Report no: O3.6

TECHNICAL REPORT ON BIOREFINERY PILOT PLANT A OPERATION IN POLAND

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1. Objective and scope of the report

The main goal of the Abowe project was to provide proof of novel technologies for biowaste treatment. One of the tested concepts was the biorefinery technology, which was implemented on the pilot scale within the installation, referred to as "Pilot A". Verification of this technology should be done based on pilot tests in 3 regions, one of which being the Lower Silesia region in Poland. Thus the polish tests contributed to Work Package WP3 **Investment and testing of a mobile biorefinery pilot plant (Pilot A).**
This paper provides a Report on pilot plant operation in Poland. The duration of the Polish testing period was two months during which time the viability of the concept was demonstrated, and a starting point for further optimization was established.

ABOWE– Implementing Advanced Concepts for Biological Utilization of Waste, which is an extension stage project of the finalized REMOWE project (Regional Mobilization of Sustainable Waste to Energy Production).
The overall objective is to of the REMOWE project was to support reducion the negative effects of carbon dioxide emissions by finding a balance between energy consumption and the use of renewable energy sources. REMOWE project focuses on the issue of bio-energy from waste collection and on actions promoting the use of energy efficient technologies in the Baltic Sea region. ABOWE, as a direct result from REMOWE, continues to work with two promising technologies, unlocking investments with support from the Baltic Sea Region programme. Two mobile pilot plants are being built and tested in several BSR regions. Pilot plant A is based on a novel biorefinery concept from Finnoflag Oy, Finland, pilot Plant B is based on the dry fermentation process, developed by Ostfalia University in Germany. The pilots form the basis for compilation of investment memos and upcoming Investor events.
Moreover the regional model, developed in REMOWE is used, to evaluate the new processes' economic and climatic impacts in each region depicted in Figure 1.

The development of **Pilot A** is a direct continuum to REMOWE main stage Innovation processes' outcome. Plant A has been designed and built within Abowe project to allow pilot scale testing of a novel biorefinery process proposed by Dr. Elias Hakalehto, Finnoflag Oy. This was evaluated in REMOWE to have potential as a novel technology for organic recycling of biowaste in the BSR. In the start-up phase of Pilot A the process was tested in Finland with forest industry waste water sludge of Savon Sellu Oy/Powerflute Oy.
In the next step the pilot plant was transferred to Poland where the purpose was to test it especially with waste from a potato chip factory. The second intended substrate for the Polish testing was separatlly collected biowaste from a commercial kitchen (restaurant waste). The pilot A was operated in premises of Regional Waste Management Company ZGO Gac Ltd. Testing period of this new process in Poland corresponds well with manifold investment in the area of waste treatment which are under way at the moment in Poland. This provided good opportunity to promote future investments in this kind of advanded biowaste recycling technology in the Polish market.

For the last testing phase Pilot A was transported to Sweden, to the chicken farm Hagby Gårdsfågel AB, where the intention was to apply the technology for the agricultural residues (especially chicken slaughterhouse waste).

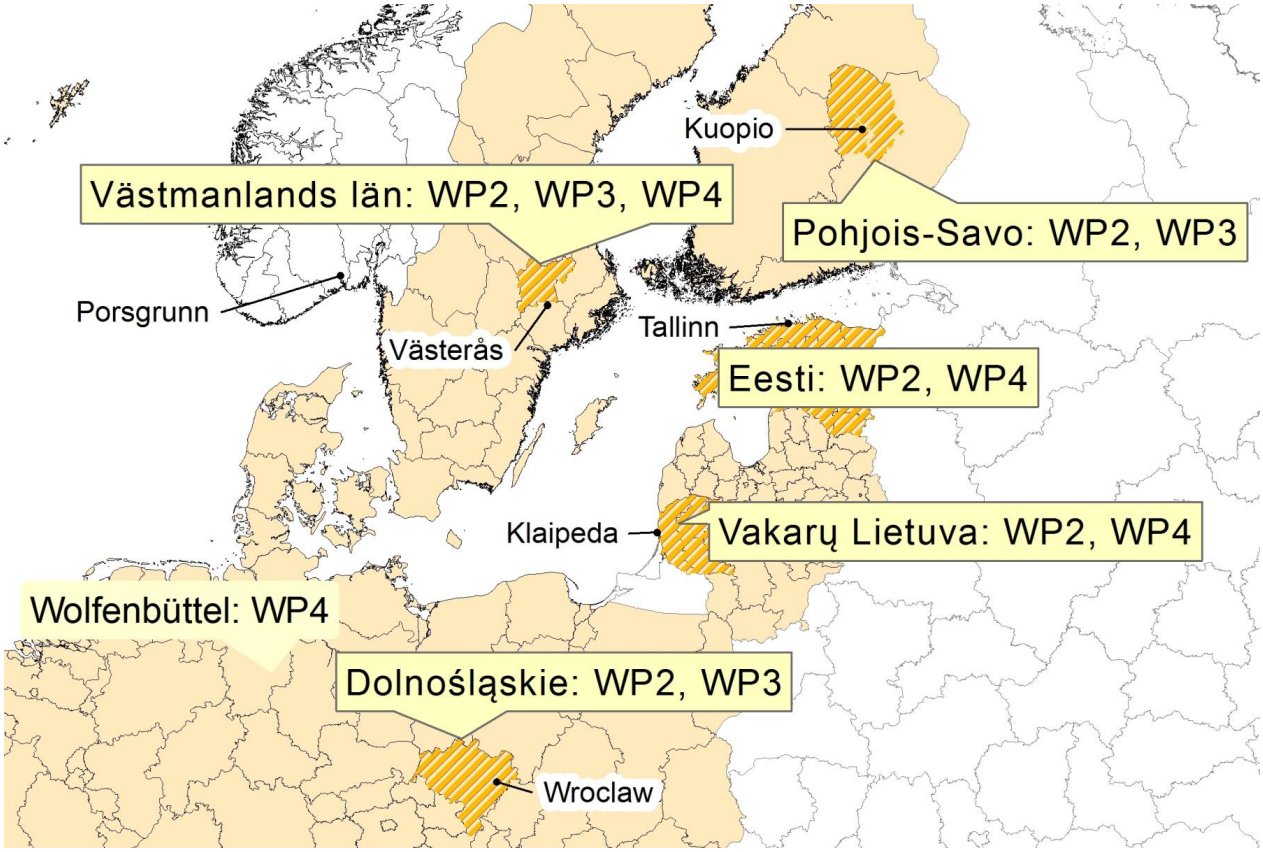

Figure 1 The regions of the ABOWE project

Thus the goals of this report are to provide proof of biorefinery technology for potato/kitchen biowaste. The results should encourage investments in this kind of technologies in the future. The biorefinery pilot plant facilitates allow transferring knowledge of bioprocessing various waste biomasses into valuable material and energy products. Ultimate aim is to provide Proof of technology and values for economical calculations, both needed in compiling Investment memos for Investor events.

Biorefinery processes offer a clear advantage over other traditional waste treatment technologies in terms of useful products which can be obtained. The biorefinery process' novelty lies also in the improved productivity of biomass contained, in case of the ABOWE project, in waste.

The biorefinery concept principles:
Inputs are waste biomass from food industry and pulp industry, such as potato, whey and wastes from chemical pulp production. Within the treatment process the following major steps take place:

- Pre-treatment including establishing appropriate solids content, pH adjustment and other physical-chemical optimizing of the fed-in material, leading to the hydrolysis of the input material;

- Main biological process with the application of selected microorganism. Biochemical routes that the process utilises are essentially 2,3-butanediol-fermentation and acetone-butanol fermentation (ABE) as well as methane fermentation;
- Separation of process outputs, which include e.g. butanediol, butyric acid, propionic acid, ethanol, acetone, hydrogen. All these are raw materials for chemical industry and can be used as alternative fuels.

Products from this type of biorefinery are bulk chemicals, biomaterials and energy products (Hakalehto et. al. 2013). The main goal in the Polish tests was the production of 2,3-butanediol (23BD).
23BD is a commodity chemical usually produced from oil. It can be used as a precursor in the manufacture of a range of chemical products, including the solvents methyl ethyl ketone (MEK), gamma-butyrolactone (GBL), and 1,3-butadiene (Köpke et al. 2011, Celińska and Grayek 2009, Voloch et al. 1985, Xiu and Zeng 2008). Commercially, the key downstream products of 23BD have a potential global market of around 32 million tons per annum, valued at approximately $43 billion in sales. 23 BD is one of the most important "platform chemicals" for the bio-based industries.

Butanediol production takes place as butanediol fermentation by microbes. 23BD is known to be produced by a range of sugar (or citrate)-fermenting microbes, including *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, and *Clostridium ragsdalei* (Köpke et al. 2011), *Bacillus amyloliquefaciens* (Celińska and Grayek 2009), *Bacillus subtilis* (Xiu and Zeng 2008), *Enterobacter aerogenes* (Byun et al. 1994), *Klebsiella pneumoniae* (Bieblcet et al. 1998), *Klebsiella oxytoca* (Syu 2001), *Lactococcus lactis* (Hugenholtz and Starrenburg 1992), *Paenibacillus polymyxa* (De Mas et al. 1988), and *Serratia marcescens* (Zhang et al. 2010).
For example, from 2,3-butanediole it is possible to produce 1,3-butadiene, which is a raw material for synthetic rubber, plastic monomers and industrial fibers. 23BD can be used also as anti-icing agent (e.g. in airports), for instance.

Another example of useful microbe fermentations is acetone-butanol fermentation, producing butanol, ethanol, acetone and hydrogen. Butanol is almost as such suitable to cars instead of gasoline and is an important industrial chemical as like acetone. Ethanol can be utilized in internal combustion engines.

There is a growing tendency to perceive waste as raw materials for various industries, as they can be converted to various forms of valuable material and energy products. The bioprocess can deliver these kind of products, utilizing different kinds of waste. This is in accordance with the principles of sustainable development and the European energy and waste management policy.

The bioprocess which was verified in the Pilot A had been previously tested in laboratory scale in volumes from 1 L to 15 L. Within Pilot A testing phase the volume of input materials was scaled up to 200 L or more, which amount is big enough for scientific and to give technical and economical proof but still does not require too much investment or instrumentation.
The reactor type is fed-batch which means semi-continuous process in which substrate is added in intervals. This approach is popular in the bioindustries. Many fermentation products are results from the secondary metabolism, which is switched on after some more

easily metabolizable substrates have been exhausted from the batch. Each production run lasted 2-3 days.
Milestones and outputs achieved:
M3.8 Pilot plant transported to testing region in Poland and taken into operation
M3.9 Testing in Poland finalized
O3.6 Report on pilot plant operation in Poland to be part of WP2 Investment Memo for Polish Investment Event.

2. Experimental setup

The tests described within this report ware performed in the Pilot A, which is a pilot scale installation implementing the concept of biorefinery process. In the following some characteristics of the input materials are provided as well as the experimental setup (step-by-step procedures) and the main test parameters of the process.

2.1 Location and time of testing

The Pilot A has been located on the premises of Waste Treatment Plant ZGO, in the village of Gac. It was settled on a sealed surface of the composting/stabilization area. Electricity and water was provided by the plant infrastructure, while wastewater was collected and discharged (after hygienisation at high temperature) into plant wastewater tank. The testing site has been selected within the project planning stage, due to its advantegous location in the vicinity of potato chips plant.
The testing period lasted from 5th of May 2014 (transfer from Finland, see Figure 2) to 18th of July 2014 (transfer to th following testing site in Sweden).

Figure 2 Transfer of Pilot A to ZGO Gać

2.2 Input materials

For the biorefinery process tested within Pilot A various biodegradable waste can be considerd as starting raw material, including waste and by-products of food industry, waste management centers, sewage sludge treatment plants, large food markets, etc.
Feedstock needs for the Pilot A in the Polish tests were predefined within the REMOWE project. These were potato waste in the first trials, enriched with the municipal, separatelly collected biowaste in the further trials.

Potato waste

Potato waste was selected as the main input stream due to vast availability of these biowaste originating fram a chips and snacks producing factory, located in the vicinity of the testing site (Stanowice, near to Olawa). Potato waste was the single input stream in five out of eight tests performed (runs 7 - 11).
The waste stream selected for the experiments were waste potato peels, which are normally utilized in an anaerobic digester (biogas reactor). This waste stream comes directly from the peeling line of the prewashed potatos Thus, it contains very limited quantity of mineral contamination, which is beneficial for the biorefinery process. The waste was delivered to the testing site in barrels (see Figure 3).

Figure 3 Potato waste used for testing of biorefinery technology in Pilot A

Potato waste was collected freshly from the chips plant. Characteristics of the potato waste stream can be found in Table 1. It can be seen that the potato waste, despite its bulk consistency could be characterized by very high water content (over 82% w/w). The size of particles was relatively small and homogenous, so it did not necessitate any further mechanical crushing. Also the organics content as well as some nutrients concentrations (Nitrogen and Phosphorus) render waste suitable for the biochemical process, although Nitrogen levels were not as high as for the restaurant waste. Also the Calcium level in potato waste was relatively low, whereas a relatively high Potassium content was measured. The levels of contaminants (heavy metals) were quite limited in both substrates.

Table 1. Characteristics of waste input meatrials to Pilot A (average)

Parameter	Unit	Potato waste	Kitchen waste
Water content	%	82,34 (±2,31)	85,99
Ignition loss (organic matter content)	% d.m.	96,96 (±3,59)	81,945
AT4	mg O2/g d.m.	156 (±10,39)	189
Total Phosphorus	% d.m.	0,14 (±0,03)	0,09
Total Nitrogen	% d.m.	0,52 (±0,11)	1,08
total K	% d.m.	1,39 (±0,24)	0,78
Ca	% d.m.	0,28	4,60
Mg	% d.m.	0,09	0,14
Cd	mg/kg d.m.	0	0
Cu	mg/kg d.m.	15,0	24,4
Zn	mg/kg d.m.	83,3	166,8
Ni	mg/kg d.m.	0	0
Pb	mg/kg d.m.	0	0

Kitchen biowaste

In the subsequent tests (runs 12-14) the second stream was included. It consisted of separately colleted kitchen waste from a local restaurant in Wrocław (Figure 4).
The waste was collected by the kitchen personnel and stored in a barrel for a few days (usually for 5 days), so at the moment of delivering it to Pilot A it contained a mixture of very fresh and slightly older biomass. It was composed of various ingredients, including mostly vegetable residues, both fresh and boiled, as well as residues of soups, some meat, fruits, fats etc. Due to some larger particles and especially larger bones present in this waste stream a manual check of it was needed (mainly removal of bones) and mechanical crushing. Characteristics of the kitchen waste is provided in Table 1.

Figure 4 Kitchen biowaste used for Pilot A tests

2.3 Microorganisms

The micro-organism for the test runs were delivered by Finnoflag Oy, and included two species: *Klebsiella mobilis* ATCC 13048 and *Escherichia coli* E 17. The microbes were cultivated and stored on ChromAgar plates. The growth medium used to enhance bacterial growth enhanced before feeding ihe inocula to the reactor was TYG: trypton, yeast extract and glucose medium.

2.4 Experimental setup

The experiments were comprised of the following steps:

1. Potato waste was fed to the pretreatment reactor to adjust the solids content to 10% dry mass. The 10% ratio was aimed at, however due to consistency of the potato waste, it was pumpable only at lower solids content of app. 5-7%.
2. The mass was fed to the hydrolyser, in which the temperature was to be risen to over 80°C in order to achieve pasteurization in the initial waste mass, which contained some inherent microorganisms. After that material has been cooled down to 65°C, pH adjusted to 5,5 and enzymes added to enhance hydrolysation step.
3. Transfer of the hydrolysed mass to the main bioreactor. The transfer of the mass occurred normally app. 24 hours after start of an experiment. Before transferring the mass to the bioreactor, pH was adjusted to 6,5 and temperature to 37°C. The mass transfer occurred either at once (runs 7 and 8) or in portions, starting with 100 dm^3 and continuing with the residual mass at the later stages of the process.
4. Transfer of microbial inoculum to the bioreactor. The microbes were cultivated in the TYG broth. After initial innoculation, the samples were incubated for 6-8 hours in in the Portable Microbe Enrichment Unit (PMEU) (Hakalehto & Heitto 2012). In each of the test at least 200 ml of *Klebsiella* inoculum and 200 ml of *E. coli* inoculum was incubated in the PMEU. From there the innoculum was transferred to three seed fermenters, where the inoculum was further incubated in a larger volume. Each of the fermenters contained 7 litres of TYG broth. Temperature was controlled at 37°C. For optimal growth, the fermenters were constantly aerated, at the flow rate of app. 1 l/minute. *Klebsiella* was inoculated into 2 fermenters and *E. coli* into the third one. After moving the hydrolysed biowaste mass to the bioreactor first the contents of two fermenters with *Klebsiella* culture were moved to bioreactor. The content of the third fermenter with *E. coli* was transfered at the later stage, letting sufficient time to the *Klebsiella* to populate the bioreactor.
5. Process control. After transferring the innoculum to the bioreactor, process parameters were configurated and controlled. Mixing of the reactor mass was ensured by the recirculation pump as well as by the aerating gases. Some other parameters were controlled automatically, by the computer based Process Control, while some parameters had to be adjusted manually, based on the measurement information. The main parameters to be controlled included:
    a. temperature (controlled automatically)
    b. pH value (controlled by the operator, with the use of the base/acid solution pumps)

c. glucose level (controlled by the operator, with the use of manual glucose meter, Glucose meter On Call Vivid)
d. dissolved oxygen content, measeured by Luminescent Dissolved Oxygen (LDO) sensor (controlled by the operator by adjusting the aeration rates by air, N2 or CO2)
e. composition of the outflow gases (controlled by the operator, with help of the Fresenius gas meter, providing O2, CO2, CH4 and H2S concentrations)

6. Sampling of the bioreactor output has been performed by the plant oparators every hour after the inoculation of the bioreactor. Samples taken from the reactor were analysed by manual pH sensor and glucose meter, as well as the Gas Chromatography calibrated to measure the concentrations of acetone, ethanol, propanol, butanol, acetic acid, propionic acid, butyric acid and 2,3-butanediol.
7. Process closure took place after the level of glucose fall below detection limits and no further production of 2,3-butanediol was expected. The mass was moved back to the hydrolyser for the hygienisation before being discharged.

2.5 Final products

Below the picture of final product obtained in biorefinery process is presented. The samples were stored at temp. 4 deg.C. the preparation and analyses with gas chromatography took place directly at Pilot A (Figure 6). The analyses were performed with Agilent 7820A GC.

Figure 5 Final product fluids/mixtures of biorefinery process stored for further analyses

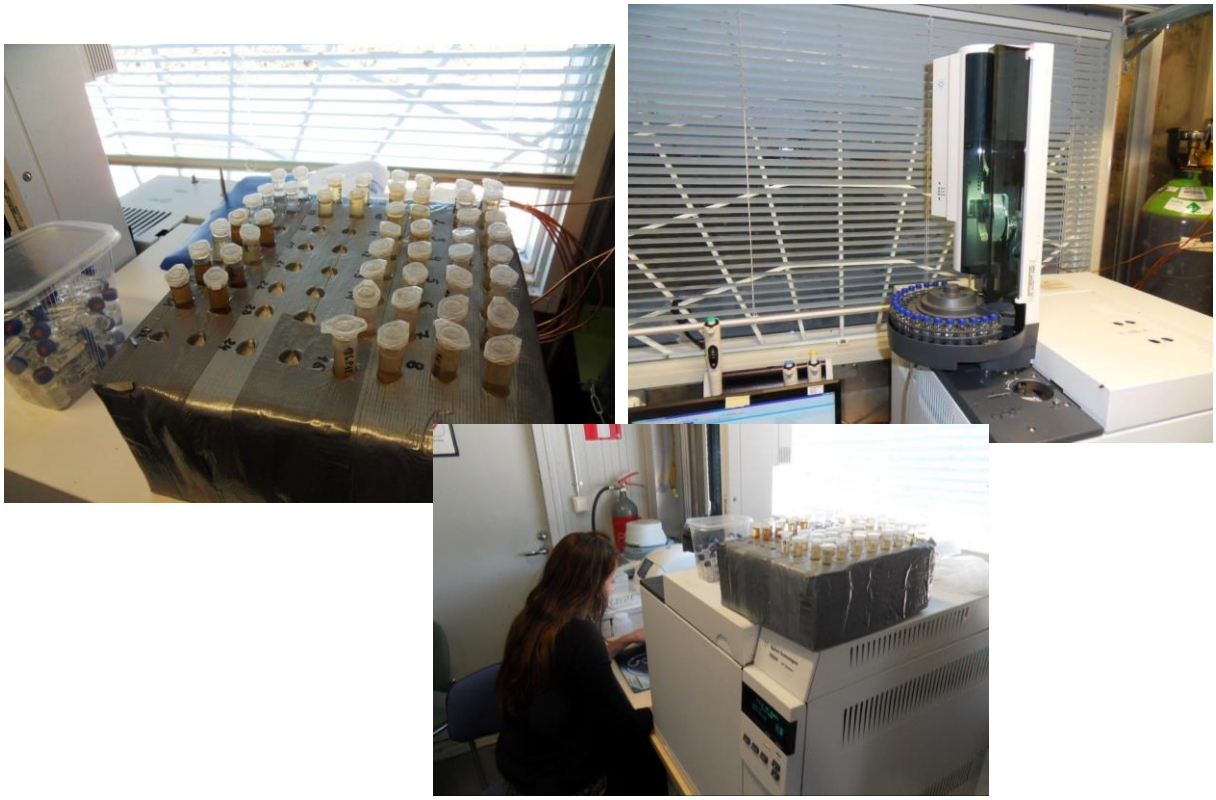

Figure 6. Gas chromatography analyses were performed in the small laboratory of Pilot A

3. Results

Within the testing periods eight full process runs were performed, which are referred to as runs 7P to 14P, where the number states for the consecutive run since the construction of Pilot A and "P" for Poland.
In the following the results of these runs are presented. The data related to process parameters such as pH and LDO, as well as to the composition of gases generated during each run comes from the datasets of the process control system.

3.1 Run 7P

Input in the Run P7 was potato waste. Two barrels of potato waste was fed into the reactor. It was then filled with water to the total volume of app. 200 l. The dry mass content was app. 6,2 % d.m., which was necessary to bring waste into pumpable state. Figure 7 shows the values of main parameters which were used to control the process. The reactor was aerated with pressured air which allowed mixing of the reactor content as well as to maintain aerobic conditions (LDO at the level of app. 1 mgO2/dm3). pH value was controlled by adding NaOH ocassionslly to keep the pH of 6,5. Additionally, glucose was measured every hour by manual glucometer, in order to control the level of readily available hydrocarbons for the microorganisms.
The gaseous products of the process are shown in Figure 8. The maximum level of CO2 reached app. 5%. At the same time the production of H2 reached the maximum level of app. 7500 ppm.

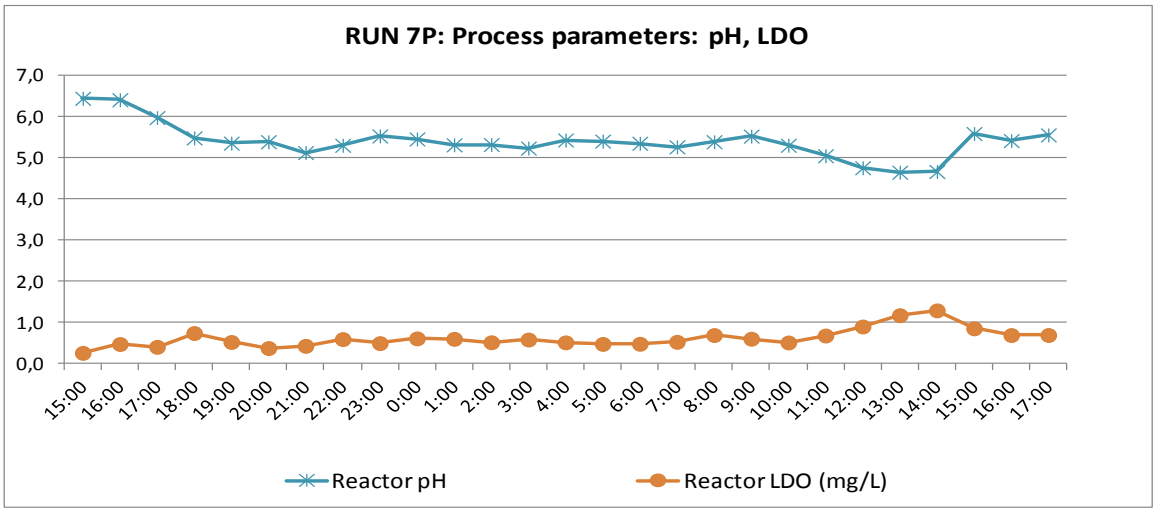

Figure 7. Process parameters run RUN 7P

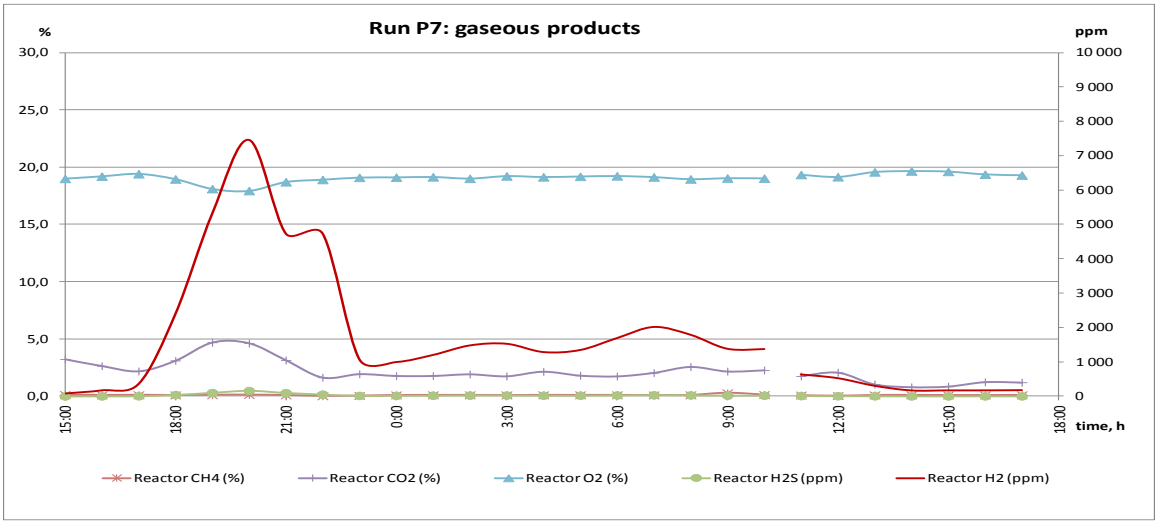

Figure 8. Gaseous products of run P7

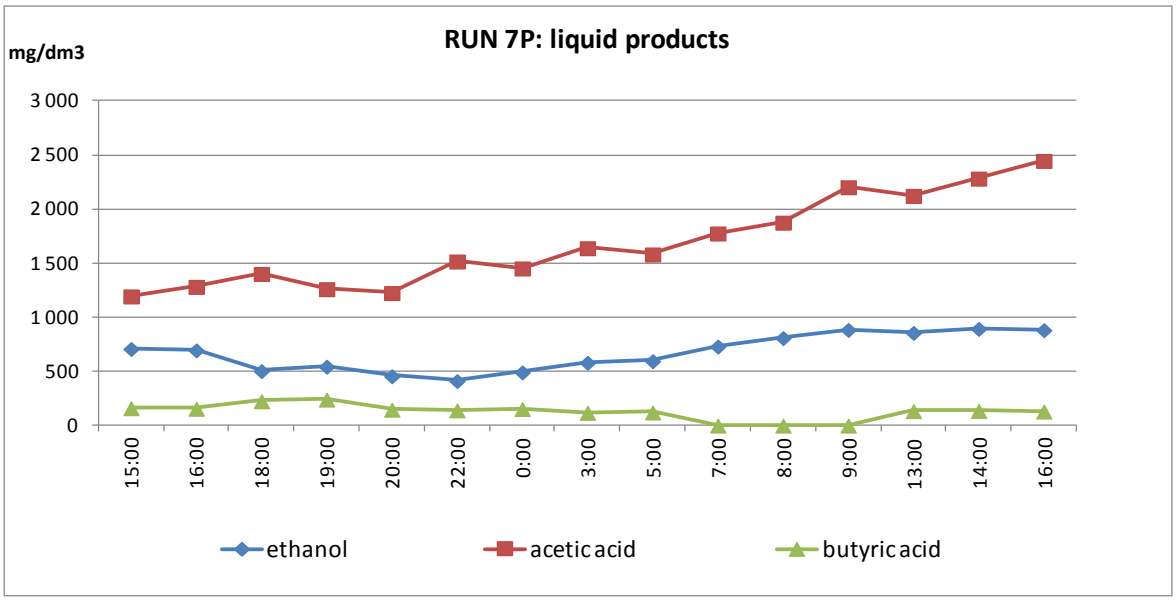

Figure 9. Liquid products of run 7P

After feeding the reactor with potato waste and the inoculum, sampling of liquid product war perfomed every full hour. Liquid samples were prepared for further analyses by double centrifugation. The solution was directly analysed with gas chromatography (GC). Results obtained from these analyses are presented in Figure 9. The main products were acetic acid, ethanol and smaller amounts of butyric acid. It can be seen the the levels of these substances continued growing during the process. The maximum level of acetic acid was app. 2500 mg/dm^3 and of ethanol app. 1000 mg/dm^3. The level of glucose dropped below detection limit on at app. 15:00 of the second day of the process run. Since no other source of carbon was available, it was decided to stop the process.

3.2 Run 8P

Run 8P was also performed with potato waste. The mass was added in two portions as shown in Figure 10. This time very low aeration rate was performed to allow lower LDO values (app. 0,25 mg O_2/dm^3).
Figure 11 shows the gaseous products of this process run. It can be seen that despite low aeration rate the oxygen level in the outflowing air stayed at relatively high level during the whole process (between 15 and 20%). The level of hydrogen reached app. 4500 ppm at the time of second feeding with the biomass. It can be seen that during the second day the level of CO2 increased significantly, reaching app. 17%. From Figure 12 it can be seen that the elevated levels of CO2 coincided with the highest production of acetic acid and ethanol (app. 4000 mg/dm^3 and 2800 mg/dm^3, respectively).
The glucose level was controlled during the process run. Initially high values were maintained. However on the second day at app. 9:00 the glucose level fell below the detection limit. To maintain the process at active metabolic state 10l of sugar water was added to the reactor at 15:00 on the second day of process run.

It can be seen that after adding sugar the level of products grew significantly.

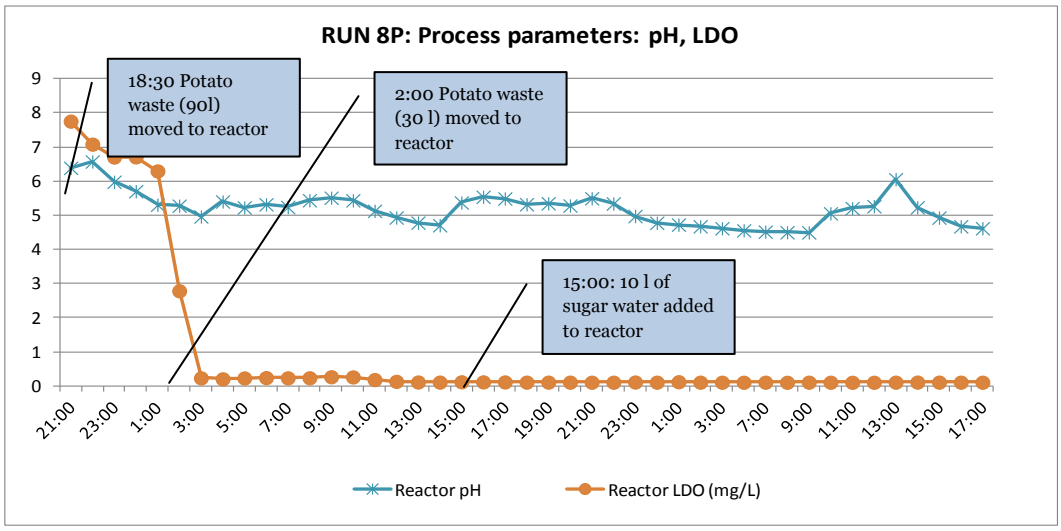

Figure 10. Process parameters run RUN 8P

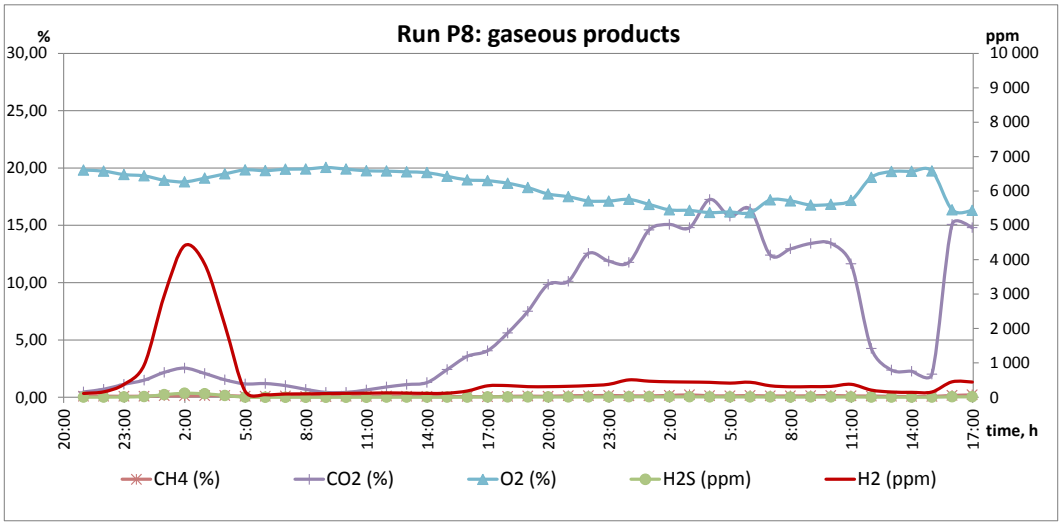

Figure 11. Gaseous products of run P7

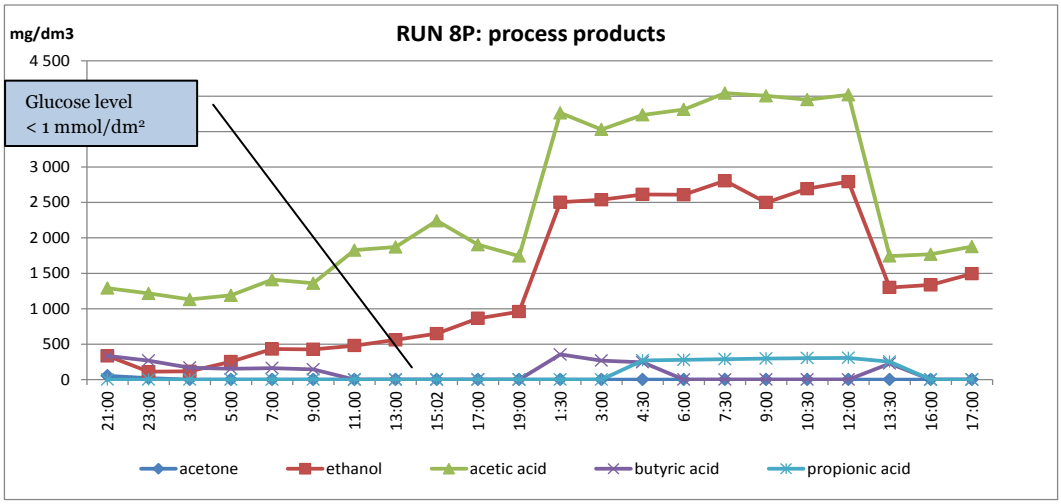

Figure 12. Liquid products of run 8P

3.3 Run 9P

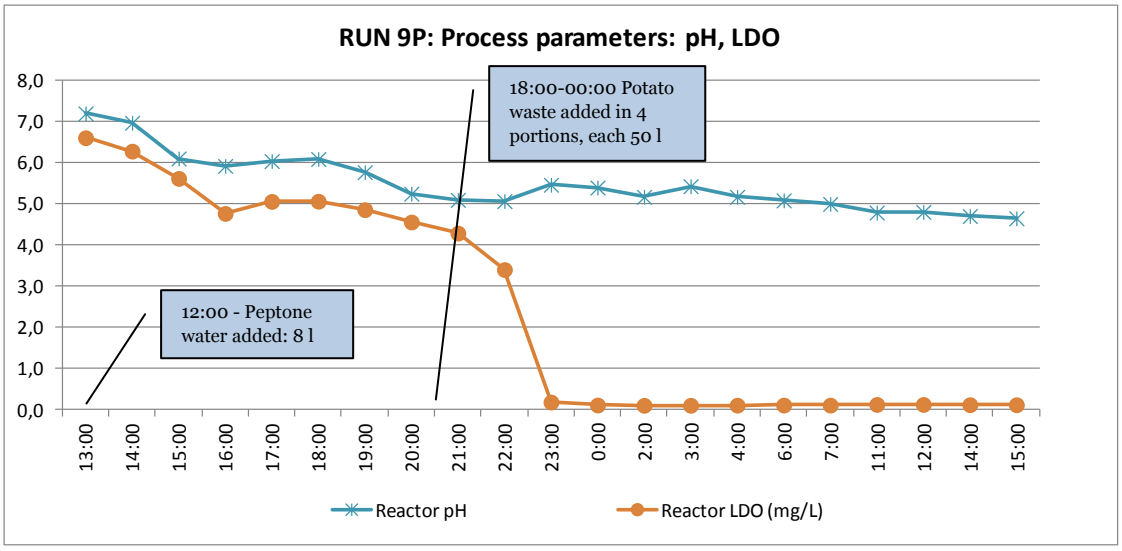

Figure 13. Process parameters run RUN 9P

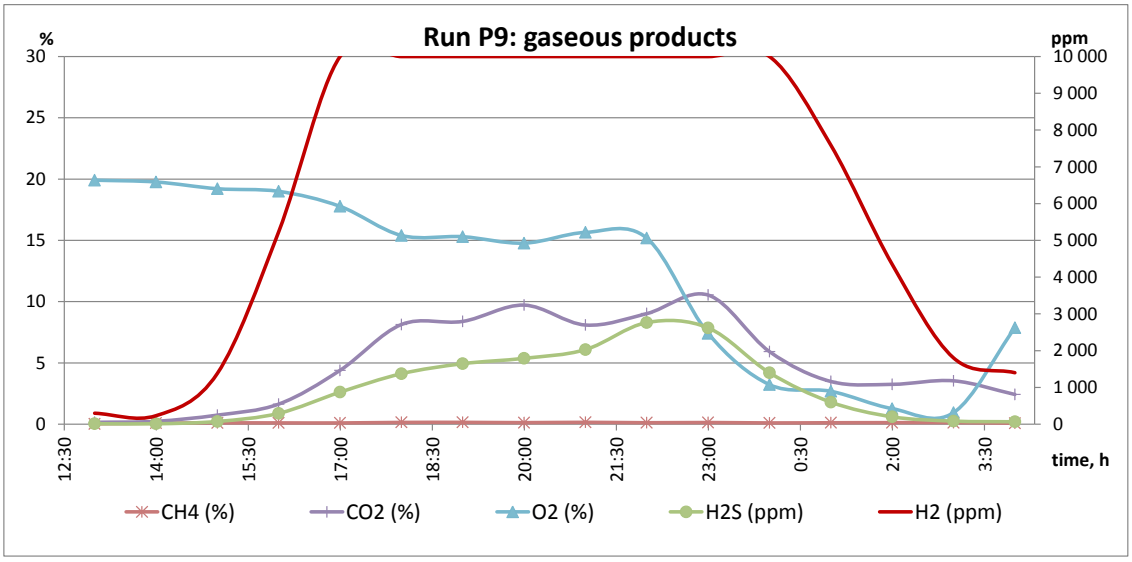

Figure 14. Gaseous products of run 9P

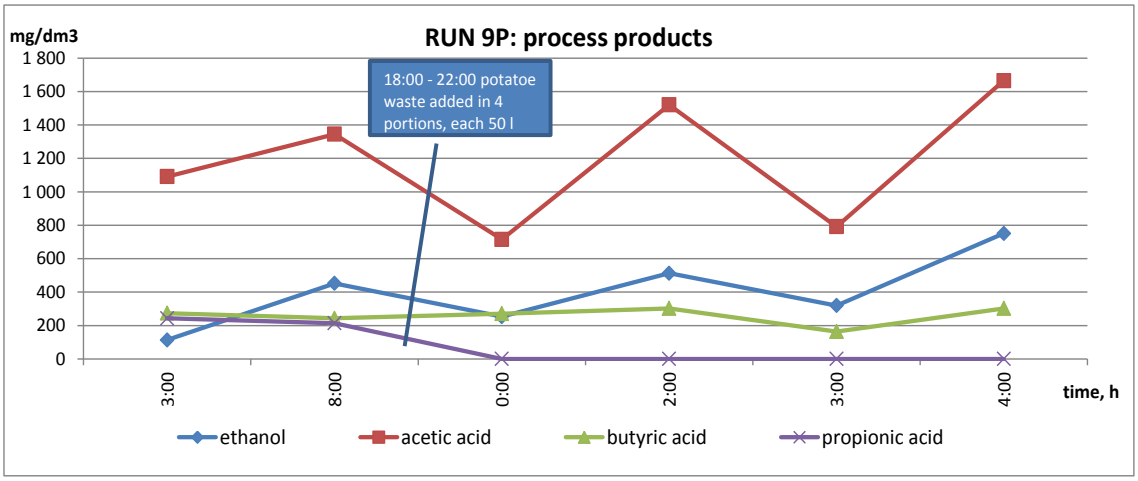

Figure 15. Liquid products of run 9P

Within run 9P, potato waste was added to the reactor in 4 equal portions. Apart from potato waste peptone water was added at the beginning of the process (app. 8 l). The process was kept anaerobic by stirring the process broth with Nitrogen instead of pressured air. From Figure 13 and Figure 14 it can be seen that after app. 14:00 pm the conditions became strongly anaerobic. It can be seen by low LOD value, as well as the composition of produced gases. Hydrogen amounts exceeded the detection level of 10 000 ppm and as it can be seen in Figure 14 the actual amount was significantly higher. A the end of the process the oxygen level had fallen to almost zero. CO_2 level remained also quite low (below 5%). Samples of liquid effluent were collected and analysed with GC. In Figure 15 the results are presented. Acetic acid was again the dominating product, reaching the level of 1600 mg/dm3. However, this time also the propionic acid and butyric acids were generated. The process had stopped after the glucose level dropped below 1 mmol/dm3. Figure 16 shows cumulative air flow within run 9P (app. 1400 l).

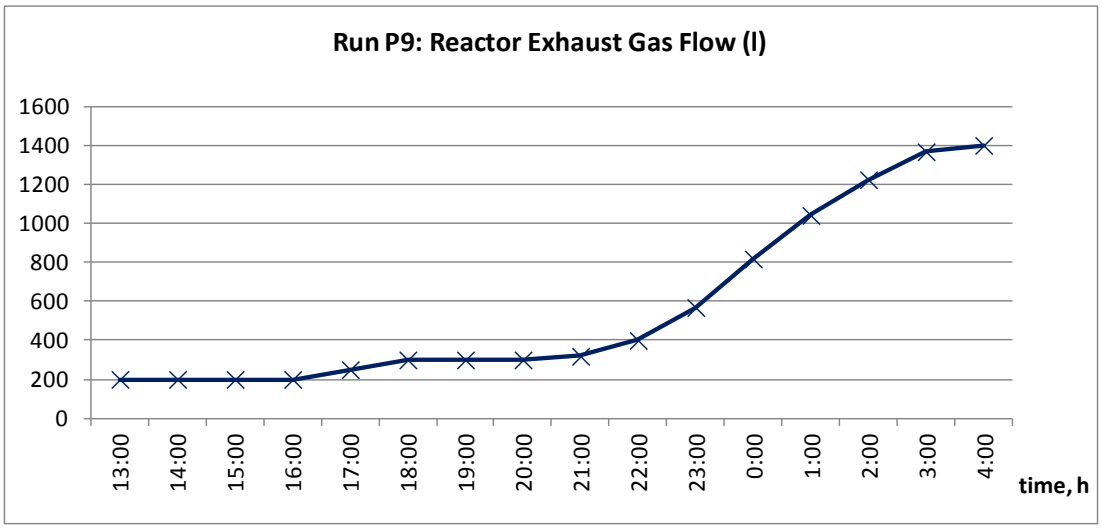

Figure 16. Cummulative curve of gas flow i run 8P

3.4 Run 10P

Within run 10P, the process was run anaerobically as well (mixing with Nitrogen). It can be seen from the composition of gases that strongly anaerobic conditions were developed in the reactor. Generation of Hydrogen reached high level again, exceeding 10000 ppm. However, for some time due to technical problems the process could not be controlled. This time also relatively high concentration of CO2 was observed, exceeding 30%. Acetic acid and ethanol were again main products, however the amounts produced were higher than before and reached app. 3000 mg/dm^3 for acetic acid and even 3500 mg/dm^3 for ethanol. The butyric acid was produced at relatively constant level of app. 400 mg/l. Moreover, for the first time a significant amount of propionic acid was generated reaching 3000 mg/dm3. The pick corresponed with the pick of acetic acid. The glucose level remained high for a long time, which means that the actual consumption was small. Normally the anaerobic processes are considered as slower than the aerobic ones. The leachate from sedimentation tank was added as additional source of carbon and nitrogen. This resulted in more anaerobic contions (H2 and H2S generation).

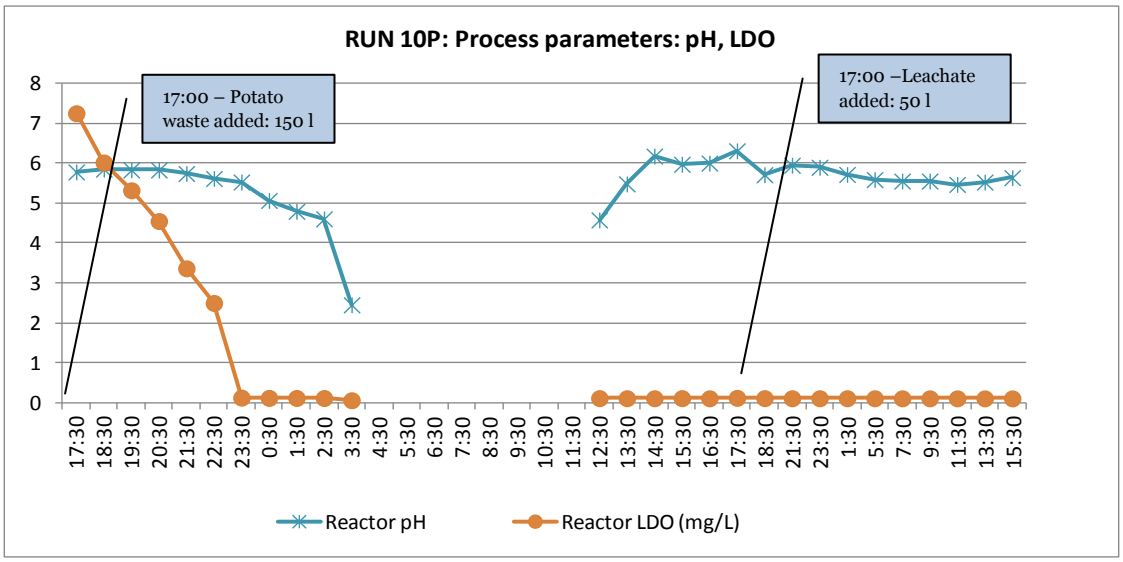

Figure 17. Process parameters run RUN 10P

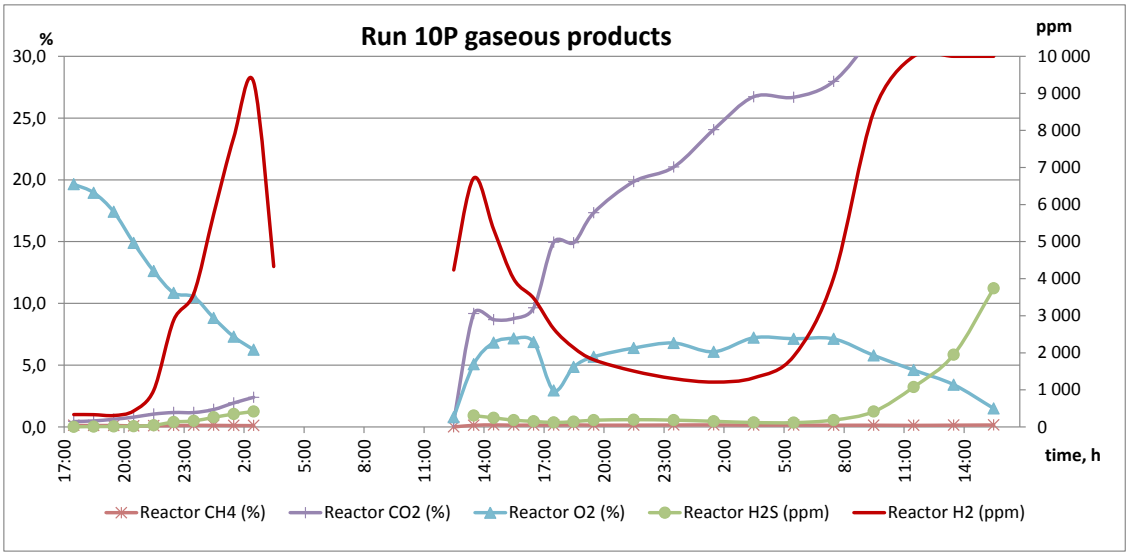

Figure 18. Gaseous products of run 10P

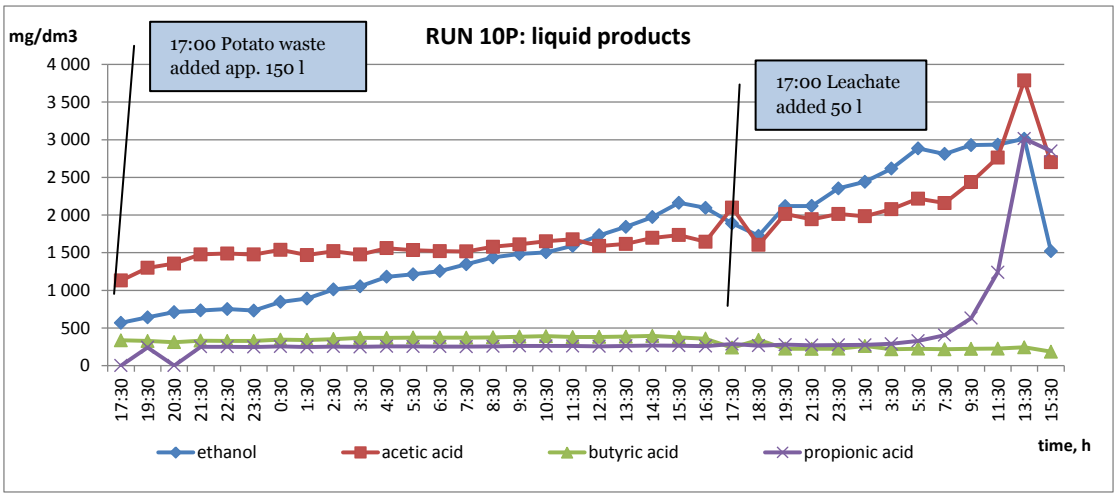

Figure 19. Liquid products of run 10P

The cumulative gas flow during process 10P has reached app. 2500 l.

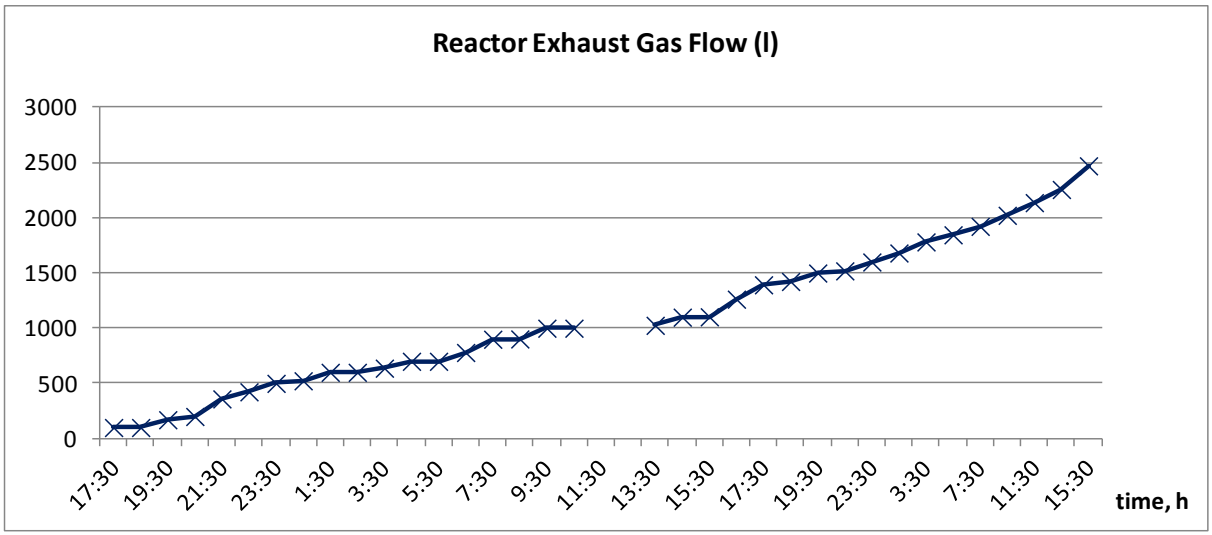

Figure 20. Cummulative curve of gas flow i run 8P

3.5 Run 11P

Run 11 P was performed with potato waste, which was added in small quantities to maintain high glucose level during the whole process. The conditions were kept anaerobic, using stirring with CO2. During this run butyric acid was produced as in run 10P. It quantity was corresponding to the quantity of ethanol. At the end of the process also some propionic acid was generated. Composition of gases indicated strongly anaerobic conditions with elevated amounts of H_2 and H_2S.

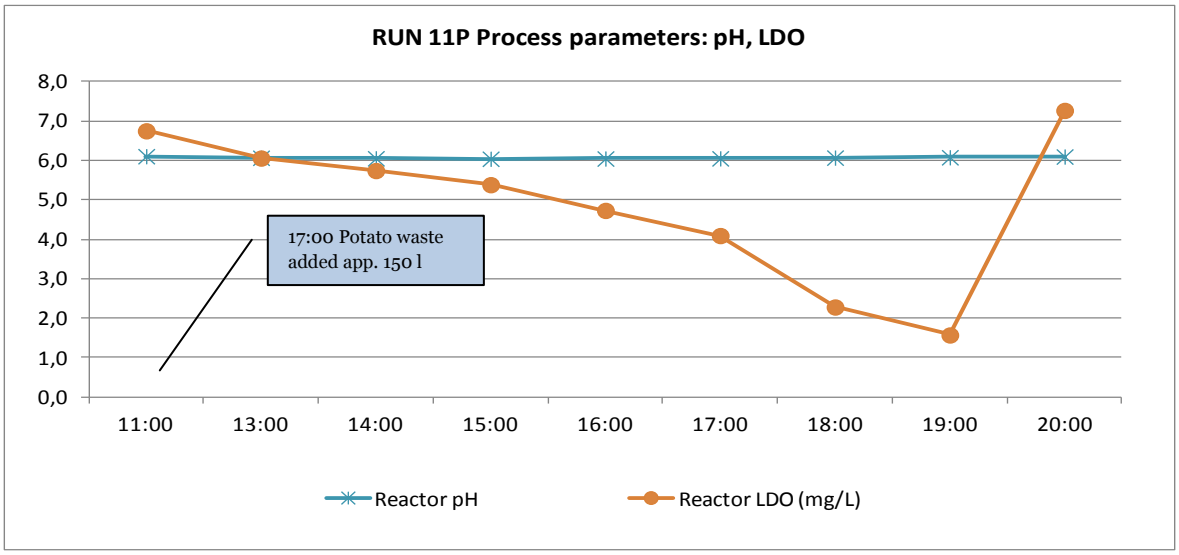

Figure 21. Process parameters run RUN 11P

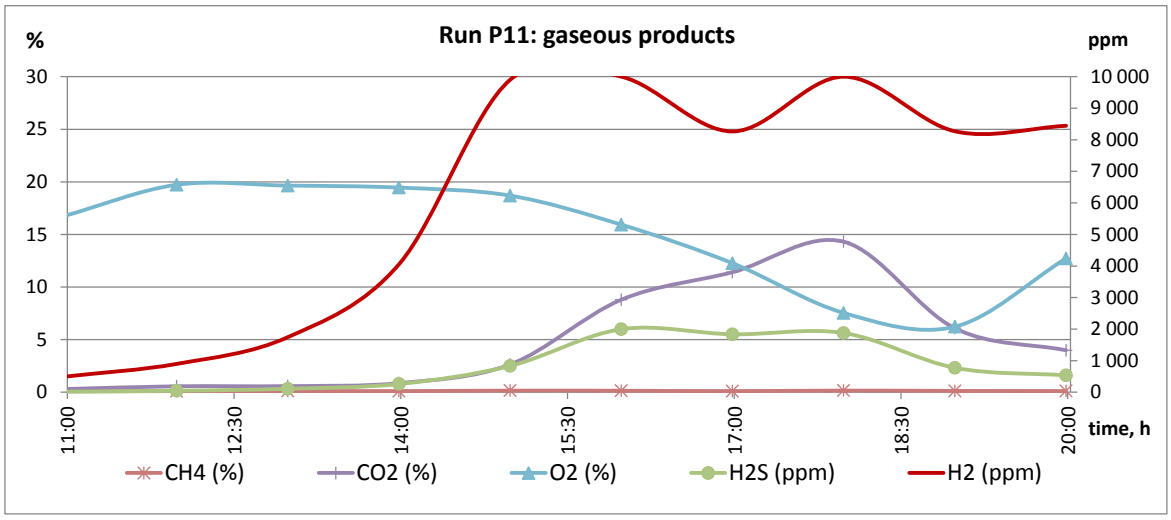

Figure 22. Gaseous products of run 11P

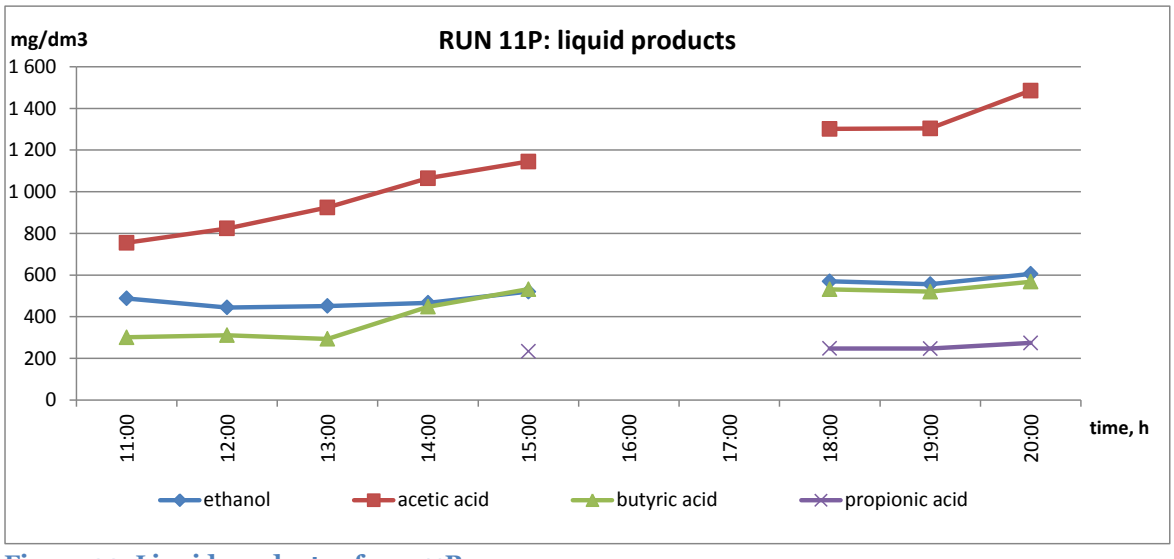

Figure 23. Liquid products of run 11P

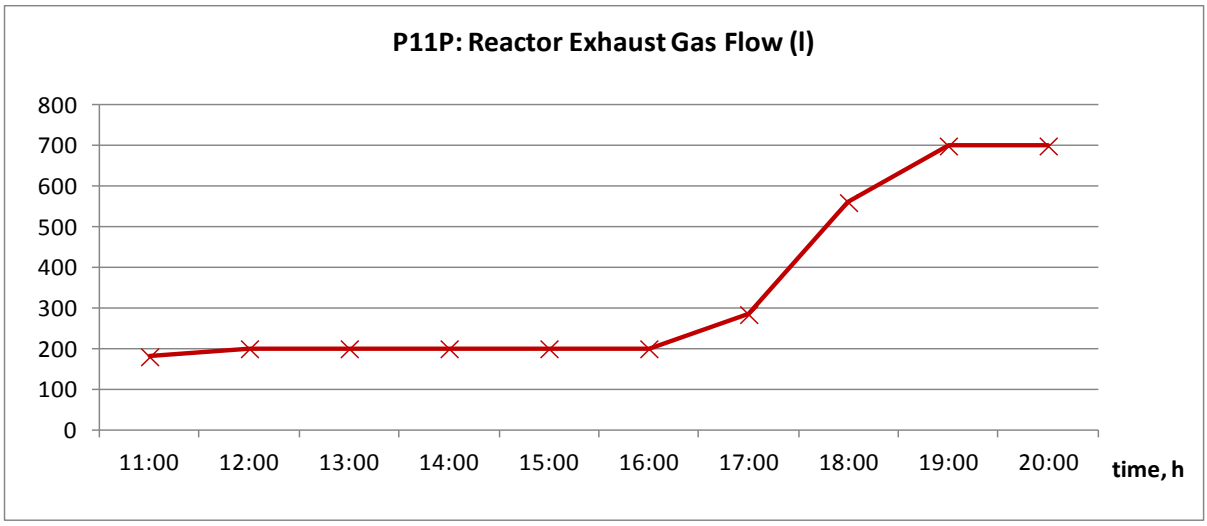

Figure 24. Cummulative curve of gas flow i run 11P

3.6 Run 12P

During run 12P a significant change took place. It was decided that since the results of the previous runs were not as good as expected in terms of the final products (especially 2,3-butanediol production), there must be some important microelements lacking in the potato waste. It was therefore decided to try first another biowaste type. Therefore for this and the following run biowaste from restaurants were collected and used as initial cosubstrate. It was typical food waste, a mixture of vegetables, residues of meals, such as soups, meat, salads, etc. App. 100 l of shredded restaurant biowaste was added to the reactor within run 12P. Run 12P was performed at aerobic conditions, as the stirring with compressed air was applied. It can be recognized from Figure 26 that the composition of gases was different – there was more oxygen present and the level of H2 and H2S was kept low. Looking at Figure 27 it can be again seen that the dominating products were ethanol, reaching the level of 3000 mg/dm3 and acetic acid (up to 4500 mg/dm3). Unfortunatelly the process was significantly influenced by the collapse of control system. Between 0:00 and 6:00 the control system was down, therefore the data for this period is lacking. It led to overheating of the substrate and there was no sence to continue the process any longer. Just before the end of the process, municipal waste leachate (rich in ammonia and organics) from leachate tank at ZGO Gać was added to test its effect on the process. It can be seen that after adding the leachate from the municipal waste treatment plant the quantity of products increased. This can be explained by overcoming the limiting factor being possibly Nitrogen content in potato waste. Leachate is rich in Nitrogen and therefore could compensate the lacking amount. It can be seen from Figure 28 that this time the amount of air flowing through the system was higher.

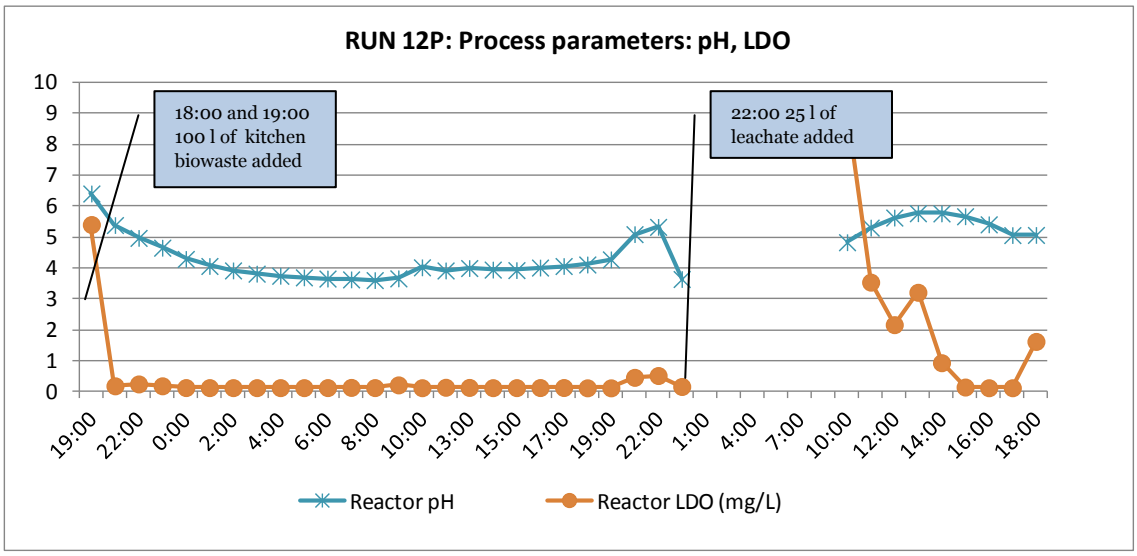

Figure 25. Process parameters run RUN 12P

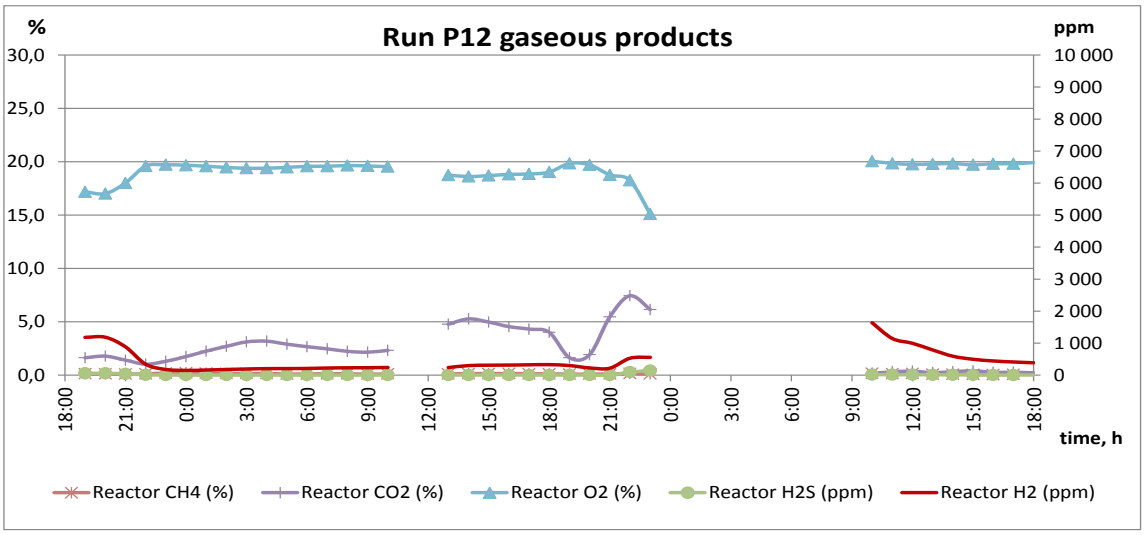

Figure 26. Gaseous products of run 11P

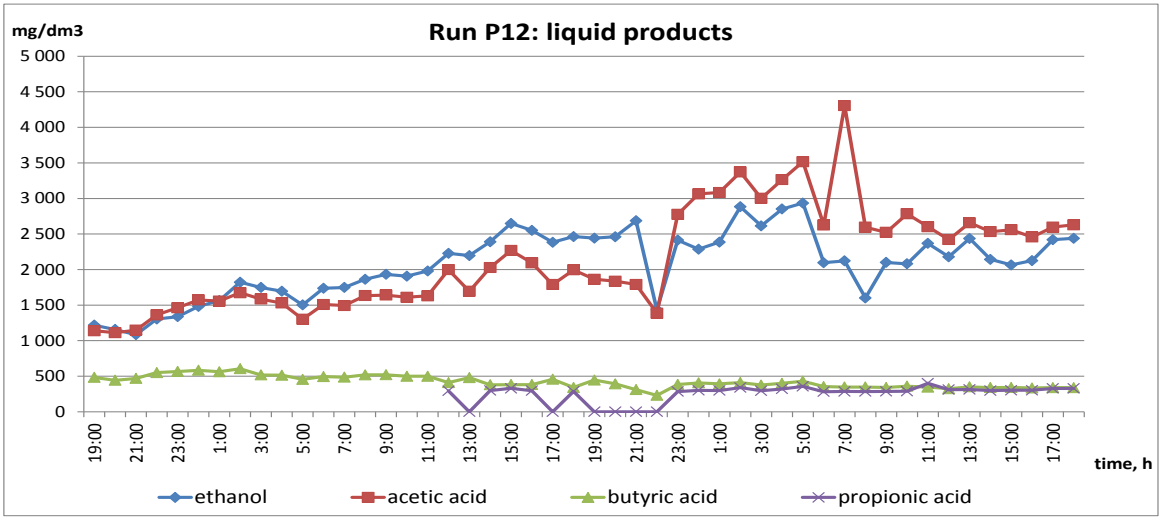

Figure 27. Liquid products of run 12P

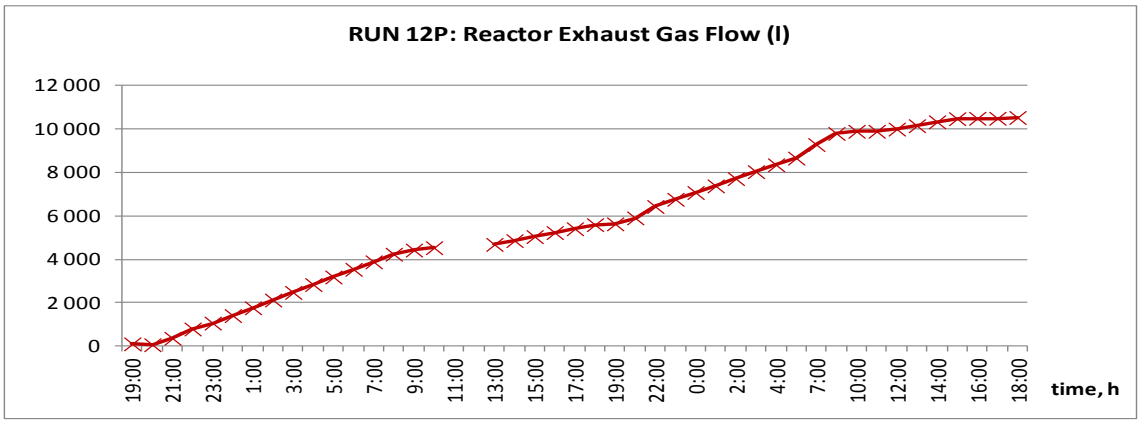

Figure 28. Cummulative curve of gas flow i run 12P

3.7 Run 13P

In run 13P the experimants with kitchen waste were continued. The first input to the reactor was 50 l of biowaste. The initial glucose level amounted to 216 mmol/dm^3. After app. 5 hours the glucose level fall below detection limit. The next day at 9:00 two barrels of potato waste were added. In this run the pH remained relatively high and thus the usage of NaOH to keep the pH at 6,5 was significantly lower than in the previous runs. The process was carried out at microaerobic conditions, by stirring with compressed air. Nevertheless, as it can be seen in Figure 30 a significant amount of H2 was generated (exceeding the measurable level of 10000 ppm). On contrary to the previous runs it coincided with the high content of oxygen. The CO2 level was also high, exceeding 30%. Also significant amounts of H2S were produced, reaching almost 4000 ppm (especially during a short break down of the control system). Figure 31 shows different production pattern than the previous runs. For the first time very high level of 2,3-butanediol (2,3BD) was measured by the GC. The maximum production reached over 7000 mg/dm^3. The generation of 2,3BD was correlated with high levels of butyric acid (reaching over 8000 mg/dm^3). At the same time the production of acetic acid and propionic acid were correlated, with propionic acid at a lower level. During the whole process run the ethanol level was at 1000 mg/dm^3.

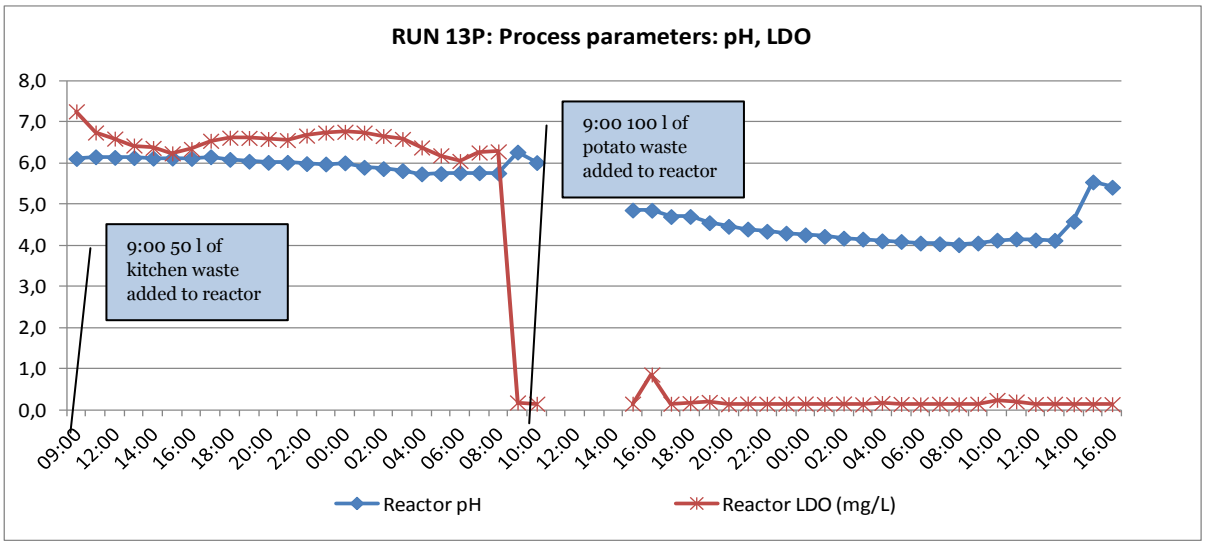

Figure 29. Process parameters run RUN 13P

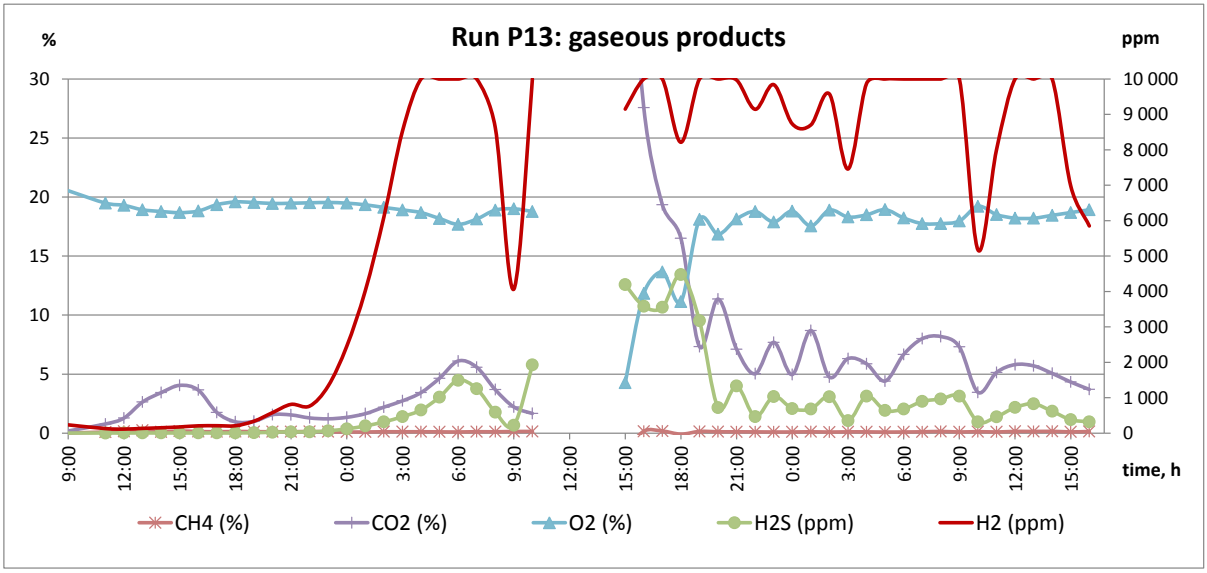

Figure 30. Gaseous products of run 13P

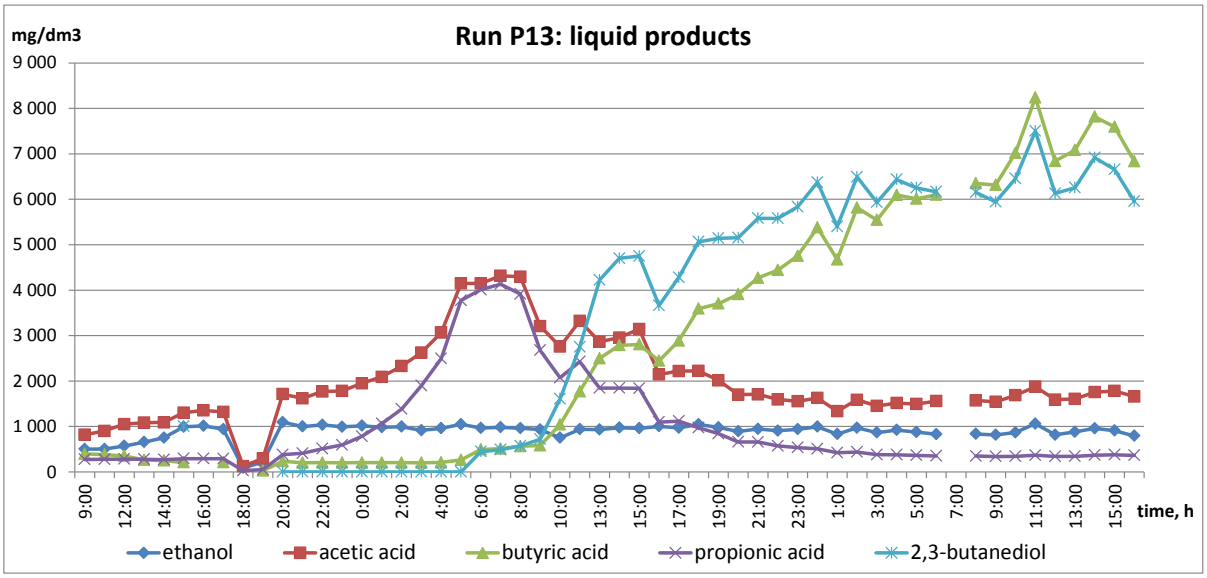

Figure 31. Liquid products of run 13P

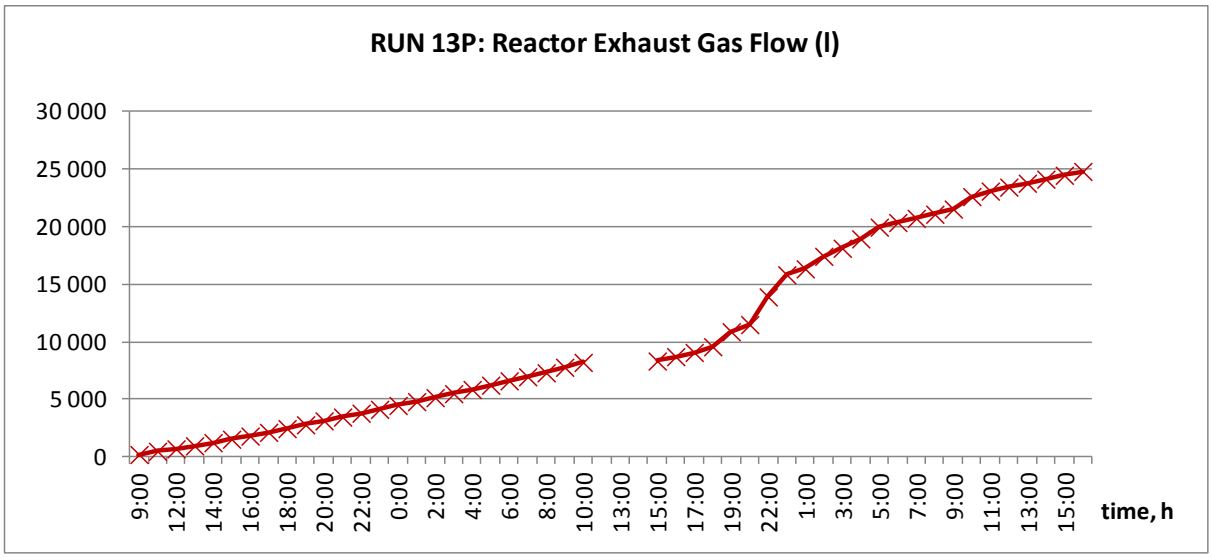

Figure 32. Cummulative curve of gas flow i run 13P

3.8 Run 14

Run 14P was similar to run 13P. At first kitchen waste was added to the reactor (100 l). The glucose level increased to 104 mmol/dm^3. The glucose was consumed within app. 8 hours. At the later stage of the process potato waste was added twice to provide carbon source.
Figure 34 shows the composition of gases which were generated during run 14P. It can be seen that high level of H2 was reached again exceeding 10 000 ppm. This was again coinciding with high oxygen level (app. 20% during the whole run). Moreover, from Figure 34 it can be clearly seen that the two later peaks in generation of CO_2, H_2 and H_2S took place just after new portions of potato waste were added. At the same time the level of oxygen dropped. Figure 35 depicts generation patterns of the final prioducts. Again, the analyses with GC indicated very high levels of 2,3-BD (up to 16 000 mg/dm^3) and almost 14 000 mg/dm^3 of butyric acid. Generation of acetic acid and propionic acids are correlated in the first part of the run, while in the later phase there is a better correlation of propionic acid with ethanol. The summed up production rates are significantly higher than in all the previous runs.

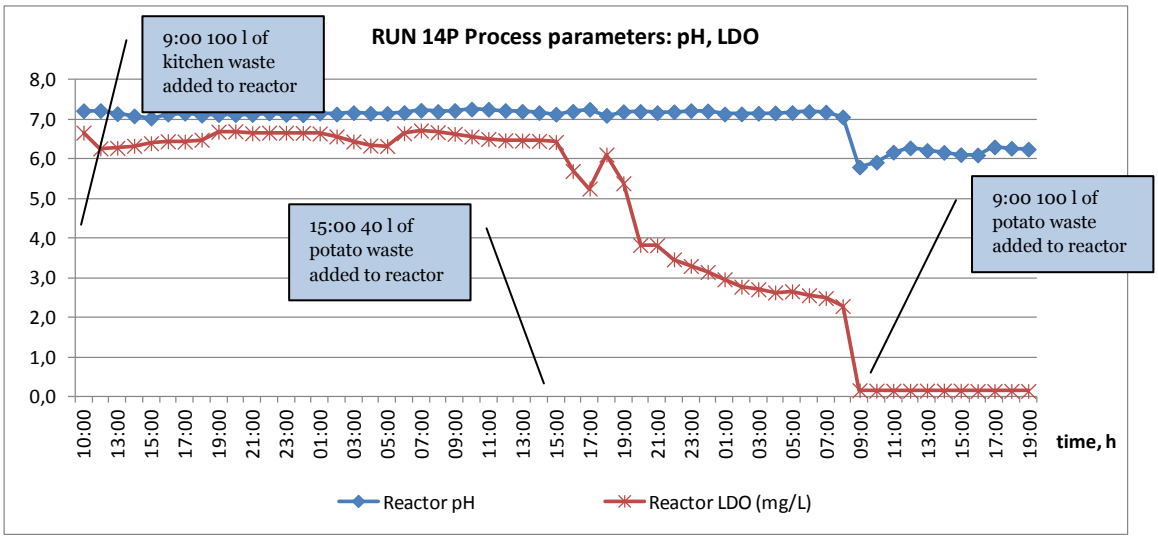

Figure 33. Process parameters run RUN 14P

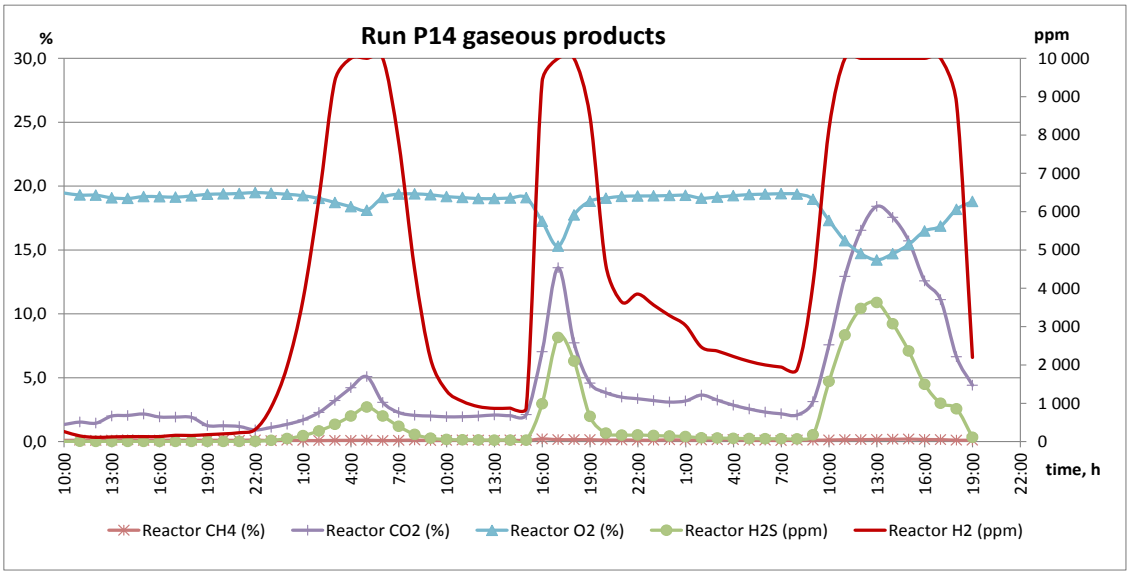

Figure 34. Gaseous products of run 14P

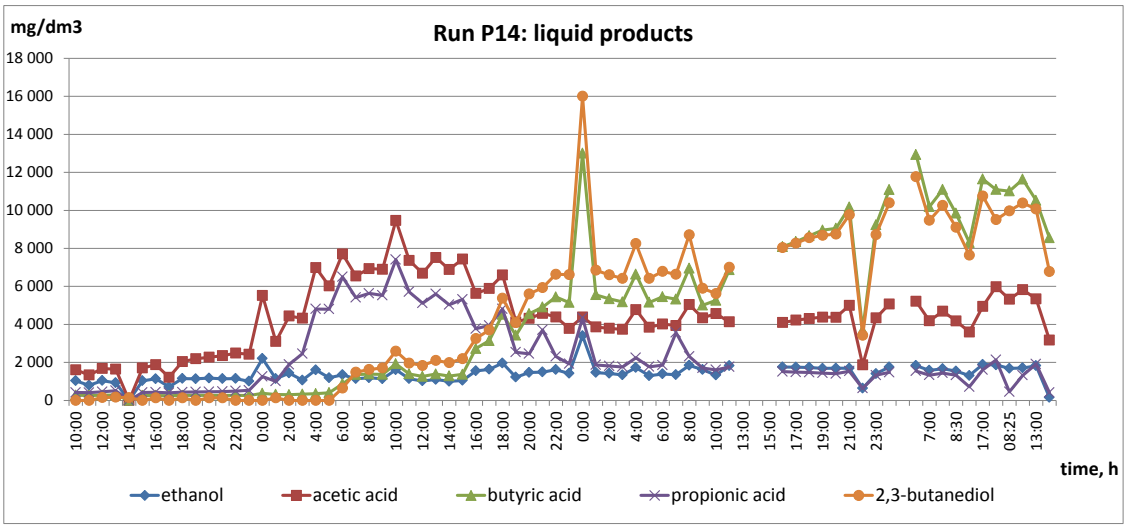

Figure 35. Liquid products of run 14P

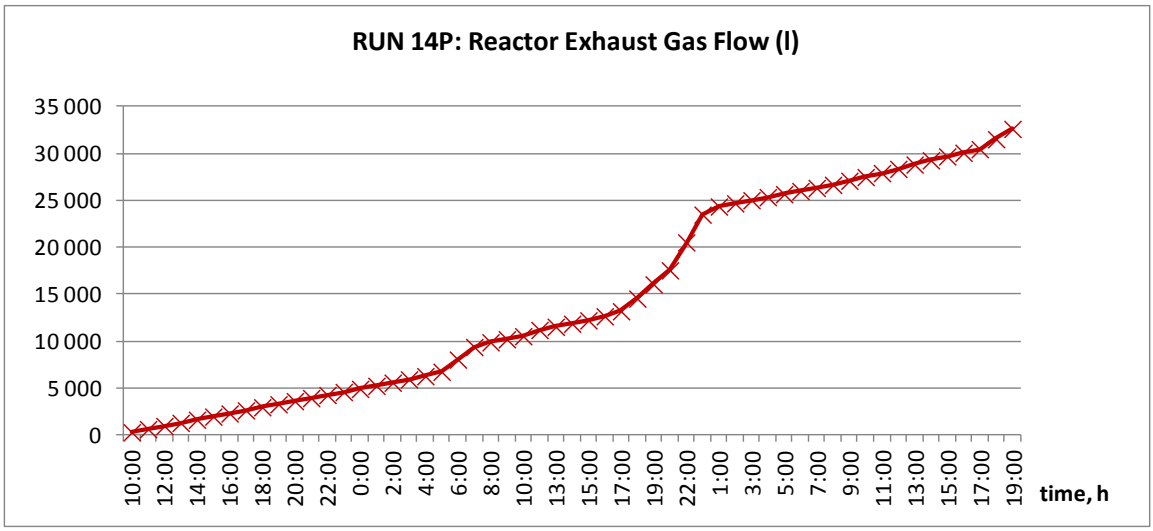

Figure 36. Cummulative curve of gas flow i run 13P

4. Results summary

In this chapter results of runs 7 – 14 have been presented together and compared. The yield of each compound is related to the amount of glucose in the input waste (mol product/mol glucose). Here especially in runs 13P and 14P high rates of 2,3-BD amounting to 0,25 and 0,25 mol/mol glucose were determined.

Table 2. Liquid products generation mol/mol glucose

	Ethanol	Acetic acid	Butyric acid	Propionic acid	2,3-butanediol
RUN 7P	0,04	0,08	0,01	0,00	0,00
RUN 8P	0,18	0,20	0,01	0,01	0,00
RUN 9P	0,05	0,08	0,01	0,01	0,00
RUN 10P	0,12	0,12	0,01	0,08	0,00
RUN 11P	0,08	0,15	0,04	0,02	0,00
RUN 12P	0,17	0,14	0,01	0,01	0,00
RUN 13P	0,07	0,10	0,30	0,02	0,26
RUN 14P	0,08	0,17	0,27	0,04	0,25

Figure 37 shows the cumulative yields of all runs. It can seen also here that the yield in run 13 and run 14 are highest, especially with regard to butyric acid and 2,3BD. Total yield in run 14P was over 0,8 mols of products per mol glucose (input). It is remarkable that in these runs the oxygen content was remining on a relatively high level, which was not preventing the formation of the products. Ethanol, however, was not produced in high quantities in these runs due to the prevailing aerobic or microaerobic conditions.

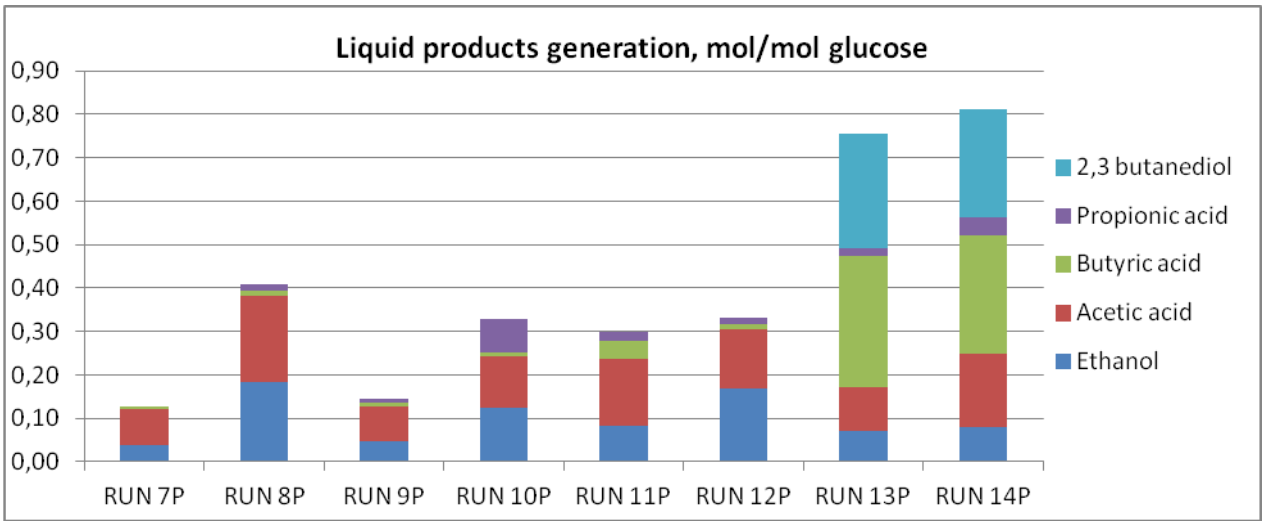

Figure 37 Liquid products generation mol/mol glucose

Table 3 shows the yields data expressed as g/kg glucose. Here we can see that the highest value was obtained in run 13. Both butyric acid and 2,3BD were produced at high level in this run as related to the total mass of glucose which was determined in the input waste substances. Therefore although in run 14 higher concentrations of product substances were achieved, it was run 13P in which the highest rate as related to the mass of input glucose was achieved (Figure 38Figure 1).

Table 3. Liquid products generation g/kg glucose

	Ethanol	Acetic acid	Butyric acid	Propionic acid	2,3-butanediol
RUN 7P	9,9	27,2	2,7	0,0	0
RUN 8P	46,6	67,0	5,9	5,1	0
RUN 9P	12,0	26,6	4,8	3,9	0
RUN 10P	31,6	39,7	4,1	31,6	0
RUN 11P	21,0	51,6	19,7	9,5	0
RUN 12P	42,9	46,2	5,9	5,8	0
RUN 13P	18,2	33,4	148,7	7,0	131,6
RUN 14P	9,6	26,9	63,7	8,2	58,9

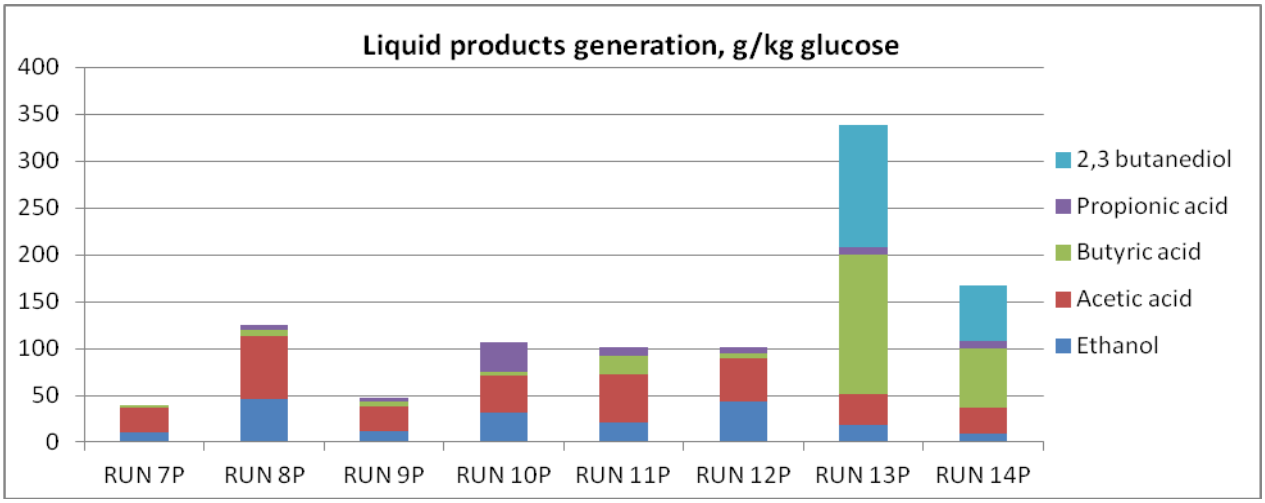

Figure 38Liquid products generation g/kg glucose

Table 4 shows characteristics of waste samples during processing, with regard to the content of organic carbon, biological oxygen demand (BOD5) total nitrogen and total phosphorus. For those analyses samples were centrifuged and filtered, so that the results pertain mostly to the dissolved forms. The last collung presents BOD5 data for total (not filtred samples). From the presented results it can be seen that both Nitrogen and Phosphorous could be limiting factors for bacterial growth. The corresponding C:N and C:P ratios were very high, reaching C:N in the range of 130-180 (while appropriate ratio is C:N of 25-35) and C:P of 50-600 ratios (while appropriate one is C:P of 100) in case of raw potato waste. The macroelements level was better in case of mixed biowaste with potato waste (C:N in the range of 50-60) and C:P in the range of 63-70. It can be seen that especially low Nitrogen level could be the limiting factor for further improvement of product yield.

Table 4 Characteristics of process samples

Run/sample date	time	Filtred samples				Not filtred
		TOC [mgC/dm3]	BOD [mgO2/dm3]	Total N [mg N/dm3]	Total P [mg P/dm3]	BOD [mg O2/dm3]
Run 7P						
2014-05-14	15:00	5935	10 358			
2014-05-15	16:00	4566	7 859			
RUN 8P						
2014-05-21	11.00	5965	10 452			
2014-05-22	1:30	11011				
2014-05-22	13:30	10259	17 020			
RUN 10 P						
2014-06-03	17:30	8852	13 791	48	14,7	13 000
2014-06-04	15:30	7960	13 333	42	11,5	21 000
2014-06-05	13:30	7196	12 492	<d.l.	18,2	19 500
RUN 11P						
2014-06-10	10:50	3983	6 174			
RUN 12P						
2014-06-16	18:50	7616	14 394	42	113	30 000
2014-06-17	22:00	5453	10 236			
2014-06-18	18:00	10507	17 584	28	109	50 000
RUN 13P						
2014-06-24	9:30	3171	5 535	68	45	50 000
2014-06-24	18:00	3148	5 658			
2014-06-25	7:00	2669	4 868			
2014-06-25	15:00	4149	7 438			
2014-06-25	17:00	4336	7 761			
2014-06-25	23:00	4275	7 890			
2014-06-26	13:00	4528	8 002			
2014-06-30	9:00	4888	8 626	<d.l	16,8	30 000
RUN 14P						
2014-07-01	11:00	4314	7 243	72	68	67 000
2014-07-01	12:00	3593	6 089			
2014-07-02	13:00	4127	7 234			
2014-07-02	17:00	5804	8 958			

Run/sample date	time	Filtred samples				Not filtred
		TOC [mgC/dm3]	BOD [mgO2/dm3]	Total N [mg N/dm3]	Total P [mg P/dm3]	BOD [mg O2/dm3]
2014-07-03	9:00	8181	14 329			
2014-07-04	7:30	8703	14 715			
2014-07-04	17:00	7537	12 896			
2014-07-07	9:30	8318	14 251	28	103	10 000

5. Comparison of NMR results and GC results

Gas chromatography results are limited only to the substances for which the calibrations were performed. It is useful to determine whether the targeted substance is available in the product mixture and what is its concentration. Typical CG spectrum of runs 8P-11P is shown in Figure 39. It can be seen that ethanol, acetic acid and butyric acids have been identified here and the concentration for these substances has been provided.
Figure 40 illustrates a GC spectrum of run 14P. I can be seen that the peaks here are higher – which means higher process yields and also we can clearly distinguish an additiona substance which was identified as 2,3BD. There is also a number of small peaks which were not identified.

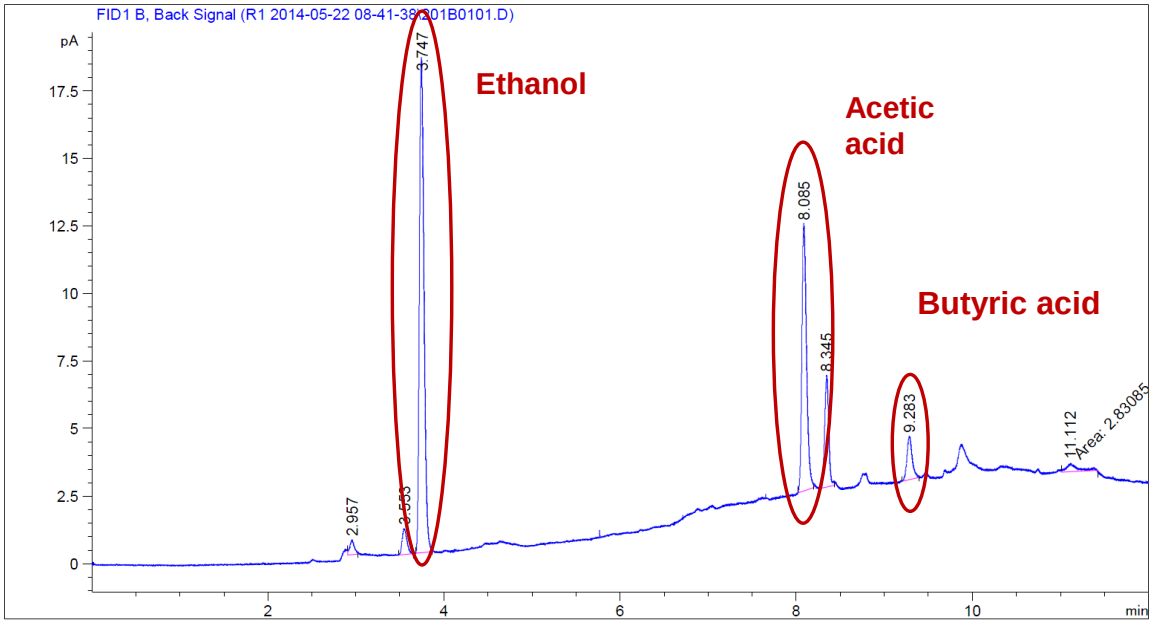

Figure 39 GC spectrum of run 8P

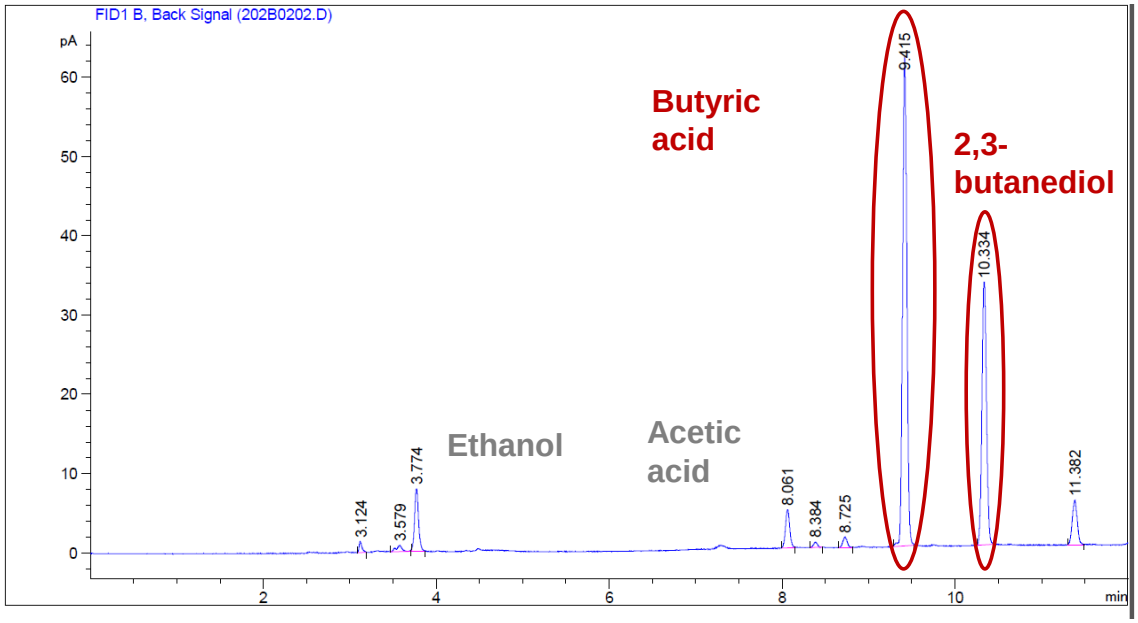

Figure 40 GC spectrum of run 14P

The applications of gas chromatography are very limited ones when it comes to the determination of unknown substances. Therefore the GC analysis is s very good screening method. However, more advanced methods are needed for more detailed analyses. For this purpose it is helpful to used more advanced methods to determine the actual compostition of product fluids. Therefore, within the ABOWE project the Nuclear Magnetic Resonance (NMR) Spectroscopy was used for selected samples in order to confirm the GC results and to obtain the full picture of received products. The analyses were performed by Prof. Reino Laatikainen from the Department of Pharmacy, University of Eastern Finland.

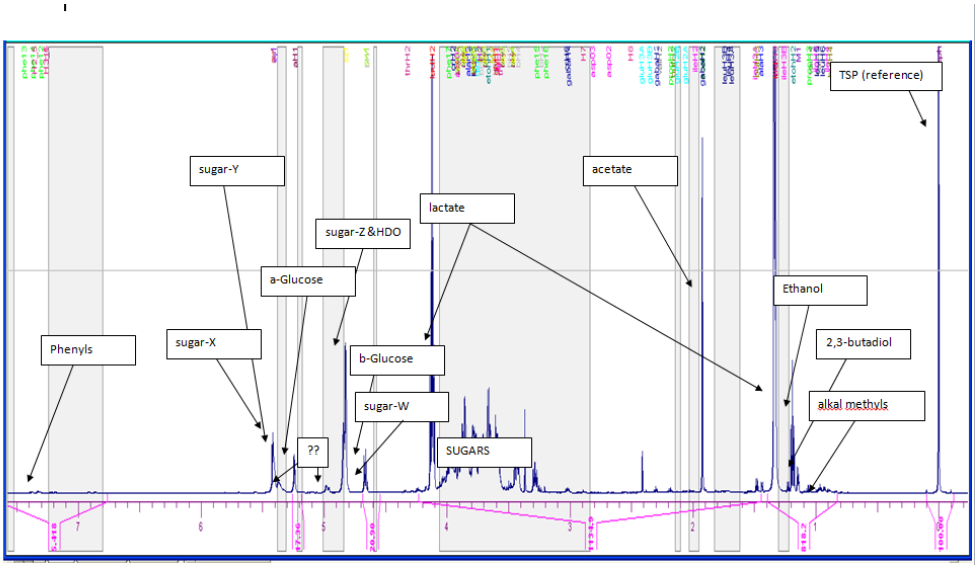

Figure 41 NMR spectrum of a sample of products of run 9P

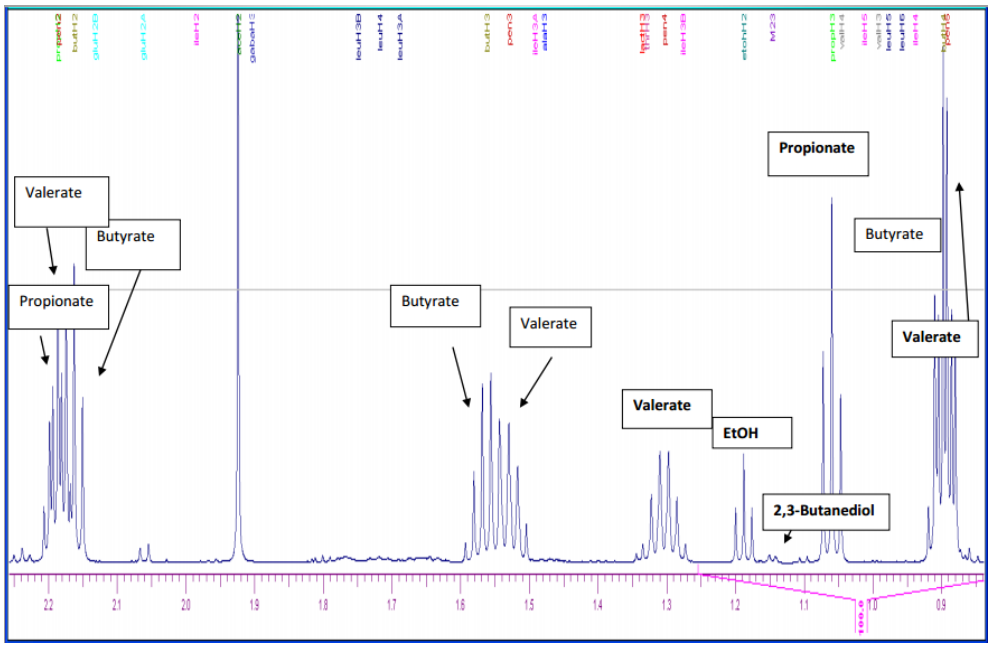

Figure 42 The NMR spectrum of analysed sample – run 13P (Laatikainen 2014)

In Figure 41 an exemplary spectrum of one sample from process run 9P is shown. It can be seen that the lactate is one of the substances which occurred with high peak but was not identified by GC. In Figure 42 one of results of run 14P is presented. There additional products are shown, especially in the form of valerate (pentanoic acid) are present. It also shows that the actual concentration of 2,3BD is not high as opposed to the results obtained by GC method. To clarify all these issues selected samples obtained within run 14P were analysed with NMR method. Below in Figure 43 these results are summarized and compared with the results of GC (Figure 44).

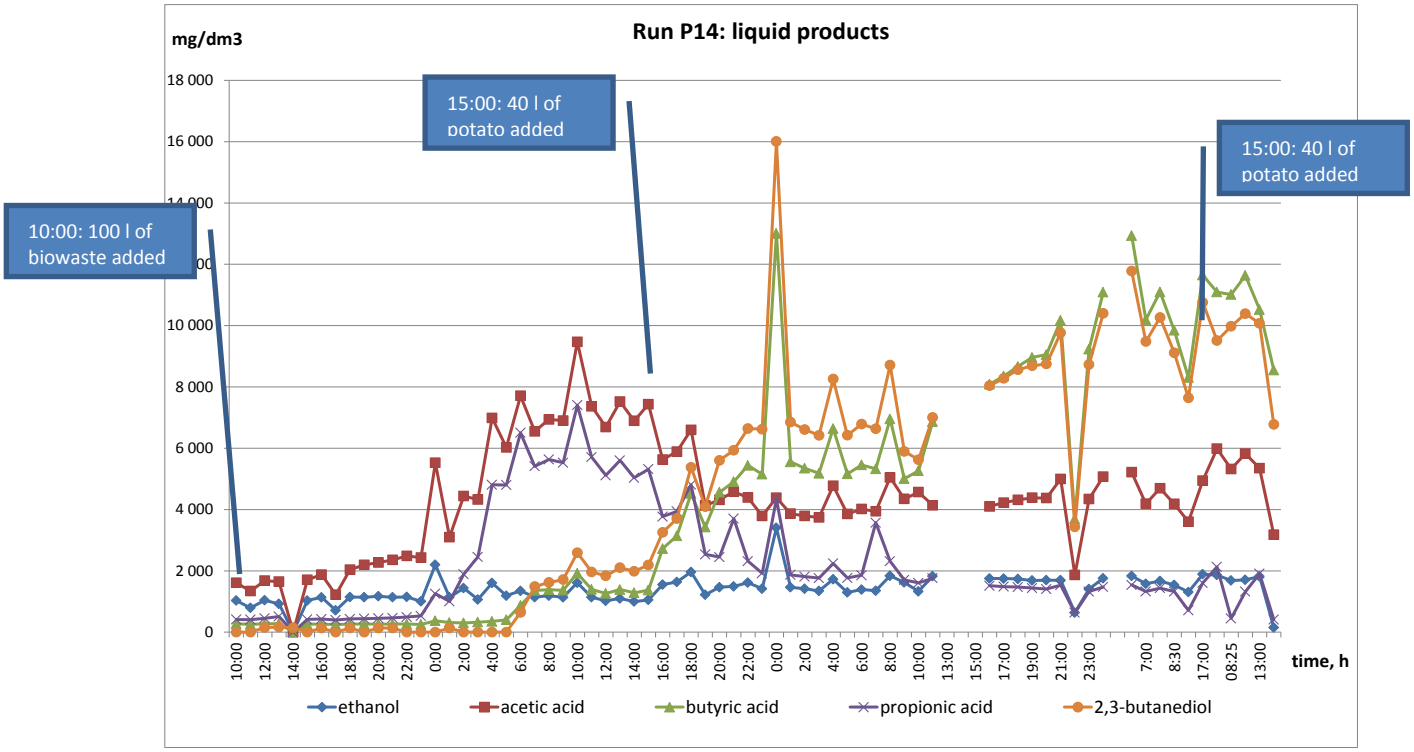

Figure 43. Results of run 14P – summary of GC results

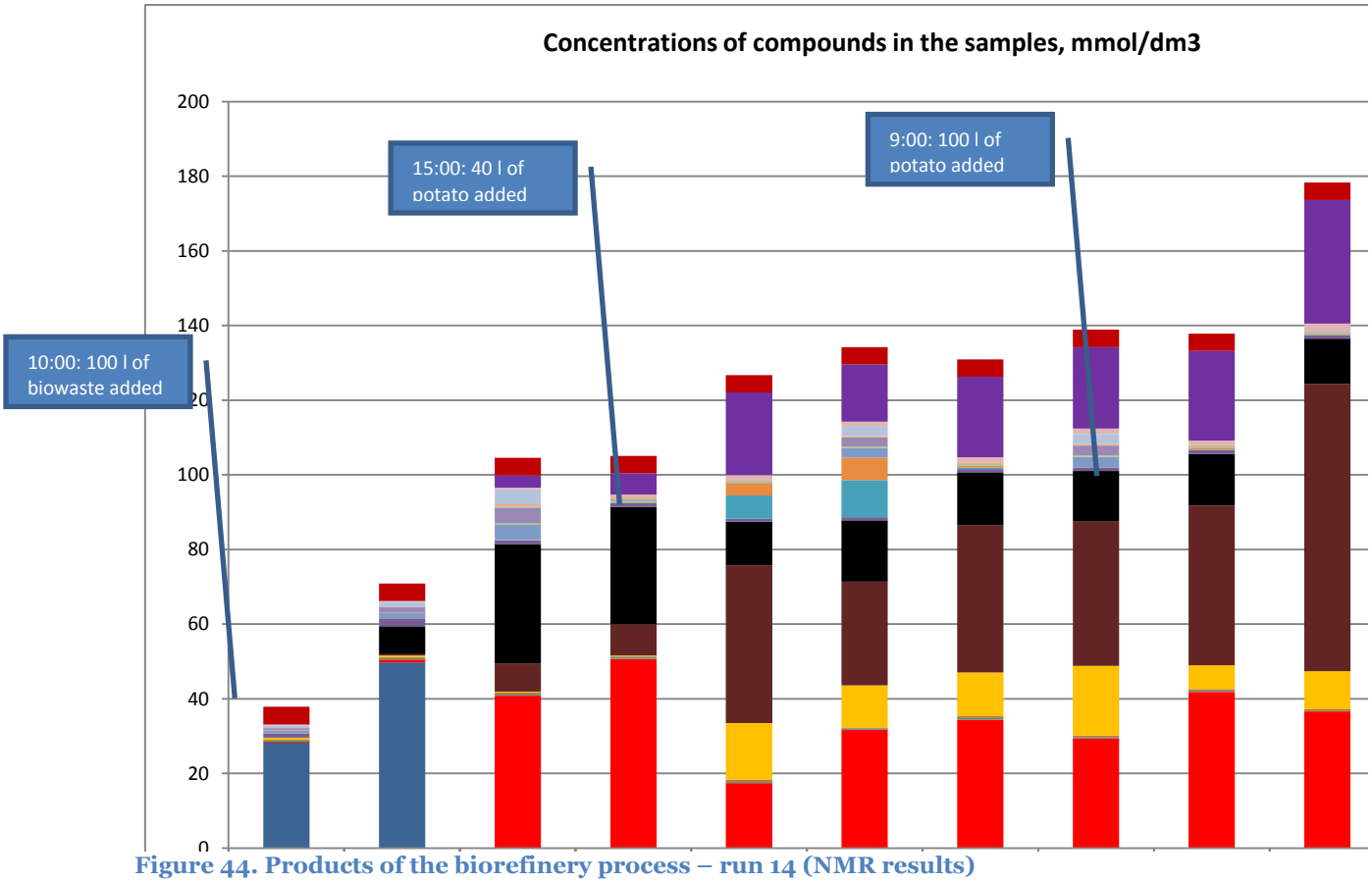

Figure 44. Products of the biorefinery process – run 14 (NMR results)

A number of additional substance were determined with NMR method. They are listed in Table 5.

Table 5 Substances identified in samples of run 14P with NMR method

Abb.	Full name	PROTONS	Chemical formula
lactate	lactate	4	CH3CH(OH)COO$^-$
acetate	acetate	3	C2H3O2$^-$
ala	alanine	4	$CH_3CH(NH_2)COOH$
valine	valine	8	2CCH(NH2)CH(CH3)2
leu	leucine	10	HO2CCH(NH2)CH2CH(CH3)2
ile	isoleucine	10	HO2CCH(NH2)CH(CH3)CH2CH3
etoh	ethanol	5	CH3CH2OH
butyrate	butyrate	7	C4H7O2$^-$
propio	propionate	5	C3H5O2$^-$
glu	**Glutamic acid**	5	$C_5H_9NO_4$
beta	**beta carbon**	7	
alfa	alfa carbon	7	
gly	glycine	2	NH2CH2COOH
phe	Phenylalanine	8	$C_9H_{11}NO_2$
3pheprop	3-Phenylpropionate	9	C6H5CH2CH2COO$^-$
creatine	creatine	5	$C_4H_9N_3O_2$
gaba	**gamma-Aminobutyric acid**	6	$C_4H_9NO_2$
asp	Aspartic acid	3	HOOCCH(NH2)CH2COOH
mannitol	Mannitol	8	C6H8(OH)6
23bud	2,3-butanediol	8	C4H10O2
pentano	Pentanoic acid (valeric acid)	9	C5H10O2
tsp	Trisodium phosphate	9	Na3PO4

In Figure 44 the results of NMR analyseds are provided – the concentrations of all substances are provided in mmol/dm^3. In can be seen that at the initial stage of the process (with only kitchen biowaste added) one of the prevailing substances was lactate (not included in the GC method). Afterwards the majority of it has been transformed to acetate and propionate. Also some butyrate was formed. After the potato mass was added a significant increase of butyrate could be obtained. Also some ethanol was formed (which has proved one of the major products of potato waste fermentation in runs 8P-12P). We observed also pentanoic (valeric) acid formation at much higher level than before. It was probably the valeric acid which was wrongly identified by the GC methos as 2,3BD. In fact the formation of 2,3BD was rather low. The molar composition of the products at different stages in shown in Figure 45.

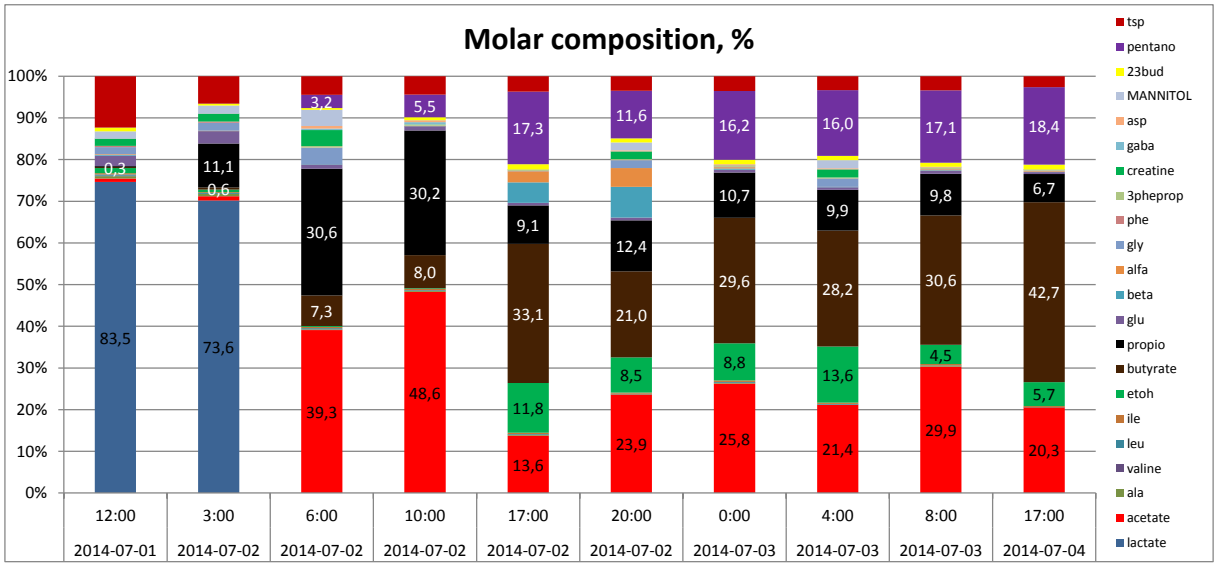

Figure 45 Molar composition (% moles) of substances generated within run P14

Figure 46 provides the cumulative quantitative amounts of substances obtained with GC analyses and NMR analyses (both in mmol/dm3). It can be seen that the corresponding amounts determined by NMR methos were significantly lower than those determined with GC method. The differences were significant. The quantities were lower for NMR analyses. The explaination can be partially degradation of biorefinery products with time. The GC analyses were performed directly at Pilot A with fresh samples. The NMR analyses were performed after storing the samples for two months. The samples had to be sent to Finland and then they were stored in a freezer. Organic acids are volatile substances. They could also be used by the remaining microorganisms and transformed into gaseous products.

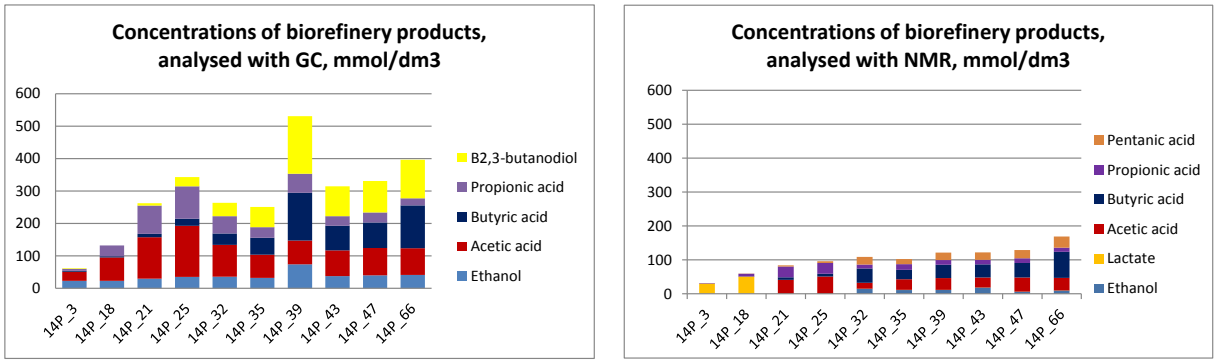

Figure 46 Comparison of quantitative results obtained with GC and NMR

Samples from run 14P were also analysed with respect to total organic catbon content (TOC). The analyses were performed at Instutute of Environment Protection Engineering Wroclaw University of Technology. However, it was also done after initial storing and transport of samples from Pilot A.

The results of these analyses correnspond well with NMR analyses. In Figure 47 the TOC results are shown and against the cumulative amount of organic carbon calculated from NMR results (taking into account the carbon content of each compound and its concentrations). It can be seen that at the beginning of run 14P there were additional carbon containing compunds in the solution. This can be glucose and other sugars which were not analysed.

At the later stages the TOC values are very much similar with the cumulative carbon amounts determined in NMR data.

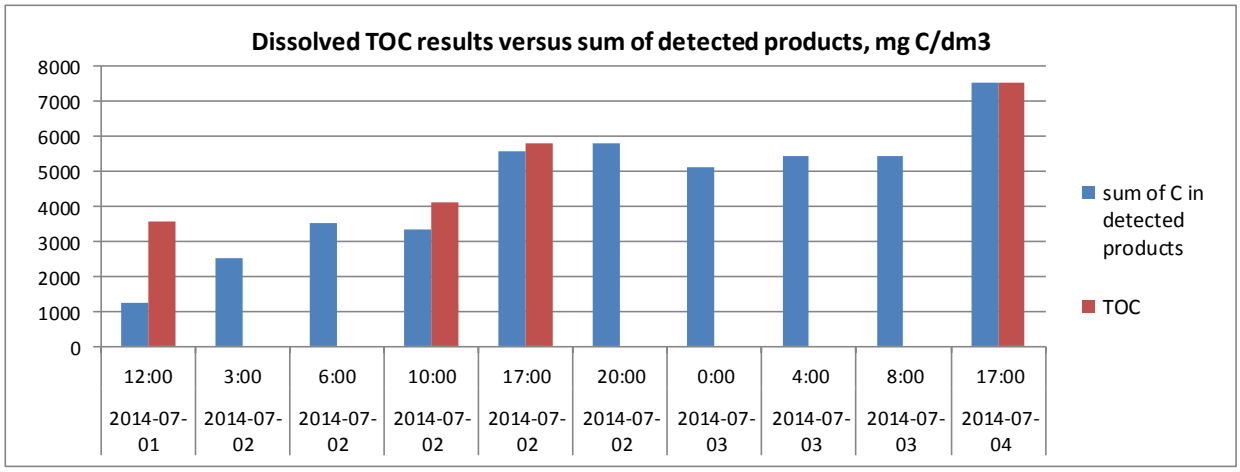

Figure 47 Comparison of TOC and NMR results

6. Final conclusions

In this report the results of Polish test of biorefinery technology experimented in plant A have been summarized.

The report presents results obtained in 8 process runs which took place in summer 2014. The initial 5 runs were performed using potato waste as a single substarate. In these experiments significant levels of ethanol and acetic acids were produced. Among the gaseous products hydrogen generation took place at elevated levels.

However, it seems that the potato waste did not contain sufficient microelemens to maintain generation of high level products. From the data provided in Table 1 it seems likely that the Nitrogen level as well as the content of Calcium could be limiting factors for the microbes. A significant improvement could be obtained when the kitchen biowaste were used as the initial substrate. This has been testes in runs 13P and 14P. It could be seen that the levels of final useful products increased significantly. With GC analyses the presence of such substances as 2,3BD, butyric acid and propionic acid were generated at elevated levels.

Especially in the runs 13P and 14P high rates of 2,3-BD amounting to 0,25 and 0,25 mol/mol glucose were determined. However from data provided in Table 4 it can be seen that even in the last two runs the low Nitrogen and Phosphorous levels coud be the limiting factors for further improvement of product yields.

Finally the results have been evaluated with NMR method, which is more reliable method for mixtures of numerous organic compounds than GC and allows more precise qualitative analyses of the generated products. It was determined that the final products of run 14P constituted of butyric acid and pentanoic acid, also known as valeric acid. The total quantities of products were lower than initially determined by the GC method. It can indicate that part of the products was unstable and was lost before the analyses with NMR were performed. However, even these lower amounts are very promising results. They give a an excellent platform to continue biorefinery research with the methods and know how developed during the ABOWE experiments. It is also very incouraging that the good results which were obtained in run13 could be replicated in the final experimental run in Poland (run 14).

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