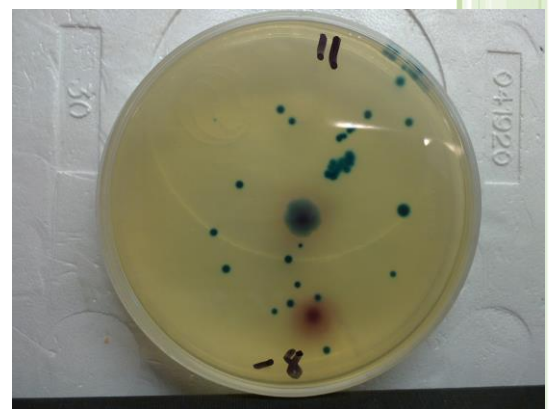


# PROOF OF TECHNOLOGY - SUMMARY OF NOVEL BIOREFINERY (PILOT A) TESTING PERIODS IN FINLAND, POLAND AND SWEDEN



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**PROOF OF TECHNOLOGY**

**SUMMARY OF NOVEL BIOREFINERY (PILOT A) TESTING PERIODS IN  
FINLAND, POLAND AND SWEDEN, OUTLINED ON 15.3.2015 BY:**

Key Technology Provider and Innovator:  
Elias Hakalehto PhD  
Adjunct Professor in Biotechnical Microbial Analysis  
University of Eastern Finland  
Finnoflag Oy  
Other Work Group in ABOVE countries

# 1. GENERAL ESTIMATION OF THE PILOT A PROCESS CONCEPT

## 1.1 General background

Microbial action is one of the driving forces behind the circulation of matter in the ecosystems. Metabolic potential of the micro-organisms is consequently well known, but its implementation into waste treatment, although used in many applications, still requires more scientific and technological input. If the microbiological means for producing energy gases, biofuels and bulk, platform and special chemicals as well as organic fertilizers could be fully exploited, this could lead also to the better avoidance of the adverse effects of global human actions on the climate. (Dahlquist *et al.* 2013, Hakalehto 2015a).

The principles of sustainable biorefinery technology includes the option of using undefined microbial cultures (UMC) which in practice derive from the Nature or from the biomass wastes used as raw materials for the processes. Even though these microbial cultures are undefined, their metabolic actions and joint biochemical regulation can and need to be elucidated. In the biorefineries these activities could form the basis for process set up. The composition of the microbial cultures could be modified or fortified, for example by the addition of known microbial producers of some valuable chemicals. This technology has been developed and tested in the laboratory of Finnoflag Oy for some twenty years.

## 1.2 Planning principles

During the construction of the Pilot A movable pilot plant and experimental station the leading principle has been the ability to give proof of concept on the usage of variable biowastes as raw materials. The pretreatments of the wastes took place in the first and second reactor tanks, the homogenizer and the main goal hydrolyzer, respectively. Enzymes were used for the hydrolysis, which was expected to partially continue in the third tank, the actual bioreactor, according to the ideas of consolidated bioprocessing (CBP) (Hakalehto 2015). The technology provider has pending patents related to the bioreactor construction.

During the Pilot A runs various gas flows can and were used for boosting the bioreactions. Thus formed liquid substances are then collected from the removed fermentation broth on a batch or semi-continuous basis. The actual product purification needs to be carried out in another unit. The downstream process for 2,3-butanediol has been under development in the Ostfalia University of Applied Sciences as a part of ABOWE and a separate report (ABOWE Report O3.8) has been published about that. In future projects, the simultaneous product recovery during the upstream process is to be included into the project plan. Any biorefinery process waste could then be further used for combined biochemical and biogas production, for example. The biogas formation could take place in the ABOWE Pilot B or in another equivalent reactor.

### 1.3 Microbes as biocatalysts

Microbial cells are small but relatively complex biological entities. They have sophisticated metabolic regulation, and form intraspecies and interspecies communities, in which the cells signal to each other, and strains form balanced mixed cultures. A phenomenon called cometabolism is also occurring in the degradation of larger molecules, or in breaking down recalcitrant or complex molecular structures.

The hydrolysis of the biomass can be accelerated by various enzymes. In the Pilot A hydrolysis tank temperatures are first elevated to 70-80 °C for some time which serves also for hygienization purposes. Although pathogens can be eliminated, many members of the natural flora withstand this treatment. Because of the non-aseptic approach of the Finnoflag biorefining system, some miscellaneous strains can find their way into the bioreactor. Since the enzymes are not inactivated, the hydrolysis process will continue during the bioreactor step. Then some external inoculation of natural origin, or of some more defined production organisms, could be added. In case of the ABOWE Pilot A runs, the following bacterial inoculations were used in various runs (Table 1).

**Table 1. Waste type and major microbial strains in the Pilot A experiments.**

	Country	Waste type	Additional microbe strain
1.	Finland	Cellulosic waste, sludges from the waste water treatment plant	<i>Klebsiella mobilis</i> <i>Escherichia coli</i> <i>Propionibacterium acidipropionici</i> <i>Clostridium butyricum</i> <i>Clostridium acetobutylicum</i>
2.	Poland	Potato industry waste Sorted domestic biowaste	<i>Klebsiella mobilis</i> <i>Escherichia coli</i>
3.	Sweden	Chicken slaughterhouse waste Chicken manure Sawdust	<i>Klebsiella mobilis</i> <i>Cellomonas</i> sp. <i>Clostridium butyricum</i> <i>Clostridium acetobutylicum</i>

## 1.4 Outlines of the test runs

Specific features of the test runs at various locations are summed up in Tables 2 A-C.

**Table 2. A-C. Main problems, solutions and potential products from the three experimentations.**

A. Savon Sellu fluting factory, Kuopio	
BIOMASS	Cellulosic waste
PROBLEM	Too high H <sub>2</sub> S production
SOLUTION	Nutrient bed with seed bacteria
PRODUCTS	2,3-butanediol, ethanol, butanol, butyrate, propionate, hydrogen
B. ZGO Gac Ltd waste management centre, Lower Silesia	
BIOMASS	Potato industry waste, municipal source-sorted biowaste
PROBLEM	Initial conversion to lactate, H <sub>2</sub> S
SOLUTION	Optimization of the fermentation conditions
PRODUCTS	2,3-butanediol, butyrate, propionate, valerate, hydrogen
C. Hagby farm (slaughterhouse), Enköping	
BIOMASS	Slaughterhouse waste, chicken manure, saw dust
PROBLEM	Inefficient hydrolysis, low soluble carbohydrate
SOLUTION	Conversion to organic acids
PRODUCTS	Propionate, valerate, butyrate, butanol, hydrogen, ammonium salts

## 1.5 Microbial bioprocess technology operations – general considerations

Since the ABOVE Pilot A plant was not intended or planned for the treatment of any particular biomass waste material, its specific features were not preliminary “tuned” for optimization of any process type. Instead, this experimental station was designed to give proof of concept type of provisional results from two months’ testing periods in each of the three countries, and consequently make a platform for future ideas on any biowaste utilization. During these very limited periods the actual purpose was fulfilled in the technical and preliminary bioprocess runs in Finland, in Poland and in Sweden. The potential targets for technical improvements and user experiences are reviewed later in this report and in the Appendices.



## 2. INTRODUCTION

This report is the final output of ABOWE project (Implementing Advanced Concepts for Biological Utilization of Waste) which belongs to EU Baltic Sea Region Programme 2007-2013. ABOWE is an extension project for REMOWE project (Regional Mobilization of Sustainable Waste to Energy Production 9/2009-12/2012) to continue with two promising technologies to unlock investments.

### 2.1 Purpose of the work

The novel biorefinery concept innovated and developed by Adj. Prof. Elias Hakalehto, Finnoflag Oy and University of Eastern Finland is one of the two bases for ABOWE project. The purpose of this report is to gather together essential information from Finnoflag biorefinery technology test runs with Pilot A in three Baltic Sea countries during 2014.



**Figure 1. Pilot A was presented in the Finnish Science Centre Heureka, Vantaa on 25.4.2014 in an introductory seminar.**

Objective was to test

- Effective pretreatments and hydrolysis of various industrial and municipal wastes.
- Enhanced natural microbial bioprocess for the upstream production of fuels and chemicals.
- Preliminary planning of the simultaneous product collection.

The goals of the ABOVE project and the movable Pilot A manufactured in Finland by Savonia University of Applied Sciences is to provide "proof of concept" on the ways, how biomass waste materials could be used as raw materials. The products are biofuels, organic chemicals, fertilizers and nutrients. These products are to be produced in an economically feasible way.

A coherent objective is to achieve industrial action which is implementing same principles that are maintaining the ecosystems. In Nature there are not e.g. landfills anywhere, but all organic material is recycled.

Novel production principles have been tested in three countries on various different wastes. One bioproduct alternative, 2,3-butanediol, is used for producing synthetic rubber, plastic monomers, anti-icing chemicals, textiles, cosmetics and other commodities. Downstream techniques have been developed in cooperation with Ostfalia University of Applied Sciences from Germany. Ethanol and hydrogen are valuable fermentation by-products.

The biorefinery process' novelty is in improved productivity, low initial investment costs and versatile product repertoire. The production exploits results by the PMEU enhanced cultivation unit (Portable Microbe Enrichment Unit), and in larger vessels in the Finnflag laboratory since 1997. When products are produced faster, the facility size reduces enabling lower investment. Moreover, end product concentrations can be increased and the total duration of the process shortened.

Overall designing of novel biorefinery pilot plant's (Pilot A) bioprocess was conducted by Adjunct Professor Elias Hakalehto (Finnflag Oy and University of Eastern Finland). The Pilot A engineering team consisted of Finnflag Oy experts and Savonia University of Applied Sciences' engineering teachers, project engineers and engineering students. Versatile knowledge of process and instrumentation, layout, electrical, mechanical, automation, IT, environmental and manufacturing was combined for Pilot A during 2013.

Lead partner manufactured Pilot A in its educational workshop. In Pilot A manufacturing were participating as component suppliers many locally operating industrial and commercial enterprises. Also many trainees from Savo Vocational College, Finland participated in the manufacturing of Pilot A. Altogether over 50 persons participated in the Pilot A engineering and manufacturing.

## 2.2 Results

Products from the test runs in Finland were:

- Ethanol
- Butanol
- 2,3-butanediol
- Organic acids
- Hydrogen



- Fertilizer biomass
- Biogas
- Purified water
- Decreased waste treatment expenses
- Lesser environmental and climate load

In case of food industry wastes, maximum productivities of 8-10 g/l/h of 2,3-butanediol have been achieved in laboratory tests before ABOWE by Finnoflag Oy (Hakalehto et al. 2013). During the ABOWE, the international teams learned to cooperate in this milieu, where biological components (biomass, microbes and enzymes) meet with the metal hardware, sensors and the computerized control. Industrial levels of various organic acids, alcohols and energy gases were produced. In the Polish test runs they were produced from potato industry waste and sorted municipal biowastes. In Sweden the tedious protein and lipid wastes from a chicken slaughterhouse and farm were converted into energy and chemicals. The experimental period of two months at each testing site gave good start for future optimization.

## 2.3 Conclusions

Due to the decreasing supply of cheap fossil fuels effective use of biomasses has become more feasible. Tightening regulation upon discarding organic masses will boost development. Large scale microbial biotechnology offers then solutions. The Pilot A was introduced to the public during two seminars in Helsinki region (Fig 1 and 2), as well as presentations at testing sites in Finland, Poland and Sweden.

ABOWE project was chosen as the winner among the projects of all Finnish universities of applied sciences in the series Applied Research Knowledge and Innovations in the national Kärjet (SPEARHEADS) competition in 2014.

Videos from ABOWE Pilot A Final Seminar in Helsinki on 30.10.2014 can be seen from [www.abowe.eu](http://www.abowe.eu)



**Fig. 2.** Another public presentation of ABOVE Pilot A was during the concluding seminar at Helsinki University Viikki campus on the 30<sup>th</sup> of October, 2014. Adjunct Professor Elias Hakalehto (left) and Project Manager Ari Jääskeläinen.

### **3. DESCRIPTION OF FINNOFLAG BIOREFINERY TECHNOLOGY**

There are four main tanks in the Pilot A biorefinery process:

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),
- 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. 3). The entire microbiological and biotechnical process control is designed by Finnoflag Oy.



**Figure 3. PMEU – Portable Microbe Enrichment Unit.**

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 were 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.

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



## 4. FINNISH TESTING PERIOD AT SAVON SELLU

### 4.1 Site and feedstock description

The first testing site was Savon Sellu cartonboard factory's waste water treatment plant in Kuopio, Finland during February-March 2014. Because of the harsh climate conditions with temperatures between  $-25^{\circ}\text{C}$  and  $-30^{\circ}\text{C}$  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 4 and 5). Another raw material for the experiments was incoming waste water (Figures 6 and 7).



**Figure 4. Pilot A in its first testing site at Savon Sellu Oy, during the coldest days of the winter 2015.**





**Figure 5. Dried waste water treatment sludge piled at Savon Sellu Oy's waste management area.**



**Figure 6. Waste water in the Homogenizer tank of Pilot A.**





**Fig. 7. Waste water effluent at Savon Sella**



The waste waters of this factory are treated with a sophisticated purification plant comprising the first forest industry active sludge process in Finland at its time when it was implemented.

## 4.2 Test runs, results and conclusions from the Finnish test runs

Products from the test runs in Finland were:

- Ethanol
- Butanol
- 2,3-butanediol
- Organic acids
- Hydrogen
- Fertilizer biomass
- Biogas
- Purified water
- Decreased waste treatment expenses
- Lesser environmental and climate load



**Figure 8. Savonia's Project Engineer/Field Manager Tero Kuhmonen in front, and Engineer Kevin King of Finnoflag Oy.**

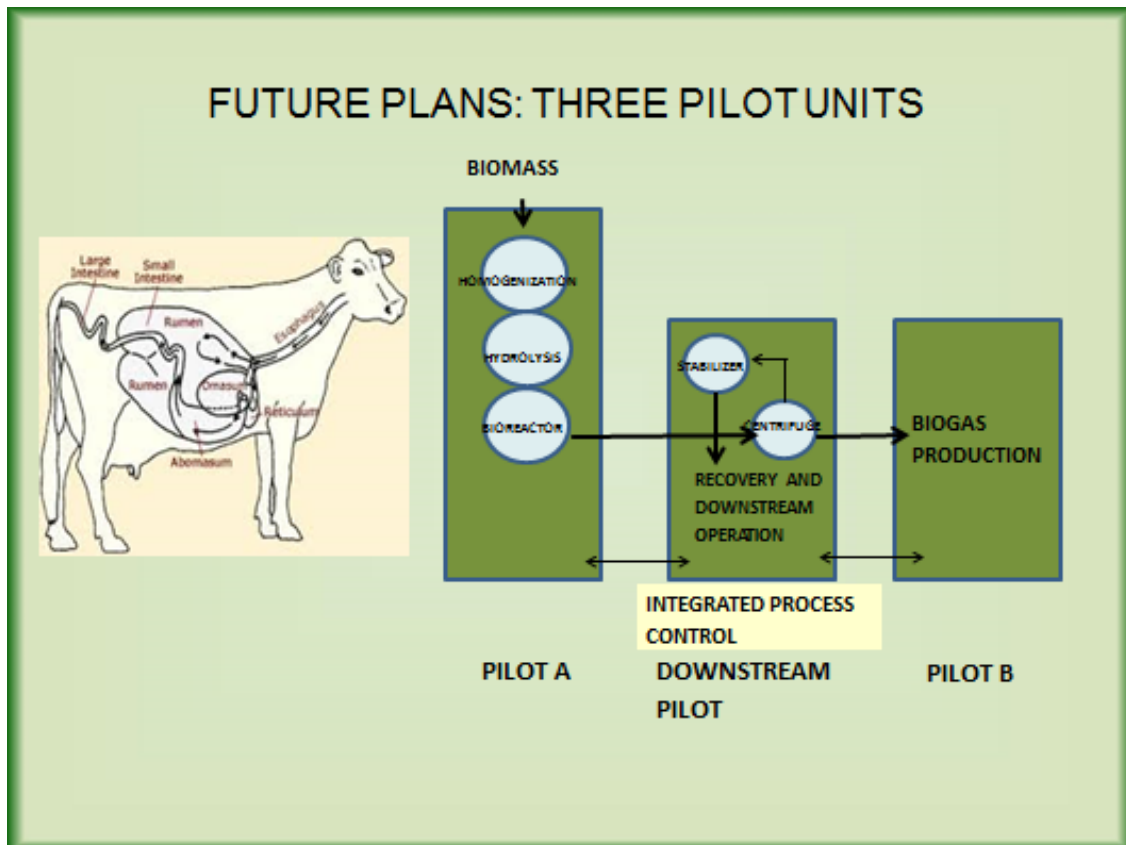


**Fig. 9. Laboratory Manager Anneli Heitto, of Finnoflag Oy, using the Gas Chromatography equipment.**

During the testing at Savon Sellu waste water treatment site, the initial break-in test runs and international training periods were accomplished in January and February 2014. The first 3-4 runs were targeted, besides these objectives, for pretreating the available biomass material in the Pilot A installations. The test runs were both anaerobic and aerobic ones. In all cases, regardless of the hygienization during the hydrolysis step, the natural microflora from the activated pools, especially the sulphur bacteria, contaminated the bioreactor broth. They were then restricted by the inoculated *Klebsiella/E.coli* strains which were preincubated in the reactor as a nutrient bed type of inoculum. This same strategy was later used in the two anaerobic cultivations with *Clostridium* sp. In these runs considerable amount of hydrogen were formed, besides several biochemicals (see the ABOWE Finnish Technical Report O3.5). However, the gas measurement on H<sub>2</sub> was restricted below 10 000 ppm which was exceeded many times during the runs. Therefore, the substantial potential of the biohydrogen production could not get estimated during ABOWE. Results from the last (sixth) Finnish run were analyzed by Harri Niska (University of Eastern Finland) using the Self-Organising Map (SOM) technique. These results are described as graphs in the Finnish Technical report.

According to the GHG (greenhouse gases) analysis on the climatological consequences of the biotechnological processes, a combined production strategy including both biorefining of chemicals from biomasses and biogas process based on its residues could add value, if technologies applied together. This approach could also bring along an effective solution for eliminating the waste problems. In this case the biorefining and the downstreaming should take place preferably in a consolidated bioprocess (CBP) where the waste macromolecules would be hydrolyzed simultaneously with the actual upstream process. In case of Pilot A the hydrolysis was partially going on also after the transfer of the pretreated biomass waste from

the hydrolysis tank into the bioreactor. Fast moving of the broth, where the biochemicals have been collected using the CBP principles, into the Pilot B type of biogas production unit from a Pilot A type of biorefinery, could contribute to the optimal result in the lowering of any climatological effect of the waste treatment (see Fig. 10) Then the remaining organic acids in the solid fraction could boost the biogas process. Also the elevated biochemical and gas production levels after optimization of the piloting and scale up trials would produce improvement in GHG reduction.



**Fig. 10. Future plans: three pilot units (Hakalehto 2014).**

During the tests at Powerflute Oy’s Savon Sellu plant in Kuopio the main focus was in the technical runs of the equipment. It was not possible to concentrate the raw material enough, but the dry weight remained at between 10-15%. Increasing this value would increase also the yield and productivity.

The main purpose of the ABOWE Pilot A tests was to give a reliable proof of concept on the industrially important substances producible in a sustainable way. This goal was achieved successfully, and several overall difficulties were overcome during the testing in three countries, from which Table 3 shows the Finnish cases.

**Table 3. Description of process development outlines in the Finnish runs at Savon Sellu factory site.**

	GENERAL PROBLEM	PRACTICAL SOLUTION	POTENTIAL SOLUTION
1.	DIFFUSION LIMITATION	<ul style="list-style-type: none"> <li>- GAS FLOW ADJUSTMENT</li> <li>- HOMOGENIZATION WITH EFFECTIVE HYDROLYSIS</li> </ul>	<ul style="list-style-type: none"> <li>- IMPROVED REACTOR DESIGN FOR FULL SCALE PLANTS</li> </ul>
2.	DISTURBING ORGANISMS	<ul style="list-style-type: none"> <li>- SPEEDED UP INOCULATIONS</li> <li>- NUTRIENT BEDS</li> <li>- HYGIENIZATION OF WASTE</li> </ul>	<ul style="list-style-type: none"> <li>- CONSOLIDATED BIOPROCESSING</li> </ul>
3.	PRODUCTIVITY PROBLEMS	<ul style="list-style-type: none"> <li>- MIXED WASTES</li> </ul>	<ul style="list-style-type: none"> <li>- SIMULTANEOUS DOWNSTREAMING FOR BLOCKING BIOLOGICAL DOWN REGULATION</li> </ul>
4.	TOO LOW RAW MATERIAL CONCENTRATION	<ul style="list-style-type: none"> <li>- PROCESS FLUID CIRCULATION</li> <li>- NUTRIENT BEDS</li> </ul>	<ul style="list-style-type: none"> <li>- BETTER PUMPS AND VALVES</li> </ul>

The Finnish tests have been reported in more detail in the ABOWE Report O3.5.



## 5 POLISH TESTING PERIOD AT ZGO GAC LTD

### 5.1 Site and feedstock description

In the biomass processing runs at GAC waste treatment unit near Wroclaw, in Southern Silesia, the main raw materials were potato peels from chips factory, and sorted domestic and restaurant biowaste. These substances were more easily degradable than the activated sludge derived from wood industry waste waters in Finland, or the protein- and lipid-rich slaughterhouse wastes in Sweden. The potato starch had been readily degradable source of hydrolyzable biomass in the previous experiments carried out by Finnoflag Oy in Finland (Hakalehto *et al.* 2013). Then record levels of 2,3-butanediol productivities had been achieved (8 g/l/h).

### 5.2 Test runs, results and conclusions from the Polish test runs

In the experiments with sole Polish potato waste (consisting mainly of potato peels, see the ABOVE Polish Technical Report O3.6), ethanol was the principal chemical product, besides the high amounts of hydrogen produced from the waste for extended periods of time. The latter one was not measurable due to the limited capacity of the gas measurement unit. In any case, the hydrogen production exceeded 10 000 ppm for long periods during each run. It should be taken into account that this flow of volatiles was produced into a carrier gas flow which was not diminished in the calculations.

In the beginning of the test runs in Poland it was believed that the heterogenous composition of sorted biowaste would disturb the process set up and control. However, this did not turn out to be a remarkable problem. Instead, the additions of miscellaneous food waste clearly boosted the production of various organic chemicals which reached a few percent of the total volume, and 15-20% of the dry weight. During these tests, as like at other experimental sites, the highest yields were not achieved or even tried to get achieved due to time limitations. In the future, efforts should be made also to concentrating the raw materials into adequately high substrate concentrations. However, with more time and some technical improvements into the Pilot A equipment, still much higher levels and productivities could easily get achieved. This is deducible also from the amounts of unused substrate in the process residues. However, even by the current experimentation several industrial levels of biochemicals were obtained.

The analysis results from Gas Chromatography (GC) and the Nuclear Magnetic Resonance (NMR) studies produced somewhat different results, in the former the levels being about 2-3 times higher. This is possibly due to the fact that samples for the NMR were stored in cold and transported to Finland, where they were analyzed much later on. It is then quite expectable that some changes could occur. Otherwise the NMR gave clear identification of the substances whereas the GC seemed to give some peaks close to each other which caused difficulties in identifying the compounds. This was the case especially with 2,3-butanediol and valeric acid, the latter of which was not expected to come out in the fermentation in large

quantities. However, it was produced in high amounts. The same occurred in Sweden where it was also measured by NMR. This organic acid was probably resulting from the condensation of acetic acid (two carbon molecule) and propionic acid (three carbon molecule). Both 2,3-butanediol and valeric (pentanoic) acid could be valuable products for producing butadiene (plastics, synthetic rubber) and in cosmetic products.

Otherwise GC turned out to be a rather reliable method and it was successfully used in the Pilot A in all three series of experimentations (in Finland, Poland and Sweden). In all sampling and sample treatments it was important to separate the solids quickly enough for preventing any degradation caused by bacteriological activities. The amount of products bound to the precipitating solid fraction could not be analyzed, and in future applications this issue related to the separation of the products in the liquid forms needs to be studied further. In any case, the Polish experimentation indicated clearly the potential of the biorefinery concept for producing soluble chemicals for industrial raw materials, as well as for the hydrogen gas generation from these processes. In fact, the hydrogen production started quickly, and it was produced on remarkable levels even though that this flow was integrated into the carried gas.



**Fig. 11. ABOWE representatives at ZGO Gac Ltd's waste management centre in Olawa, Poland.**

## 6. SWEDISH TESTING PERIOD AT HAGBY GÅRDSFÅGEL AB

### 6.1 Site and feedstock description

During the tests at Hagby farm, some 30% of chicken slaughterhouse waste was mixed with other biomass. The latter were chicken manure from the farm, some saw dust used as litter in the bird shelters, and occasionally some waste apples available from the farm. Also some potato flour, sugar or blueberry soup were occasionally used as additives. During the testing considerable problems emerged due to the inability of the pumps to work on the sticking feathers, as well as with the small stones originating from the bird digestion. Even though the pumps had enough capacity for forwarding the biomass, they got easily stuck with these miscellaneous particles or substances. Therefore, the final density and dry mass content was too low for higher product yields. However, the proof of concept was clearly demonstrated, and valuable products formed within the limits of the raw material offered. It was possible to convert a tedious mixture of protein and lipid wastes first into yellowish milky broth where no particles were detected practically in overnight, and further to a solution of organic acids and alcohols. This could take place without significant loss in the dry weight of the soluble substances.



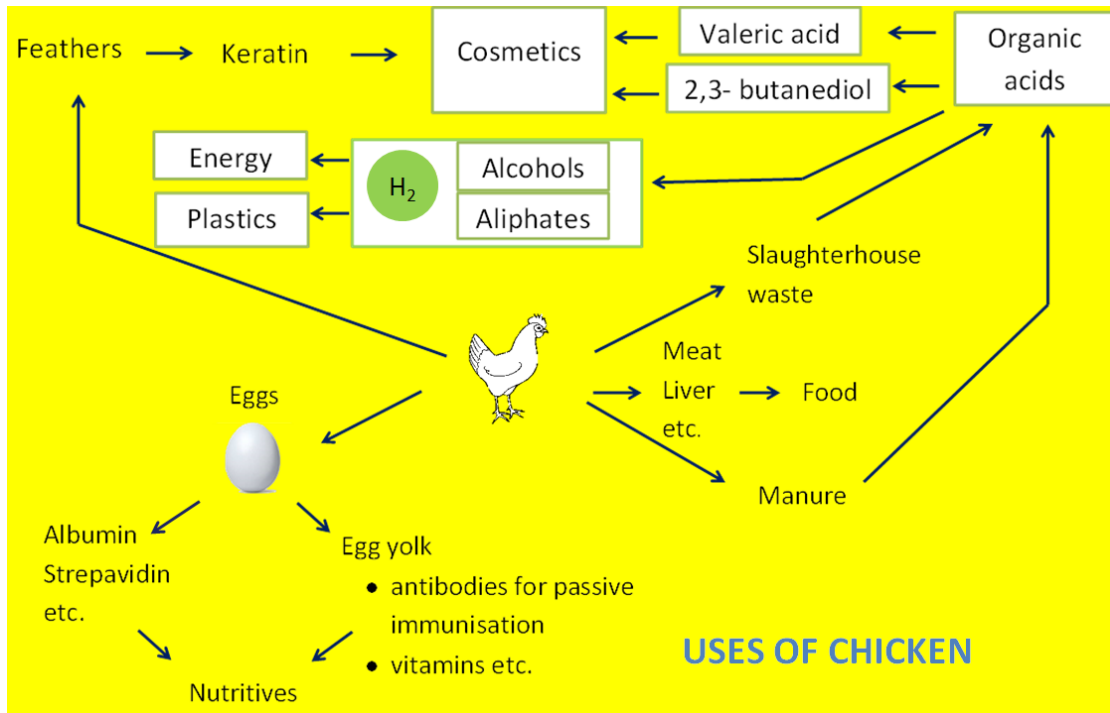
Fig. 12. ABOWE representatives at Hagby farm in Enköping, Sweden.

## 6.2 Test runs, results and conclusions from the Swedish test runs

The slaughterhouse located some 40km from the testing site. Therefore the chicken inner organs and other remaining parts were cooled for transportation. This cooling was probably fast or not effective enough, and provided time for the mixed flora to develop too far for the optimal raw material use in the biorefinery. In order to boost the biochemical production after the hygienization (in the hydrolyzer), strains of *Clostridium acetobutylicum* and *Clostridium butyricum* were inoculated. It is noteworthy that these bacteria have been reported to withstand some oxygen occasionally, even though they are generally considered as obligate anaerobes. They also could stay active under 100% oxygen flow (Hell *et al.* 2010). This was used as a selective factor during the experimentation. Earlier it had been reported that the clostridial growth was boosted by CO<sub>2</sub> as well, which has been exploited for the rapid onset of growth (Hakalehto and Hänninen 2012; Hakalehto 2015a). In some runs subsequent inoculations seemed to initiate the production of some chemicals which is implying to some quorum sensing type of signaling in the bacterial cultures. Also, addition of blueberry juice into some runs clearly had a positive boosting effect which is an indication of the need for some trace elements and minerals for best production levels.

The combination of 1. biorefinery process with subsequent 2. biogas production turned out a promising approach for treating this type of agricultural biowastes. Methane production from the biorefinery digestate was increased compared with the direct biogas production from the substrates. Hence, this combination of unit processes turned out a useful solution for the processing of biowastes.

Besides the expected products, short chained organic acids, hydrogen and some 2,3-butanediol, analysis by the NMR in Finland revealed some additional products such as valeric (pentanoic) acid and amyl alcohol. They were obtained partially from the apple waste, but could get produced also without the apples. In an overall consideration, the Swedish testing period gave a proof of concept on a reasonable method to deal with tedious wastes from slaughterhouse and bird farm in a short time. Also, a multitude of products could be obtained potentially from the chicken farm (Fig. 13).



**Fig. 13. Bioprocess and other uses of chicken. (Hakalehto 2015c).**

The Swedish tests have been reported in more detail in the ABOWE Swedish Technical Report O3.7.



## 7. PROOF OF TECHNOLOGY IN SEVERAL COUNTRIES

During the ABOWE Pilot A experimentation it was possible to give a three time proof of concept with various biowaste. It could be documented that the process optimization could take place in the next phase with some tested principles. Also several improvements to the equipment were suggested. Consequently, it could be stated that Pilot A biorefinery pilot could operate as an upstream biorefinery for all kinds of organic wastes. The microbiological processes are operable. Best results could be obtained by connecting this unit to simultaneous downstream processing , and biogas production, such as ABOWE Pilot B. This overall concept of Pilot A turned out functional at all three different Baltic Sea countries (Sweden, Poland and Finland), and technological cooperation was established between different institutes, also with Ostfalia University of Applied Sciences in Germany. The Pilot B was successfully tested in Lithuania, Estonia and Sweden (see the ABOWE reports of Pilot B). Many potential testing sites for future studies have emerged during the testing period, and this concept of microbial biorefinery technologies has given the proof of technology during the ABOWE project. The sustainable aspect was also fulfilled. Joint efforts of the Baltic Sea Region's ABOWE community in six countries was taking part in refining the technologies and in estimating their impacts.

### 7.1 Aspects of sustainability in ABOWE biorefinement

Since the biorefinery trials with Pilot A and Pilot B used local waste materials as substrates, no transportation or combustion of fossil fuels was required for that part. Also other transportation of substrates becomes unnecessary, provided that the sources were converted into energy at site (Hakalehto 2015b). Therefore, the actual idea forming about the energy balances according to the piloting experiments could be divided at least into five basic parts:

1. utilization of biomethane and biohydrogen from the wastes
2. solvents and organic mixtures from biorefining are combustible
3. co-combustion of some solid fractions from or outside the process
4. recirculation of the incineration outflow gases into bioreaction, e.g. carbon oxides
5. collection and reuse of the thermal energies from the industrial or waste treatment processes

These above-mentioned novel ways should be implemented into the planning and implementation of any new facilities planned to deal with industrial (or municipal) organic wastes. In such arrangements, the microbes are circulating, besides the substances, also in a way the chemical energies bound to them. This makes the emissions, their climatological consequences and environmental burden all the way declining. The ABOWE piloting has revealed the potential of total planning in biotechnical waste utilization and bioprocess design. For instance, the residues from the Pilot A type of biorefinery could be effectively used as raw materials in the Pilot B type of dry digestion biogas unit. This was confirmed e.g. by the results from the Swedish tests. Any solid fractions could then be used as organic fertilizer, composted or combusted, depending on the type of the particular fractions.



Gaseous emissions could be at least partially redirected into the biorefinery, with the recollection of their thermal and chemical energies. The carbon oxides, and some volatile nitrogen compounds, for example, could then be bound into the biomass in the Pilot A type of biorefinery.

Besides the industrial or municipal biowastes, also agricultural wastes could be recycled according to the ABOWE experiences. Then the organic fertilizers potentially are produced from the biological process e.g. as precious wastes could be returned back to the cycles in the fields and forests. This type of mineral addition is associated with the organic molecules, thus being slowly liberating source of power and building blocks for the farm or forest vegetation. In future, the industries will be interlinked with the agriculture and housing on the basis of the networks of circulating substances and of liberating chemical energies, as well as reducing gas emissions.

In case of recycling the biomasses in the ABOWE way from industrial, municipal or agricultural sludges, also the microbial cell mass load to the environment could be restricted, as the microbial biomass is being reused effectively in the combined biorefinery, biogas production, organic fertilizer output and combustion operations. This would further lower the effects of human activities on the ecosystems (Hakalehto 2015a).

## **7.2 Ecologically sustainable is also economically feasible - proofs of ABOWE**

Besides the intensified energy production from the organic wastes, which are facilitated by interconnected, interlinked and intercontrolled biological units, the biorefineries produce utilizable chemicals, whose value could be as high as up to 2000 € per ton in the case of 2,3-butanediol, or up to 5-7000 € per ton in case of valeric (or pentanoic) acid. These types of chemicals are being produced together with many other alcohols or organic acids, which could be converted into energy or into useful platform chemicals for the industries.

On the basis of above-mentioned economic values, theoretical calculations could be facilitated. For example, if some biorefinery broth resulting from glucose-rich biowaste could be used as a raw material for 2,3-butanediol production with possible recycling of carbon oxides, this could facilitate roughly 30% conversion of the dry weight substances (15%) into the product. This sums up to about 5-10% of the dry weight into the products (e.g. ethanol and hydrogen besides the 2,3-butanediol). If 5% is 2,3-butanediol, every volumetric ton of waste could produce 50 times 20€ during the process time (approximately 24 hours). This productivity had been enhanced to 8 grams per liter per hour in previous experiments with potato waste in the Finnoflag laboratory (Hakalehto *et al.* 2013), and it could be further elevated by:

1. consolidated bioprocessing (hydrolysis combined with biochemical synthesis)
2. simultaneous downstreaming
3. recycling of volatile emissions

During ABOVE Pilot A runs in Poland some 2,3-butanediol was formed, but it was approximately 10% of the concentration of the valeric acid at best according to the NMR (nuclear magnetic resonance) imaging. Butyric acid and ethanol yields were also remarkable. The reason for the relatively low yields when compared with earlier studies was the dilute biomass raw material from which almost all utilizable glucose was exhausted too swiftly. In any case, a remarkable proportion of the dry weight (15-20 %) was collected as useful products. In the Finnish and Swedish tests, the 2,3-butanediol was also found in relatively small concentrations due to the same reason. In Sweden, higher levels of valeric acid were measured, besides some other organic acids.

In fact, the valeric acid (being more valuably priced than 2,3-butanediol) could also be used as a platform chemical from waste utilization bioreactions. As like 2,3-butanediol, it could be also used as substrate for 1,3-butadiene (leading to synthetic rubber and plastic monomers). Valeric acid is microbiologically formed as a condensate of acetate and propionate. If we repeat the theoretical calculations above, and carefully estimate its potential yield to be 2% of total process suspension, the resulting economic output could reach 20 times 50€, making also 100€ per ton in a day, as purified chemical substrate. This equals with the theoretical economic output of 2,3-butanediol production. This output was given a proof in the Polish tests at the GAC waste treatment site near Wrocław in Southern Silesia during two month testing period as a result of cooperation of Polish and Finnish teams.

In budgeting bioprocesses, these economic calculations should take into account the relatively high recovery and purification costs (downstream processing) of the biochemicals. During ABOVE, this was also paid attention to in a parallel downstream experiment in Ostfalia University of Applied Sciences, Germany, where novel method for 2,3-butanediol downstream process was developed and successfully tested.

The rapid conversion of relatively difficult organic mixtures, e.g. proteins and fats in Swedish tests, or lignocellulosic substances with high sulphur content in the Finnish tests at Savon Sellu factory in Kuopio, was drafted, carried out and reported during the approximately two month testing period in each country. In Enköping, Sweden, similar productivities for valeric acid as measured in the Polish tests were achieved by cooperation between Swedish and Finnish teams. The valeric acid measurement, as well as other confirmation of the on site chemical tests in Kuopio, Silesia and Enköping by Pilot A gas chromatography was carried out at the School of Pharmacy of the University of Eastern Finland by Prof. Reino Laatikainen.

In the biowaste homogenisation and pretreatment, commercial enzymatic products were utilized besides the natural activities of the biomass microbes. The results were promising, with the formation of homogenous solutions or suspensions practically in overnight. However, the attempts to elevate the levels of glucose and other sugar monomers in the broth were left underway at each testing site during the two month testing periods. In any case, the inadequacies seemed to be possible to get overcome by careful planning of waste mixtures, better transport and storage conditions for it, as well by improved construction of the piloting/full scale production units. These could be further developed in future experiments.

One main purpose of the ABOWE project was to introduce some unique and innovative features into microbiological biorefining, such as

- use of undefined microbe cultures (UMC)
- fortification of the inocula with UMC with known production organisms
- selection of active microbial strains with gas applications
- search for novel products by the NMR (Nucleic Magnetic Resonance)
- rapid conversion of mixed organic wastes into useful substances
- consolidated bioprocessing (CPB) involving hydrolysis directly linked with the actual bioreaction
- remote satellite controlled processes
- simultaneous collection of products with the upstream production

In fact, the two last objectives were not fully achieved, due to technical obstacles. For example, IT connections to the testing sites in Poland or Sweden did not work without disturbances.

The product level optimization during the two month testing periods at each location would not have worked out properly. Therefore, main focus was in giving a proof of technology on the basic principles of the novel technologies.

*Summa summarum*, during ABOWE experimentation in three Baltic Sea countries, it was demonstrated that biorefining and biogas production from biowastes could be planned as an economically feasible integrated process from miscellaneous organic sources. This could be achieved regardless of the country or waste type, and the outlines of the production reactions were documented in the various test runs in different countries. Naturally, there is a lot of room for technical improvement of the facilities, biomass pretreatments, and other arrangements of the runs, but the limitations in time for the testing periods left optimization work to be carried out in future projects.

### **7.3 Possibilities to optimize the pilot plant process**

It is important to pay attention to the preliminary nature of the experiments. Nevertheless, a Proof-of-Concept actualized in all three countries with three different biowaste mixtures. This gives a sound basis for future developmental work on the basis of the novel biorefinery technology concept using the undefined microbes together with some known strains for the production of chemical goods in a low cost non-aseptic environment. Such arrangement underlines the sustainable values combined with reasonable investment and operation costs.

In order to enhance further the yields and productivities of the biorefineries specific attention should be paid to the bioprocess on the following points based on the experiments with ABOWE Pilot A:

- increasing dry weight
- improved bioprocess storage of the raw materials
- consolidated bioprocessing: simultaneous hydrolysis with the upstream reactions

- combining upstream and downstream activities (see also Fig. 1)
- integrated R & D system for 1. upstream process, 2. downstream process, and 3. biogas production (Pilot B)
- elevating the concentrations of readily usable carbohydrates in the bioprocess

For further consideration based on the Polish and Swedish test runs, see Appendices 3 and 4.

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## **APPENDICES**

- APPENDIX 1. ABOWE Pilot A Introductory seminar program 25.4.2014 at the Finnish Science Centre Heureka, Vantaa, Finland
- APPENDIX 2. ABOWE Pilot A Concluding seminar program 30.10.2014 at the University of Helsinki Viikki campus, Helsinki, Finland
- APPENDIX 3. Suggested technical improvements of Pilot A based on the Polish tests
- APPENDIX 4. Possibilities for optimization of biorefinery process





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**abowe**  
Implementing Advanced Concepts for Biological Utilization of Waste

**Novel ecologically sustainable biorefinery technology developed by Finnoflag Oy and the Pilot plant constructed on the basis of it is presented in an**

**INTRODUCTORY SEMINAR AND INVESTOR EVENT**

**at The Finnish Science Centre Heureka in Vantaa (Kuninkaalantie 7)**

**on Friday 25.4. between 9.00 and 15.00**

## **Programme**

9:00 Reception

9:30-9:45 Welcome and overview of Savonia University of Applied Sciences RDI activities, Research manager Eero Antikainen, Savonia University of Applied Sciences

9:45-10:00 Overview of ABOWE project, Project manager Ari Jääskeläinen, Savonia University of Applied Sciences

10:00-11:10 Finnoflag biorefinery technology and its impacts, Elias Hakalehto, Microbiologist and Biotechnologist, Finnoflag Oy and Adjunct Professor in Biotechnical Microbial Analytics, University of Eastern Finland

Break

11:25-11:45 Potential feedstocks for fermentation processes, Researcher Tuomas Huopana, University of Eastern Finland

11:45-12:00 Discussion

Lunch at Café Einstein (subject to a charge)

12:00-15:00 Visit inside the Pilot plant at Heureka outdoor exhibition area (in groups)



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## **ABOWE BIOREFINERY FINAL SEMINAR**

**Biorefining Around the Baltic Sea and Global Ecodevelopment  
at Viikki Campus Area, University of Helsinki  
30.10.2014**

**Venue: Lecture Hall 1, B-Building (Latokartanonkaari 7-9)**

### **8:30 Reception**

**9:00-9:10 Opening** Harry Ekestam, Counselor of Regional Development, Ministry of Employment and the Economy, Helsinki, Finland.

**9:10-9:50 Keynote Lecture 1. Biomass Utilization - Next Industrial Revolution.** Erik Dahlquist, Professor of Energy Technology, Research Director of the School of Business, Society and Engineering, Mälardalen University, Västerås, Sweden.

**9:50-10:20 Keynote Lecture 2. Concepts and Applications of Biotechnologies: Importance of Plant and Microbial Interaction in Phytoremediation.** Zhi-Qing Lin, Professor in the Department of Biological Sciences and the Director of the Environmental Sciences Program, Southern Illinois University - Edwardsville, U.S.A.

### **10:20-10:40 Coffee Break**

**10:40-11:00 Biomass Potential in North Europe and Globally.** Tuomas Huopana, Researcher of Environmental Informatics, University of Eastern Finland, Kuopio, Finland.

**11:00-11:40 Biocatalysis in Finnoflag Biorefining – Outlines of ABOWE Results and Future Perspectives.** Elias Hakalehto, Adjunct Professor in Biotechnical Microbe Analytics, University of Eastern Finland, Kuopio, Finland and CEO, Finnoflag Oy, Siilinjärvi, Finland.



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**11:40-12:40 Lunch (subject to a charge)**

**12:40-12:55 ABOWE - Arrangements of an International Baltic Sea Region Testing Program.** Ari Jääskeläinen, Project Coordinator, Savonia University of Applied Sciences, Kuopio, Finland.

**12:55-13:10 Nucleic Magnetic Resonance in the Biorefinery Result Evaluation.** Reino Laatikainen, Professor of Chemistry, School of Pharmacy, University of Eastern Finland, Kuopio, Finland.

**13:10-13:20 Finnish Testing at Savon Sellu.** Anssi Suhonen, Senior Lecturer of Mechanical Engineering, Savonia University of Applied Sciences, Kuopio, Finland.

**13:20-13:30 Polish Testing with Food Waste.** Emilia den Boer, Assistant Professor of Environment Protection Engineering, Wrocław University of Technology, Wrocław, Poland.

**13:30-13:40 Swedish Testing with Animal Waste.** Erik Dahlquist, Professor of Energy Technology, Research Director of the School of Business, Society & Engineering, Mälardalen University, Västerås, Sweden.

**13:40-13:55 Joint Discussion About the Economical Feasibility of the Biorefineries**

**13:55-14:10 Coffee**

**14:10-14:30 Consolidated bioprocessing - Integration of ABOWE Biorefinery Process with Simultaneous Downstreaming.** Tim Freidank, Engineer for Biotechnology and Bioprocess Engineering, Ostfalia University of Applied Sciences, Wolfenbüttel, Germany.



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**14:30-14:50 Co-Application of Biosolids and Drinking Water Treatment Residuals in Agricultural Land in the US Midwest.** Zhi-Qing Lin, Professor in the Department of Biological Sciences and the Director of the Environmental Sciences Program, Southern Illinois University - Edwardsville, U.S.A.

**14:50-15:05 Biorefineries in the EU policies.** Reljo Saarepera, Chairman of the Board, ERKAS, Estonia.

**15:05-15:20 Low Carbon Bioeconomics in Finland.** Peter Lund, Professor of Engineering Physics and Advanced Energy Systems, Aalto University, Helsinki, Finland.

**15:20-16:00 Our sustainable future - Panel Discussion**

**16:00-16:10 Concluding Remarks.** Osmo Hänninen, Professor Emeritus of Physiology, University of Eastern Finland, Kuopio, Finland.

Bus transfer

**Pilot A exhibition at Gardenia Tropical Garden (Koetilantie 1).**

Exhibition starts to groups of c. 6 persons at one time.

Cafeteria is open by the site.

Also the Tropical Garden is open by the site (subject to a charge).

### **About the event**

The event is open and free of charge for all interested stakeholders.

The event will be held in English.

Registration as soon as possible to [mervi.lappi@savonia.fi](mailto:mervi.lappi@savonia.fi)

There is more information about ABOWE project on [www.abowe.eu](http://www.abowe.eu)

WELCOME!



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APPENDIX 3

# SUGGESTED TECHNICAL IMPROVEMENTS OF PILOT A BASED ON THE POLISH TESTS

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*Władysław Kluczkiewicz*  
*Daria Lewandowska*  
Tero Kuhmonen  
Ari Jääskeläinen

March 2015

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## 1. Objective and scope of the report

The main goal of the ABOWE project was to provide the 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 were done based on pilot tests in three regions, one of which being the Lower Silesia region in Poland.

This Appendix provides a report on improvement potential of Pilot A technical details based on the Polish experiences.

## 2. Experiences from Pilot A operation

### 2.1 Recommendations for Pilot A operation

- Walls of the container heavily dissipated from the sun. In summer the air conditioning is not sufficient for the control room when operating the GC. The container should be set in the shaded place and the front door to the East to prevent getting too hot.  
An efficient cooling would require own cooling unit for the indoor air in the container. This would be c. 10 000 € cost but there is not very much room for such a unit.  
kustannus, mutta tilaa ei laitteelle kovin hyvin ole.
- The recommended pressure on the reservoir description of the balance tanks of the central heating is 1,5 bar nominally. During the start up operation it was first set at more than 3 bar (as a result they did not perform their function). From the experimental period in Poland it is recommended to set the pressure at 1 bar.  
There are separate circuits for heating and service water, it might be that the pressures of these have been mixed together somehow.
- The compressor should be drained occasionally (using the small valve at the bottom).
- The router needs to be reset occasionally, using the password for the router.
- When the process is running in the Bioreactor it is difficult to use the hydrolyzing reactor for the hydrolisation of fresh mass. Cooling is not efficient. It was found out, that it is helpful to close the valve with special gray tape. (Reason for that is that the flow of cooling and heating systems are combined, and the valves are probably not fulfilling their function properly). Closing the valve with gray tape is permitted only if the reactor did not enter cooling mode. Cooling the valve closed is causing even greater heating of the Bioreactor.  
It is not possible to use temperature adjustment in the both at the same time because the water volumes in the circuit are large.
- A simple solution to overcome overheating of tanks is to switch off the automatical temperature stabilisation and allow slow cooling down of reactors.
- pH sensors were causing much problems during the operation. The values shown were not realistic. The pH sensor in the Hydrolyzer requires permanent check and calibration. It is only possible to calibrate them with one point calibration. Frequent



discalibration results probably from the environment in which they are used: mixture of various solutions, stray currents, poor calibration, contamination of the head unit. The only solution is to check the pH of the samples manually, using a separate hand-held pH meter and on this basis controlling the readings of the pH control in the Hydrolyzer. Sensor after being sunk in pH 7 buffer or KCl had correct readings but it was not possible to eliminate the drift of the sensor during its use.

pH sensor in the Bioreactor operates mainly properly, only smaller deviation of measured values from the hand-held pH meater measured values was observed (however a few times the sensor was covered with contamination and this has caused an incorrect sensor reading).

Generally, pH sensors may have problems determining the correct values. Consultation with the technical department and the service did not give solutions to a recurring problem, probably the provider of the device should be contacted.

There might be problems in the connections of the Hydrolyzer's pH measurement. Otherwise these probably are relating to heterogeneity of masses as well as high temperatures and other loads to sensors caused by material properties.

It is in any case needed to occasionally check the measurements with a hand-held meter although the process sensors showed correct values.

- The LDO sensor requires calibration after setting the general parameters of external calibration (pressure or height above sea level or otherwise) is done in the air (in calibration bag) according to instructions provided in the manual.
- Regarding the control: Hydrolyzation process temperature relies on "temp-tank" measurement even when choosing as a dominant temp sensor "pH temp". With the stabilization process the sensor can be selected already. There is a difference of app. 8 degrees between the sensors in the Hydrolyzer for the same measurement displayed on the stored data.

In the Bioreactor there is also a difference in readings between the temperature sensors.

When mixing only with air, the "temperature-tank" sensor was not working properly, it was probably covered with the sedimentating biomass. Then the measurement was incorrect, either too high or too low.

The sensor is located lower than aerators so the mass is not mixing there.

- During hydrolysalation it is suggested to keep the heating circuit valve completely open until the temperature is reached and maintained to a set point (start of control as before). The waiting time for obtaining the set temperature has been quite long.
- Because of the large loss of heat to the environment (no insulation, partly applied thermal emergency blanket on the reactor), it was difficult of stabilise the temperature in the Bioreactor. The solution is to set the heating water temperature higher than expected for the heating water and current control for the other sensors. For example if the expected temperature was 37°C the set point was 38°C. After some time, it can be changed.

The real temperature has to be taken into consideration as this is not necessary the same as the sensor is showing. Mass mixing is mostly affecting to this.

- Accumulation of biomass in the place of outflow to the Stabilizer takes place. There is a need of thorough cleaning of this place.

## 2.2 Improvements made during the operation in Poland

- The Hydrolyzer pH sensor has been installed on a new structure in Poland, since the old one was not robust enough. However, for permanent use it should be replaced because it is made of elements with poor resistance to the conditions of use (corrosion).
- The drain pipe of the sink was replaced to allow sewage flow directly into the tank with the pump.
- Cooling system pipes were wrapped with insulation material to protect the formation of condensation of water vapor. The water condenses on the extending steel metal.

## 2.3 Defects in Pilot A detected during experiments

- Window in the control room has a small leak during the rain. The opening loft on the roof of the container also has a leak during rain.
- UPS battery needs replacing, it does not maintain operation. It will be changed before the next use of Pilot A.
- There is a need for permanent control of the parameter  $p_{10} \Rightarrow 0$  of inverters.
- Screw pump serving as a reject pump in the Bioreactor does not start automatically, it need to be moved several times by hand.  
This has been cleaned from crushed stones and other particles.
- Electrical protection system detected three times damage to the electric insulation, although everything was working properly.
- A few times there were problems with control system. The Profi Bus needed to be reset when one of the processes network Profi Bus crashed which caused the system to be blind to the actual measured values. (Temperature stabilization based on temperature sensor pH. Value read by Lab VIEW was in this case  $0^{\circ}$  C. Provoked the opening of the valves with warm water and warm up of the Bioreactor to more than  $60^{\circ}$  C.) There were also other troubling situations associated with defects of the network Profi Bus.
- Clogging of valves for sampling.
- Bioreactor: There are serious doubts whether the measurement of outflowing gas concentrations is appropriate; in connection with the different densities of certain gases, accumulation can occur in the area in which the measurement is performed. A check by device provider is needed.

## 2.4 Recommended improvements to be made in Pilot A

- Installing additional sampling valve: sampling was often difficult due to the clogging of all valves. Additional sampling valve on the lower circuit, at a higher pressure could be helpful. This would make cleaning easier as the test substance is spilled on the floor immediately.  
This is actually possible to do already currently by closing the circuit valve and driving carefully with the pump with sampling valve opened.
- Add additional Svi (additional automation function), which for a given pH automatically adds acid or base at predetermined dosage, check with pH sensor provider, if the value keeps drifting (showing incorrect values after the initial

calibration). Proposed dosage of base in an amount of about 1l/h after, about 20 minutes, changing to 3l/h if necessary. Similar controls when  $\text{pH} > 6$ ; pumping the acid.

This has been the original intention as soon as it will be exactly known how the process will be run in the specific case and with longer testing period allowing optimization.

- For aeration of fermenters a separate circuit for compressed air could be added beyond the one controlled by Lab View. It could have a small rotameter at the input to determine the overall flow, or several rotameters for different outputs.
- The control system has a protection against overheating of the boiler, possibly it could be used also to control the temperature in the boiler to the set point. It is difficult to adjust all the thermostates to a specific temperature, and this causes a very large number of starts during a certain period.

Thermostates are not intended to be continuously adjusted but when the Hydrolyzer is needed then temperature is  $90^{\circ}\text{C}$  and otherwise temperature is  $60^{\circ}\text{C}$ .

- An additional measurement of the pressure in the water system with alarm on the desktop when the pressure drops below the preset value would be helpful.
- Measurement system of the pressure at the reject pipe can help to detect blockage on the outlet pipe.
- The switch of the shredder is not very convenient when it comes to continuous work, another switch type e.g. allowing switching off everything would be more convenient for operation.
- Blades in the Homogenizer mixer could be smaller, with serrated edges and fixed in a way to work as low as possible. With 300 litres' fill in the Homogeniser only the lower blades were working effectively but this does not matter however.

Regarding the process:

- The first tank (The Homogenizer) was not adequately used, all biomass was inserted into it after a while it was pumped into the Hydrolyzer. The shredder could be placed directly before the Hydrolyzer. During the hydrolysis process the mass is still mixed and homogenized. It would prevent the necessity of adding extra water to make the biomass "pumpable" from the Homogenizer to the Hydrolyzer.

This does not remove the need for adding water because mass is pumped also in other process phases and the Bioreactor cannot be used with feed material with too high dry matter content.

- Modification of the shredding process: The shredder itself works efficiently. Difficulties with larger waste feed are mostly caused by clogging of the feed pipe. For safety the longer tube could be used but without the rods. The shredder and especially the feed hopper have been installed far too high, which makes the feeding of the biomass difficult.

It is not, however, very easy to install the shredder lower.

- In the Hydrolyzer another type of stirrer could be installed, e.g. in a form of a tiltable plate skimmer (mounted on the bearing slide) to enable stirring of denser pulp mixes. Also a new way to mount the sensor of pH and temperature is needed, for example in the form of a dovetail.

- The level sensor has difficulty in determining the appropriate value under agitation. Perhaps the usual measurement of hydrostatic pressure would be a better solution. By this the liquid level in the tank could be determined.  
The vague measurement, however, is probably due to tank reflections instead of the type of the sensor. It is on the other hand presumable that the measurement of hydrostatic pressure would function even worse with the masses used.
- The flow of liquids from one tank to another could be based on gravity.
- The heating system for the Hydrolizer and the Bioreactor could be replaced with just electric heaters. Only the cooling system could remain in its current state. It would prevent the current problems with setting appropriate temperatures in the Hydrolyzer and the Bioreactor.  
This is, however, not possible because of the ATEX-restrictions.
- For the inoculum fermenters – the connection of disks could be replaced by threaded fast connection. The outflowing air could be directly fed outside the container (not through the venting system)
- Sedimentation of mass in the Bioreactor is observed, despite mixing with the gases and with circulation pump. It is proposed to modify mixing by using the lowest position valve and introducing gas under pressure.
- Better thermal insulation would be advantageous, especially regarding the Bioreactor.
- For the electrical supply: sensitive equipment such as PC analyzer could be on a separate circuit, separated from the circuit with heavy loads; heaters, inverters, motors.
- Separation could be done by two circuits for the container. The sensitivity should be shifted not to duplicate each other and interfere with system security.
- All safety systems should be outside the control of LabVIEW. At the moment additional ventilation will not start if the control system fails.

# POSSIBILITIES FOR OPTIMIZATION OF THE NOVEL BIOREFINERY PROCESS

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## 1. Introduction

The biorefinery concept has been piloted in three different locations in Finland, Poland and Sweden, respectively, with three different substrates. In this report discussions and reflections concerning optimization of the process are presented.

In Finland the biorefinery pilot plant used waste water treatment sludge from a cartonboard factory, including wooden parts, as substrate.

In Poland the biorefinery pilot plant was run on waste from potato chip industry.

In Sweden the main substrate was slaughterhouse waste from an ecological chicken farm. Also manure from the farm was added.

The first runs with a new material seem to be always a bit of a hassle in each testing site. After all, this is experimentation. The limits or the protocols of reasonable biorefining for any particular waste were set up. The result is always dependent on many factors, and like e.g. in Poland some relatively small adjustments can produce the successful result "in the end of the day".

## 2. Parameters for optimisation

### 2.1 Efficiency

The Pilot A runs done within the ABOWE project show that it is possible to produce several valuable products. What needs to be further evaluated and stated is the possible efficiency for the process. Here the possibilities for continuing separation of the products and how to upgrade them are important.

The efficiency concerns both material conversion and energy balance.

### 2.2 Process path

The main challenge for the process with a high possibility for further optimization is what process path to use for what substrate. The process path depends on the mix of substrates and the enzymes and microorganisms used.

The basic concept for the process includes additions of specified enzymes and microorganisms to enhance specific routes for substrate degradation and product formation. Here many different combinations of enzymes and microorganisms are possible and also when to add them can be crucial for the process route. Mixed cultures can for example be advantageous for mixed substrates, to make it possible to utilise both carbohydrates and proteins during the fermentation phase of the process. Some culture mixtures can also be advantageous for avoiding inhibition. The main route aimed for in the piloting has been to produce 2,3-butanediol. In the analysis of the product from the runs also other valuable compounds such as 1,2-butanediol and valeric acid, both possible to use for butadiene production, have been found. Another product of interest that formed during the pilot runs in Finland and Poland was hydrogen. The process should be optimized concerning what enzymes and microorganisms to use for what substrates. This includes several repetitions of process runs done in the same way to give proof of concept for the different process routes. Next step would be to optimize the control of the process for the different process routes.

The substrate used in Sweden was different from the substrate used in the two other piloting cases mainly concerning the content of carbohydrates. Laboratory test on the substrate used in Sweden showed enough production of carbohydrates in the hydrolysis steps but this was a very slow process in the pilot runs and for the first three runs carbohydrate sources were added to secure continuation of the test runs. However, for the last pilot run in Sweden the initial hydrolysis step was done in a different manner and for longer time and the additions of enzymes and microorganisms was also changed resulting in that products could be observed also without addition of carbohydrate sources.

To optimize the process to avoid formation of large amount of H<sub>2</sub>S is also important.

Also what parameters to measure for following the process and control it is important to develop. The glucose level is a good indicator for the carbohydrate content but when the protein and fat content is high, as for the substrate used in the Swedish case, other substances and the gas formation might be better to follow.

## **2.3 Substrate characterization and pre-treatment**

To be able to optimise the process path for the substrate it is necessary to know its characteristics but also the handling of the substrate before feeding it to the process is important.

The size of the feeding material is an important factor for providing accessibility for the enzymes and microorganisms to the material. In the pilot plant a mill was used to reduce the size. However, all substrate tested, for example feathers from the chicken slaughterhouse, was not suitable for the mill.

For the substrate used in the piloting in Sweden the handling of the waste before feeding was important. For the slaughter house waste it was crucial that it was kept cold to avoid degradation before feeding.

The hygienisation of the substrate, by heating, is very important for the process. By this pre-treatment the non-wanted microorganisms are removed and the by this the conversion process in the reactor can be better controlled.

## **2.4 Process steps and design**

There were some problems in the pilot runs due to that the design of the plant was not optimal. The size reduction part of the process needs to be optimised based on what substrates to be expected. Some of the pumps in the plant got stuck with substrate parts. This should be avoided in future process design.

The hydrolysis step is a process step with potential for optimisation. In the last run for the Swedish case the hydrolysis was done in several separate steps for part of the substrate that was then mixed in the reactor. This gave the possibility to produce valuable products without addition of an additional carbohydrate rich substrate. How long time to use for the hydrolysis is also a subject for optimisation. If the hydrolysis step is allowed to go on for too long time the risk can be that the degradation of the substrate goes too far to be optimal for the process route of interest in the reactor. On the other hand, if the time is too short the levels of compounds, for example carbohydrates, necessary for the microorganisms in the reactor will be too low.

### **3. Modelling and simulation**

To be able to control and optimise the biorefinery process in the design of the process or at process changes, as for example changes of substrate type or amount, a model of the process and simulation of the process outcomes can be a valuable tool.

It is possible to model the biological/chemical reactions of the process but there is currently no such model available for the biorefinery process tested in the ABOWE piloting. It might require more process knowledge to build up. The model probably need to be adapted to the individual substrate cases. Syu (2001) conducts a mini-review of the biological production of 2,3-butanediol including a shorter section on modelling. Previous models have been based on bioenergetics and also a neural network has been used to model the process.

It could also be possible to use a simpler model for the biological side, mainly based on empirical data from laboratory scale and pilot scale process runs. By using this approach it is assumed that the same yields will apply at a larger scale and that the amount of gas and other consumables will be the same per unit as well. Then an energy part of the model could also be added that includes heat loss based on the size of the reaction chamber and the hydrolysis unit. The energy balances could then be estimated.

## 4. References

Syu, M.-J. 2001. Biological production of 2,3-butanediol. *Applied Microbiology and Biotechnology*. 55(1). 10-18.