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*Chapter 11*

# ABOWE Project Concept and Proof of Technology

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## Abstract

In an industrial process development, novel concepts usually give rise to needs for new tools and techniques. Using those, in turn, induce further research on the basic principles of the reactivity, interactions and cascade functions of the process itself. If it is a microbiological one, the continuous testing and re-evaluation of the basics is advisable. In case of big volumes, the research and development cannot take place in the laboratories only, but larger pilot scale is required for simulation of the scale up. The technologies that were used in the ABOWE project emerge from this basis, and from the versatility of the biological systems using mixed substrates. Since non-aseptic approach was chosen, in order to lower the investment costs, this multifunction process situation described above was to be controlled by a simultaneous adjustment of several functions with coherent effects on the production of the desired substances. The project team constructed the pilot plant accordingly from the basic ideas, and it was then tested in three countries successfully.

## 1. Introduction

ABOWE project (Implementing Advanced Concepts for Biological Utilization of Waste) belonged to EU Baltic Sea Region Programme 2007-2013. ABOWE was 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.

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. In this Chapter essential information from Finnoflag biorefinery technology test runs with Pilot A in three Baltic Sea countries during 2014 has been gathered together.

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 goal of the ABOWE project and the movable Pilot A manufactured in Finland by Savonia University of Applied Sciences (UAS) was 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 was 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, was used for producing synthetic rubber, plastic monomers, anti-icing chemicals, textiles, cosmetics and other commodities. Downstream techniques were 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 Finnoflag 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 (Finnoflag Oy and University of Eastern Finland).

The Pilot A engineering team consisted of Finnoflag 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 Pilot A engineering and manufacturing. Essential information from Finnoflag biorefinery technology test runs with Pilot A in three Baltic Sea countries during 2014 have previously been gathered together in the ABOWE report (Hakalehto et al. 2015a).

## 2. 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 the era of modern reindustrialization.
3. BIOREACTOR (Figure 1) 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.

Figure 1. The BioReactor of Pilot A. Photo: Ari Jääskeläinen in ABOWE project.

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 entire microbiological and biotechnical process control was designed by Finnoflag Oy.

The microbiological inocula are produced first in the PMEU equipment (Portable Microbe Enrichment Unit) (Samplion Oy, Siilinjärvi, Finland) (Figure 2), 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 (Hakalehto and Heitto 2012). The PMEU was originally planned for environmental, clinical, food, and other hygienic control purposes (Hakalehto 2012; 2013a). For the ABOWE Pilot A, a new version designated as “PMEU 5” was planned.

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.


Figure 2. PMEU – Portable Microbe Enrichment Unit. Manufactured by Samplion Oy.

The process fluid was 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 was provisionally experimented.

Figure 3. The Process Room of Pilot A. Photo: Ari Jääskeläinen in ABOWE project.

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 Bioreactor 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. In Figure 3. is presented the Process room of the Pilot A.

## 3. Tests in Finland at Savon Sellu Oy Cartonboard Factory's Waste Water Treatment Plant

### 3.1. 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°C and -30°C the functions of the Pilot A were put into a real "climate test." Fluids tend to get iced in the tubes during their pumping into the unit. Also the raw material for the experiments, the dried sludge from the waste treatment plant was cooling down rapidly in the piles where it was collected from (Figure 4 and 5). Another raw material for the experiments was incoming waste water.

Figure 4. Savon Sellu Oy's cartonboard factory in Kuopio. Photo: Ari Jääskeläinen in ABOWE project.

### 3.2. Test Runs, Results and Conclusion 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 5. Dried waste water treatment sludge piled at Savon Sellu Oy's waste management area. Photo: Ari Jääskeläinen in ABOWE project.

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. However, the gas measurement on $H_2$ 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.

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. 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. An example of the process development at Savon Sellu testing site is presented in Table 1.

**Table 1. Description of process development outlines in the Finnish runs at Savon Sellu factory site**

| | General problem | Practical solution | Potential solution |
|---|---|---|---|
| 1. | Diffusion limitation | Gas flow adjustment<br>Homogenization with effective hydrolysis | Improved reactor design for full scale plants |
| 2. | Disturbing organisms | Speeded up inoculations<br>Nutrient beds<br>Hygienization of waste | Consolidated bioprocessing |
| 3. | Productivity problems | Mixed wastes | Simultaneous downstreaming for blocking biological down regulation |
| 4. | Too low raw material concentration | Process fluid circulation<br>Nutrient beds | Better pumps and valves |

The Finnish tests have been reported in the ABOWE project report (Hakalehto et al. 2015b). See also the chapter 10 of this book

# 4. Tests in Poland at ZGO GAC Ltd's Waste Management Centre

## 4.1. 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 hydrolizable 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). This degradative process was based on the studies with the members of *Enterobacteriaceae* family of facultatively anaerobic bacteria, particularly of the genus *Klebsiella* (Hakalehto et al. 2008; Hakalehto 2013b).

## 4.2. Test Runs, Results and Conclusion from the Polish Test Runs

24 students and six experts from Wroclaw University of Technology participated in the tests together with Finnoflag Oy. The substrate was of a “carboxylic platform” type (den Boer et al. 2016).

In the experiments with sole Polish potato waste (consisting mainly of potato peels) 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.

Figure 6. ABOWE representatives at ZGO Gac Ltd’s waste management centre in Olawa, Poland. Photo: Ari Jääskeläinen in ABOWE project.

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, the latter conducted by Prof. Reino Laatikainen, School of Pharmacy, University of Eastern Finland, produced somewhat different results. In the former the levels being about 2-3 times higher in some runs. 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. The Polish tests have been reported in the ABOWE project report (den Boer et al. 2015). See also the chapter 13 of this book, as well as the manuscript by den Boer et al

## 5. Tests in Sweden at a Bird Farm

### 5.1. 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 were 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.

Figure 7. Group at Hagby farm in Enköping at 7:00 on 9.9.2014. From left to right: Elias Hakalehto, Erik Dahlquist, Anneli Heitto, Yuying Li, Henny Andersson, Fadi Atif Fakhir.

## 5.2. Test Runs, Results and Conclusion 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 $CO_2$ 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 quarum 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.


Figure 8. ABOWE representatives from six Baltic Sea countries at Hagby farm in Enköping, Sweden. Photo: Ari Jääskeläinen in ABOWE project.

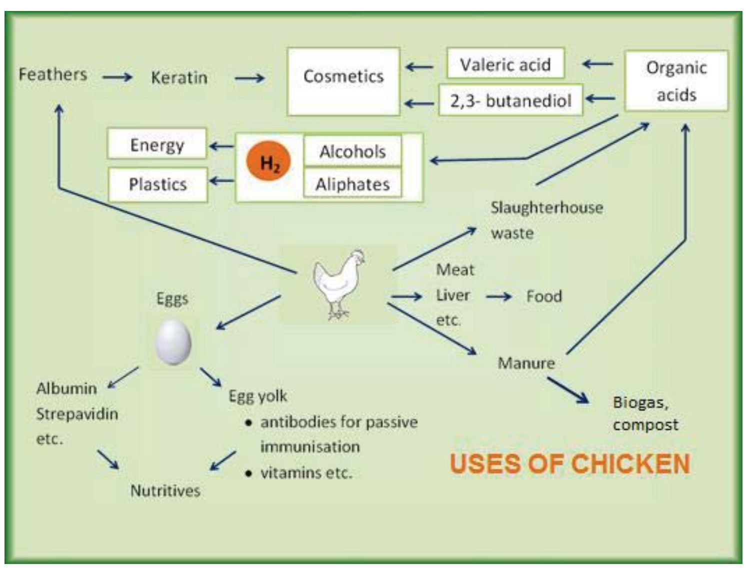


Figure 9. Bioprocess and other uses of chicken. See also Hakalehto (2015b).

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 (Figure 9).

The Swedish tests have been reported in more detail in the ABOWE project report (Anderson et al. 2015). See also the chapter 15 of this book, as well as the manuscript by Schwede et al. (2016).

## 6. Future Plans: Three Pilot Plants

One basic idea is to combine the above described upstream process with a parallel downstream operation(s). This helps to avoid biological regulation mechanisms of the production organisms, since the products are recovered at the same time as they are formed into the process broth or suspension. Then the end-product inhibition or other suppression mechanisms of the cell metabolism cannot influence the result. Therefore, it is also possible to achieve higher productivity in the bio-processing using the concept of Consolidated Bio-processing (CBP) (Hakalehto 2015a). The following figure depicts the process in detail:

Various biomass rounds need to be re-circulated in the process after the valuables have been collected from them. The entire bioprocess also needs to be operated in a continuous or semi-batch mode. New raw materials are fed in gradually. This new level of functionality requires more advanced planning, selection and construction e.g., of the pumping and other technical equipment, as well as process control system where the recovery operations for the down-streaming have to be interlinked with the real-time data on the product formation.

As a result practical solutions where any biomass waste can be converted into valuable energy products, chemical raw materials, or fertilizers can be obtained. Also thermal energies are produced in the process. In the heart of the operations is the consolidation of up- and downstream processes under effective automation and operator control. The human factor as the supervisor for the system is required during the runs, since we deal with biological materials whose behavior cannot be fully predicted. However, a practical way to convert any organic waste treatment unit into an ecologically and economically feasible industrial unit can be made conceivable and achievable using the natural microbes, their metabolism and enzymes for the process design.

Besides achieving the scientifically ambitious objective put forward in the previous passage, the solution has also to be economically feasible and viable, to obtain the desired objectives of "valuable products." Therefore also various sophisticated data-analysis and processing methods have to be used in combination with the ones previously described.

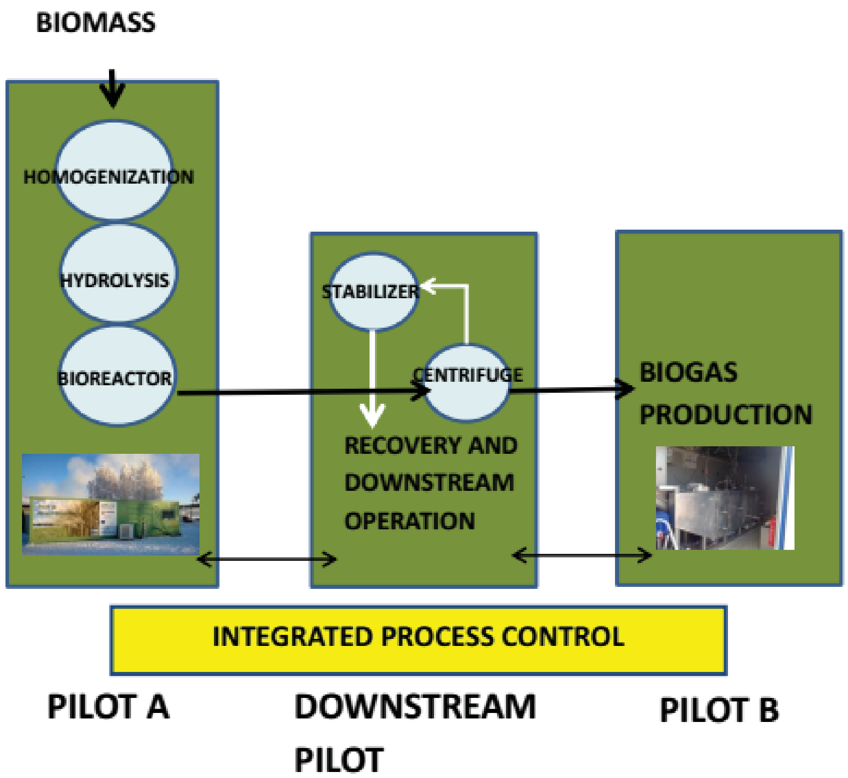


Figure 10. Schematic overview of the entire prototype of the novel biorefinery test concept.

## 7. Proof of Technology – Ecologically Sustainable Is Also Economically Feasible

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 Figure 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

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 5000-7000 € 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 produce 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 5 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, and it could be further elevated by:

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

During ABOWE 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 (nucleic 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 most 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. As such, the organic acids provide an opportunity to get converted into alcohols successfully (Perez 2012). In the Master Thesis by Cornell University in 2012, an anaerobic strain of *Clostridium ljungdahl* was experimented for that purpose.

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 2 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 Wroclaw 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 ABOWE, 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 sulfur 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 chemical on site 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 (CBP) 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 in 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.

# 8. Full-Scale Biorefinery Plant's Pre-Engineering and Investment Calculation Using Savon Sellu Fluting Factory's Waste Treatment as an Example

Pre-Engineering work of an industrial scale biorefinery was done based on Savon Sellu's annual waste water sludge amount, 30 000 t/a (25% Total Solids). The biomass which was used during the pilot tests was the dried sludge from the waste water treatment plant which consists of:

- Surplus of pulp factory's circulating water
- Hard wood pulp reject
- Fuel gas scrubber's washing waters
- Surface waters of the sludge field
- Factory's sanitary waters

The aeration process of the active sludge treatment plant reduces the amount of valuable chemicals that could be recovered; therefore this study will concentrate on a solution where the biomass will be collected from the vertical clarifier surplus, before the active sludge treatment. In the calculations, it is assumed that we can collect equal amount of dried sludge compared to collected sludge from the present waste water treatment plant.

Investment cost for an industrial scale biorefinery based on ABOWE Pilot A technology was calculated by M.Sc. Jyri Pelkonen from Pöyry Finland Oy (Table 2). Initial technical values and the process description were given by M.Sc. Anssi Suhonen of Savonia UAS. The microbiological process is invented and patented by Adjunct Professor Elias Hakalehto, Finnoflag Oy.

**Table 2. Overall estimate of the investment requirement of the whole plant (30 000 t/a)**

| | |
|---|---|
| Construction work | 2 340 000 € |
| Machinery and equipment | 1 410 000 € |
| Electric, automation and instrumentation | 550 000 € |
| Overhead costs (app. 15%) | |
| Including engineering and erection work | 650 000 € |
| Cost booking (app. 15%) | 660 000 € |
| Indirect costs | 1 300 000 € |
| Biorefinery total | 5 600 000 € (not including vat.) |

The pre-engineering was done based on the results of the pilot runs performed between February and March, 2014. The technology is new and the dimensioning of the plant is based on the data received from Savon Sellu, Finnoflag Oy and Savonia UAS. Pöyry's expertise and design experience of different bioprocesses and sludge fermentation was used in the dimensioning principles.

The crucial factors in plant operation that have risen up during the pre-engineering phase were:

- handling and pumping of sludge at 10 … 15% TS higher dry mass content
- energy consumption and recovery in sludge heating and cooling
- bioreactor design with internal chambers in pool shape reactors -> higher TS%
- air lift principle in fermentation works when dry mass concentration is at levels of 4…6% TS
- the operational costs of liquid nitrogen and carbon dioxide will be approx. 50 … 60 000 € / a
- bioreactors' mixing using only nitrogen in large reactors
- handling of sludge residue left over after the bioprocess
- collection of biogas for energy use in post treatment of sludge
- scaling up pilot A's technology and technical solutions for a full scale plant

The result of the pre-engineering has still some assumptions in technological solutions and cannot therefore be precise in all aspects. The investment calculations have approximately a 30% deviation on accuracy. The downstream process for chemical recovery has not been included in these calculations.

## 9. Prologue: Proof of Technology: Aspects of Sustainability in ABOWE Biorefinement

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 were contributing to the refining of the technologies and in estimating their impacts.

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. 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.

In case of the ABOWE Pilot A, the microbiological community, or microbiome, usually consisted of at least partially unidentified microbial cultures or strains (Undefined Mixed Cultures, UMC). It was found earlier that digester microbiomes based on bacterial sequences clustered by substrate type (Zhang et al. 2014). This result relates the studies of the microbiomic production in biorefineries around specific substrate types. However, in ABOWE the process runs were usually inoculated with specific strains regardless of the fact that they were non-aseptic ones from the substrate point-of-view. This caused a situation, in which the seeded incoming strains had to compete or adapt with the previously existing flora.

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. During the Swedish runs, it was detected that the prior bioprocess enhanced the methane production capacity of the undefined mixed cultures (Schwede et al. 2016). This finding is in line with the report of Labatut et al. (2014), stating that co-digestion of dairy manure with easily-degradable substrates increases the specific methane yields when compared to manure-only digestion.

In comparison with the Polish runs, where a strategy of using the “carboxylate platform” turned out to be highly successful (den Boer et al. 2016), the Swedish tests gave a proof that the amino acids were a viable substrate for extensive production of valuable organic acids (Schwede et al. 2016). The use of the carboxylates as starting materials was recently introduced in a review article by Agler et al. (2011).

In order to find space for improvement in the biorefinery activities with an ABOWE type of process solution, new type of process approaches should be attempted. For example, in case of wood waste conversion to short-chained fatty acids, and simultaneously to hydrogen, it was found out that alkaline conditions produced the best overall result (Dahiya et al. 2015). A versatile and flexible analysis of both bioprocess and environmental microbes is warranted (Hakalehto 2015c).

When 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.

## CONCLUSION

The ABOWE Pilot A plant fulfilled its purpose in giving a proof-of-concept on the biorefining of various biowastes into useful products. These results are introduced in other chapters of this book more precisely, but the important new front has been created, where the challenges of the microbial bioprocessing are to be solved with a combination of the seed cultures with the undefined mixed culture (UMC). Both the ecological and economic feasibilities of the process type were studied and estimated.

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