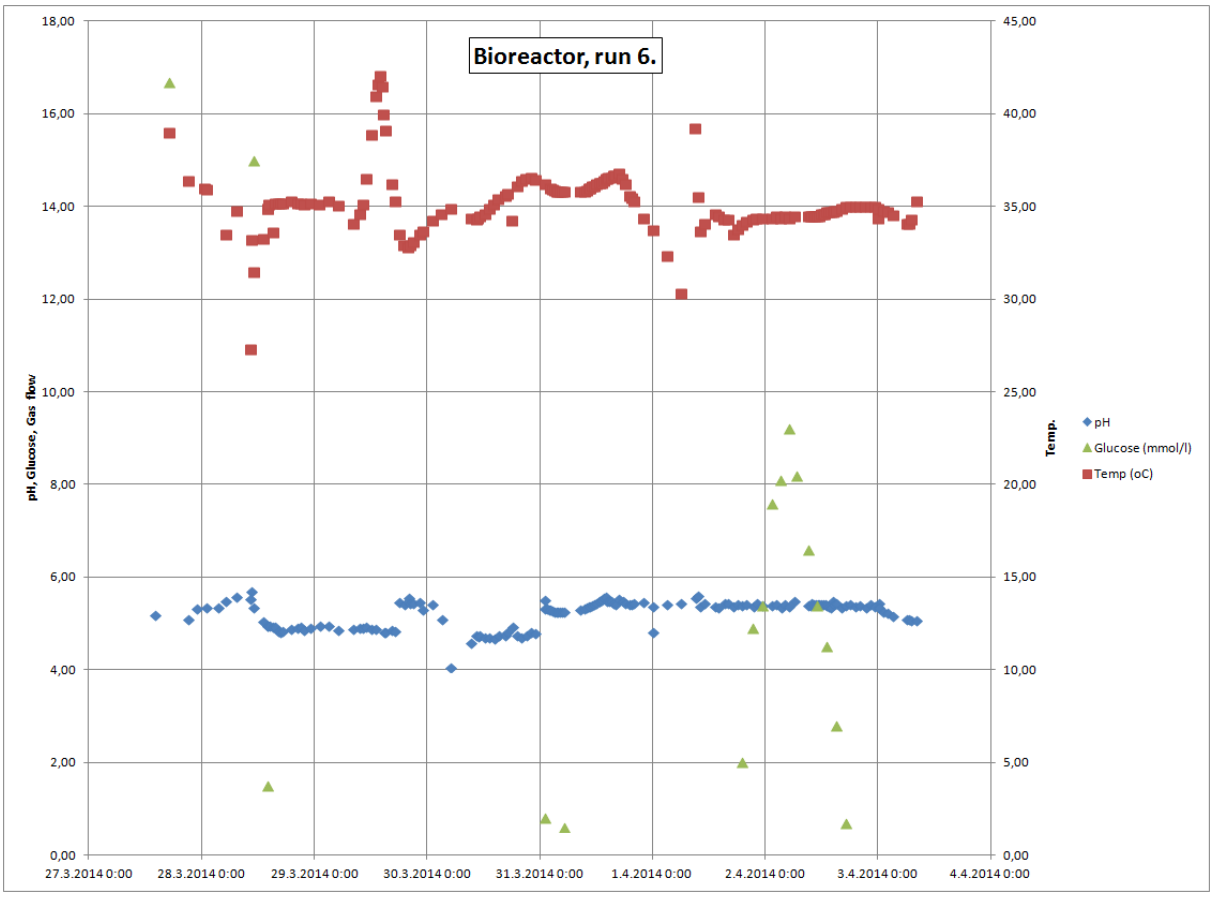

Figure 30. Temperature, pH and the amount of glucose during run 6.

5.2 SOM Analysis of the process run 6

The computer program for the analysis of the process parameters and product formation was tested by Dr. Harri Niska from University of Eastern Finland.

The process data collected from the Pilot A was analysed using the Self-Organising Map (SOM) (Kohonen 1982). SOM is an unsupervised mathematical learning technique to produce a low-dimensional representation (typically two dimensions) from multidimensional data. SOM is suitable data analysis technique for interpreting and understanding of complex ill-defined processes (such as biological processes) and phenomena behind them.

The initial analysis was performed by training the SOM map using the measured data from the Savon Sellu testing period. The resulting SOM map is showed in Figure 31.

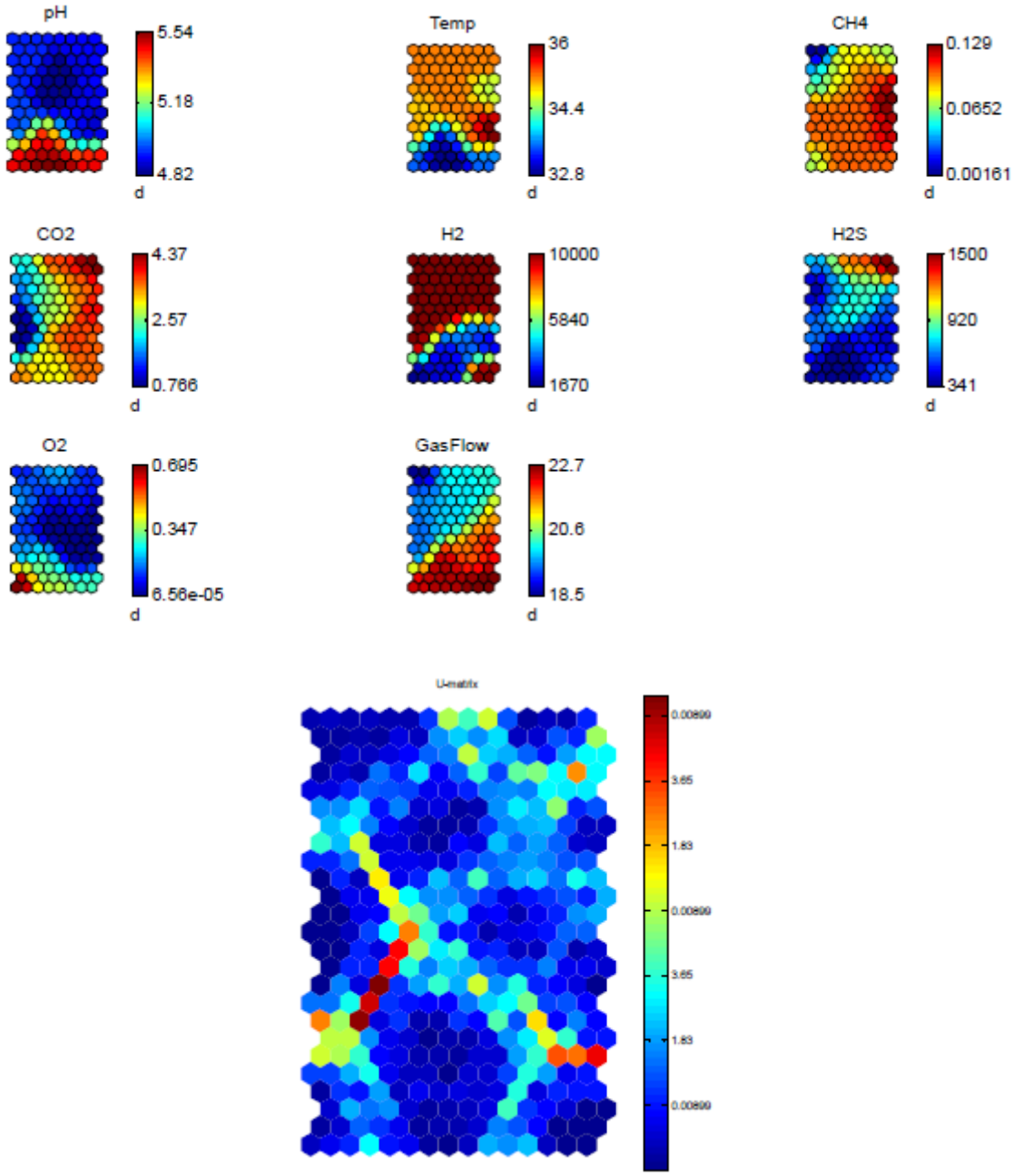

Figure 31. Process state analysis using SOM (Kohonen, 1982).

Sample ID/measurement location	Date	Time	Sample taker	pH	Aeration gas	Temp (°C)	Dry matter (%)	Glucose (mmol/l)	CO_2 (Vol%)	CH_4 (Vol%)	O_2 (Vol%)	H_2 (ppm)	H_2S (ppm)	Gas flow (m^3)
Reactor		12:15		33.6		NaN			2.21	0	0.1	10000	703	18.515
Reactor		12:40		NaN		NaN			2.52	0.08	0.1	10000	703	NaN
Reactor		13:53		4.95		34.89			0.78	0.1	0.1	10000	597	19.428
Reactor		14:17		4.95		35.10			0.75	0.1	0.2	10000	597	19.512
Reactor		15:30		4.93		35.20			1.53	0.05	0.2	10000	335	19.615
Reactor		16:45		4.82		35.20			2.68	0.1	0	10000	817	19.907
Reactor		17:30		NaN		NaN			NaN	NaN	NaN	NaN	NaN	NaN
Reactor		18:00		NaN		NaN			3.4	0.1	0.1	10000	842	20.013
Reactor		18:20		NaN		NaN			3.69	0.08	0.2	10000	1223	20.089
Reactor		20:15		4.9		35.20			4.39	0.07	0.1	10000	1509	20.209
reactor		9:30		4.9		34.60			3.95	0.13	0	10000	565	21.526
reactor		10:10		4.92		35.10			3.74	0.1	0	3727	502	21.586
Reactor	29.3.2014	16.20		4.86		36.20			3.55	0.12	0	2249	415	22.138
Reactor		18:00		5.46		33.50			3.45	0.07	0.7	1644	375	22.296
Reactor		19:55		5.56		32.80			3	0.1	0.4	2392	341	22.44
Reactor		20:20		5.45		32.90			3	0.1	0.3	2392	341	22.48
Reactor		22:15	AH	5.46		33.50			3.5	0.1	0.3	10000	562	22.665

Figure 32. Example of the measurement sheet from Run 6.

According to Figure 28 different process states and behavior of different process parameters can be visually examined on the 2D map, e.g. in terms of optimal CH_4 production. Furthermore it is possible to use clustering techniques to automate the identification of prevailing process states.

According to the results, further pilot runs are however needed in order to collect more extensive dataset and finally to make more stable conclusions about the process optimality and efficiency in respect to different feedstock and process parameters. This work is necessary to support development of the process towards an operating full-scale plant.

5.3 Examples of total solid and volatile solid contents during runs

Chemical and physical parameters of the raw materials were followed up by Finnoflag Oy, UEF and subcontractors for ensuring the reasonable starting values for the experiments. This enabled also further calculations on the basis of the results. In table 2 some total solid and volatile solid values are presented as examples.

Table 2. Total solids and volatile solids in some samples from Savon Sellu runs 1-6.

	sample	TS%	VS%	notes
SaSel RUN1	H101	8,83	8,86	input 275l wastewater, 100 kg dry sludge
	R102	6,81	6,16	
	R110	6,59	5,42	
SaSel RUN2	H201	7,61	6,99	input 275l wastewater, 104 kg dry sludge
	HY201	7,76	7,16	
	R201	7,80	7,12	
	R215	9,96	9,25	
SaSel RUN3	R305	3,0	2,54	input 120l raw cellu in water, 10 l cellu, 120 l waste water and 37 kg dry sludge,

				later on 30 l hydrolyzed cellulose
	R318	3,49	3,08	
SaSel RUN4	HY401	10,43	10,03	input to hydrolysis 140l raw cellulose in water, 30kg dry sludge
	HY403	13,36	12,37	
	R402	6,41	5,29	to hydrolyzed raw cellulose/sludgemass, 120l output sludge from previous run and 30 l hydrolyzed cellulose added
	R407	6,04	4,96	
	R412	6,11	5,02	
	R414	6,07	4,97	
	RA17	8,44	7,33	
	RA20	8,15	6,99	
SaSel RUN5	HY501	6,77	6,14	input to hydrolysis 200l water, 80kg dry sludge
	HY507	8,56	7,78	
	R501	1,45	0,94	in reactor 30 l hydrolyzed cellulose with bacteria
	R505	1,61	1,11	
	R507	1,37	0,92	
	R510	7,73	6,94	water/sludge from hydrolyzer to reactor
	R516	8,51	7,60	
	R521	7,95	6,90	
SaSel RUN6	H601	9,93	9,22	in homogenisator 97 kg dry sludge and water
	H605	10,30	9,57	
	HY601	10,21	9,51	
	R610	2,42	2,11	raw cellulose and hydrolyzed cellulose
	R612	8,09	7,48	sludge added
	R644	7,28	6,14	
	R648	7,83	6,70	
	R658	5,56	4,55	22l hydrolyzed cellulose and 6l of distilled water added
	R661	8,22	7,06	
	R669	8,94	7,54	
	R680	10,30	9,54	

5.4 Microbiological follow up in the Finnoflag laboratory

The composition and purity of the biorefinery process flora was continuously monitored during the runs by using either PMEU technology or direct plating. This control was

necessary for confirming the presence of strains useful for product formation in the enrichment broth.

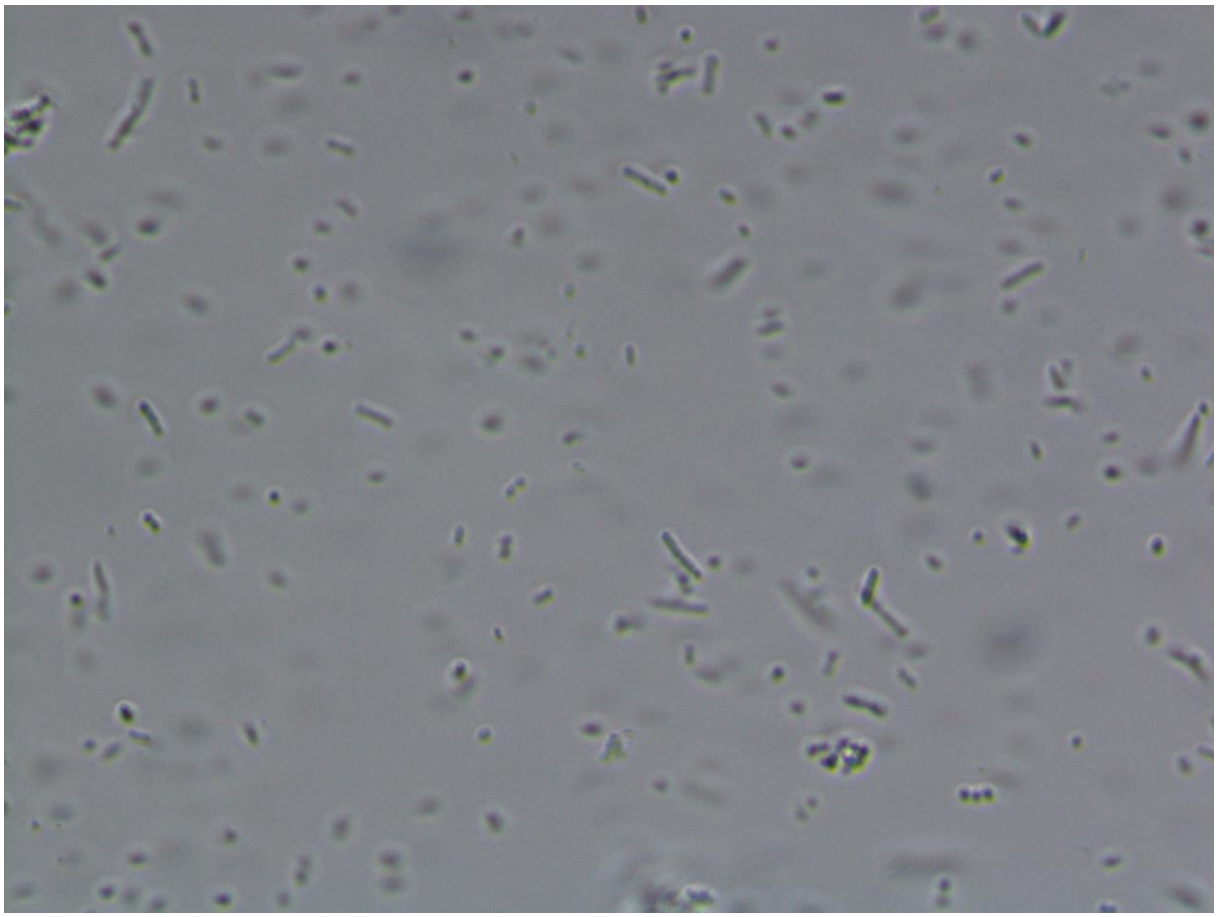

Figure 33. Microscopic image of *Klebsiella* culture in reactor during run3. The microscopic sample was collected from Chromagar plate in Finnoflag laboratory.

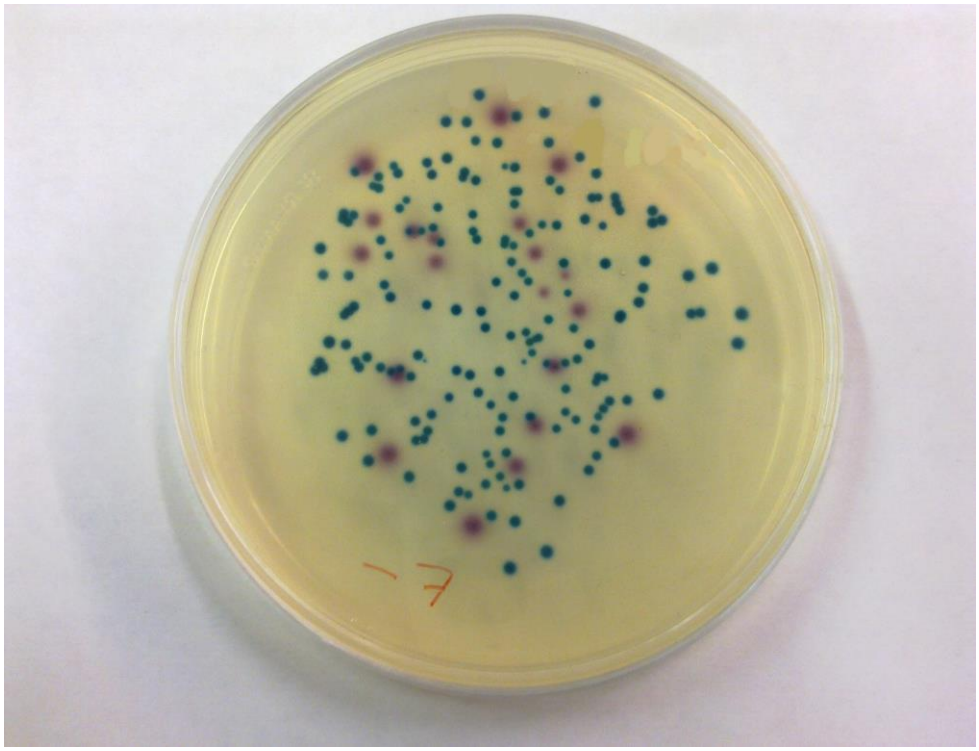

Figure 34. Colonies of *Klebsiella mobilis* (blue) and *Escherichia coli* (pink) on ChromAgar™ plates. These two members of the family *Enterobacteriaceae* have been documented to form a dualistic balance for mutual benefit (Hakalehto *et al.* 2008; Hakalehto 2012).

6. Conclusions

In the factory there are actually two separate systems, namely the pulp factory and the corrugated cartonboard machine. Waste waters to the water treatment and purification area arrive from both machines. The original raw material wood chips are treated with many chemicals during the process of their transformation into products (Figure 35).

Figure 35. End product from Powerflute Savon Sellu cartonboard factory.

Microbiologically one of the most important additions is the use of sulphuric compounds in the factory process, which eventually led to the enrichment of H_2S liberating bacteria into the gas emission flow of the Pilot A bioreactor unit when the sludge was used as raw material.

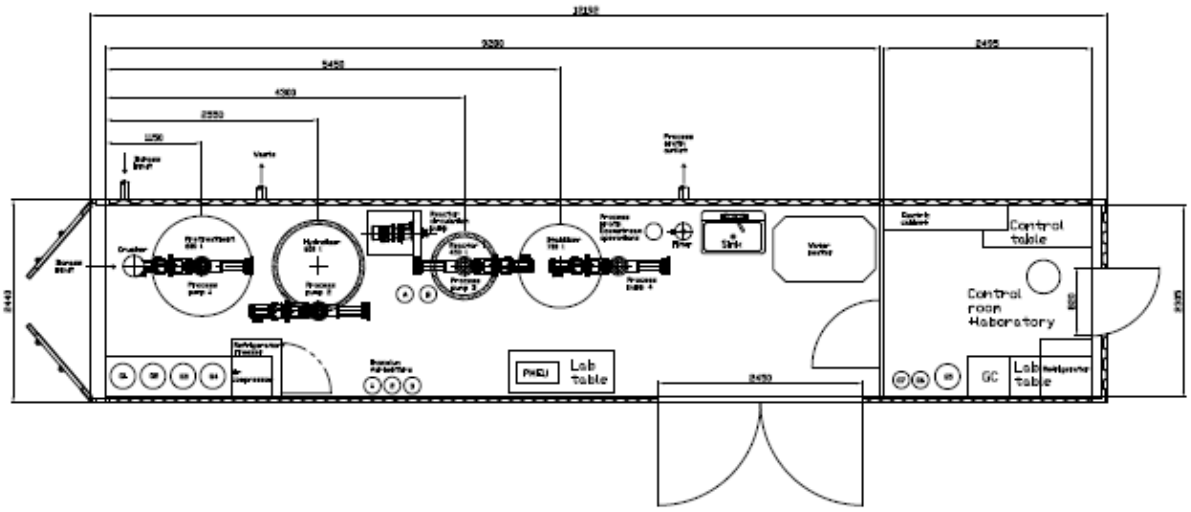

Figure 36. Lay-out of the Pilot A. Anssi Suhonen and Elias Hakalehto.

This liberation of the toxic gas flow caused some precautions, and a specific alarm system was installed. However, all gases were directed out of the pilot plant which completely prevented their accumulation inside, and thus the formation of occupational hazards. The general safety of the unit had been discussed beforehand with Regional Rescue Services of North Savo and they have been satisfied with the unit and short testing period based on discussions.

Since the H_2S formation was on a very high level in most of the process runs, the fed-batch function of the Pilot A was not possible to get tested during the short period of time at the Finnish testing site. Also, a big part of the scheduled time was needed for elaborations of the thermal regulation system of this ecologically sustainable unit.

Three out of the four main reactor tanks in the pilot were purchased as recycled industrial equipment and modified for the bioprocess use in Savonia UAS.

Even though the time window for testing was very short, promising results were obtained. The main purpose of the first tests in Finland was mainly to get familiar with the relatively complicated system of the equipment with computer control, numerous measurements, as well as thermal, pH and gas adjustments. Several sensors turned out to be useful with secondary screens in the bioreactor room itself, besides the computerized control room. The schematic presentation (lay-out) of the Pilot A is presented in Fig. 36.

In principle, as the main focus was directed to learn to run the Pilot A experimental station, the production rates were secondary in the beginning. These levels are possible to get improved in long run, during actual optimization of the process. In fact, the basic principles for steering the pilot were being learnt, and their implementation gave promises for the potential for quick optimisation of the results in future bioprocess development. In the start-up phase any result from the biological multi-variable process is giving valuable information for future trials. It was also possible to outline the potentials for future bioprocessing, as well

as to get an idea on the process improvement. Also, the national and international teams learned well to cooperate in this milieu, where biological components (biomass, microbes and enzymes) meet with metal hardware, sensors and the computerized control.

In the test runs in Savon Sellu testing site the main goal was to learn the use of the equipment and also several improvements were made according to user feedback. Further developments of the Pilot A equipment are still needed, such as the installation of pumps capable to move more concentrated biomass. On the bioprocess site, the unaceptic principle was tested, because it could at best lower the investment costs in the process to one tenth in the large units. Therefore, the natural microflora from the waste pools and the activated sludge caused several problems and produced overgrowth which almost took over the bioreactor in the beginning of the experimentation. Protocols for attenuating the background flora were attempted, but these trials were not completed during the short testing period. However, the nutrient bed approach with inoculation of the production organisms prior to the major feedstock addition seemed to be the correct way to handle with most of the problems caused by the background flora. Also, the combinations of the natural flora with some added microbial strains should be further tested in future. It would be necessary to continue the experiments with the cellulosic waste in order to demonstrate the full potential of the biorefinery principle. It could then be possible to combine the activities of the natural flora into the overall bioprocess steering. The problems with emitted H_2S can be solved by further improvement in the equipment and the process. This could make it possible to fully utilize the fed-batch application and circulation of the biomass in Pilot A. The current test was the promising start for the future implementation of the bioprocess technologies into the waste treatment.

During the short 2-3 month period of time available for the Finnish testing, it was possible to demonstrate the potential for development of the microbial processing of the dried activated sludge from the forest industry waste treatment unit. The activated sludge is as such a product from a kind of biotechnological and microbiological process, which was then further processed in the Pilot A to comprise raw material for energy and chemical production. If combined with biogas formation from the residues, the positive climatological effect and sustainability of the whole chain of treatments could be enhanced.

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