Small-scale Biorefining

Editors: Chris de Visser en René van Ree

WAGENINGEN
UNIVERSITY & RESEARCH

Small-scale Biorefining

Editors: Chris de Visser en René van Ree

WAGENINGEN
UNIVERSITY & RESEARCH

Content

1 The Potential Role of Small-scale Biorefineries to the Dutch Circular BioEconomy 7

2 Does size matter? Success factors for small-scale biorefineries 11

3 Side-stream valorisation: materials screening, fractionation and products 19

4 New value chains in the Circular Economy - valorising waste water and side streams with aquatic biomass 23

5 Direct processing of sugar beet for the fermentative production of chemical building blocks 33

6 Refinery of green leaves to produce protein products 41

7 Acidified water extraction and purification of steviol glycosides from fresh Stevia 49

8 Fatty acid and PHA production from residues 55

1

The Potential Role of Small-scale Biorefineries to the Dutch Circular BioEconomy

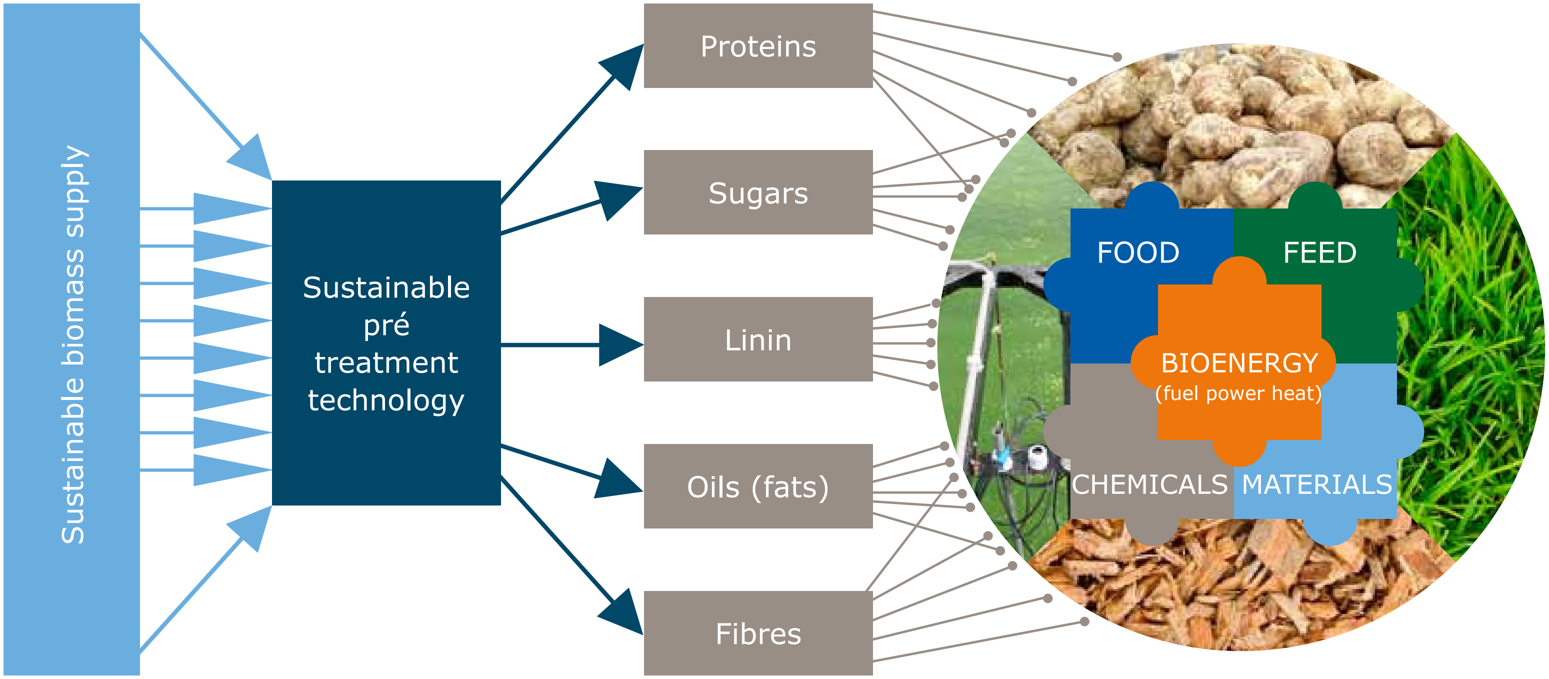
Authors: René van Ree and Chris de Visser

Biorefining Processes in the Circular BioEconomy

Efficient and sustainable use of biomass resources is one of the pillars of a future Dutch Circular BioEconomy (CBE). This is required for both primary land and aquatic crops, secondary agro, forestry and process residues, and tertiary post-consumer residues. These resources can be used for the production of biobased products (food/feed ingredients, chemicals, materials) and bioenergy (fuels, power heat), via biorefinery processes be it at either small, regional or large scale.

Biorefining processes are at the core of the "Strategic Biomass Vision towards 2030" of the Dutch government (2015)[1], and also clearly addressed by the Dutch Sustainable Biomass Commission (2015)[2]. Based on this, the Dutch government has developed an R&D Agenda Biobased Economy 2015 – 2027 "B4B" (2015)[3]. The basis for this agenda clearly recognises that the emergence of the Biobased Economy will not only be (part of) the answer to various environmental threats, but will also contribute to the growth of the Dutch Economy, potentially resulting in 4,500 new jobs and 2.5 Mt CO_2-reduction.

Figure 1.1: Biorefining approach as pillar of a Circular BioEconomy [WUR, 2016].



Current Deployment Status Biorefineries

The Circular BioEconomy is not new. It is already worth about 2,000 billion Euros in annual turnover (biobased industries: 600 billion Euros), and accounts for more than 22 million jobs in Europe[4]. Is has become an European strategic priority in recent years for its recognised potential in stimulating sustainable growth and jobs, refining renewable biomass resources in a smart, sustainable and efficient way, making Europe more self-sufficient, and in reducing global GHG emissions.

Biorefineries have already been applied for some considerable time in the food industry and the pulp & paper sector. Large-scale implementation of biorefineries for non-food (incl. bioenergy) applications, however, is still lacking.

The major reasons for this are [IEA Bioenergy Task42]:

- Readiness of technology: Some of the key technologies (e.g. fractionation & product separation, downstream processing etc.), which are part of integrated biorefinery plants, are still not mature enough for commercial market implementation.

- Uncertain economic feasibility: Due to limited pilot and demonstration plants there is a shortage of sound biorefinery business cases to prove the feasibility for broad deployment. The current low crude-oil price has created significant economic pressures with respect to biorefineries leading to a limited pull from the market.

- There is still no level-playing-field for sustainable biomass use for food and non-food applications. For example, the global mineral hydrocarbon industry still receives significant tax and subsidy advantages over biorefineries utilising sustainable biomass, selling the produce on a similar market.

- Lack of co-operation: market sectors that should co-operate from a biorefinery perspective, such as food, feed, agro, chemistry, energy, fuels, logistics, etc., are often still not working together to develop and commercialize full sustainable biomass value chains, including highly-efficient biorefinery processes, , and

- Missing knowledge/expertise: there is still a lack of knowledge and expertise on the advantages of biorefinery processes for optimal sustainable biomass use at industrial, SME and (regional) governmental level.

However, significant growth is expected from biorefineries, which can lead to new biobased industries, transforming existing ones, and open-up new markets for biobased products.

---

[1] *Biomassa 2030: strategische visie voor de inzet van biomassa op weg naar 2030, publicatie nummer: 89293, Ministerie van Economische Zaken, Directie Groene Groei & Biobased Economy, Directoraat-Generaal Bedrijfsleven en Innovatie, Den Haag, 2015.*

[2] *Naar een duurzame bio-economie, visie van de Commissie Duurzaamheidsvraagstukken Biomassa, oktober 2015.*

[3] *Onderzoeksagenda Biobased Economy 2015 – 2027 'B4B: biobased voor bedrijven, burgers en beleid', 12 mei 2015.*

[4] *European BioEconomy in Figures, Nova Institute commissioned by EU-BIC, March 2016.*

Perspectives Small-scale Biorefining

One promising way to accelerate the market implementation of integrated biorefineries is to promote small (regional) biorefinery initiatives. Small-scale biorefineries require relatively low initial investments, and therefore are often lacking the financing problems that larger facilities face (new technologies with often complicated business cases making it difficult to get proper financing conditions). They are potentially able to make use of available local resources and involve stakeholders and product markets that create a common foundation for joint development and market deployment. Furthermore, by using modular and transportable units, the refinery process potentially can be operated at several locations, increasing their operation window, and therefore their market competitiveness. Small-scale biorefinery processes seem to be specifically interesting for the efficient and sustainable valorisation for relatively wet agro-crops (grass, beets, maize, etc.), agro-residues (leaves/foliage), food processing residues and aquatic biomass (microalgae, duckweed, etc.).

Public-Private Partnership (PPP) Cooperation

Both technical and non-technical barriers exist that prevent wide market implementation of small-scale biorefineries at the moment. To facilitate the market deployment of small-scale biorefineries a Public-Private Partnership (PPP) between over 35 market stakeholders (mainly RTOs) and Wageningen Research has been established. This PPP was executed for 4 years (2013-2016), and the main results are published in this brochure. Work was done on the success factors for small-scale biorefinery that was set up to define design rules that could assess the economic feasibility of small-scale processes. Also, co-operation was implemented to design process steps that would allow companies to quickly assess the potential to valorise their organic side streams for biorefinery purposes. Furthermore co-operation was set up in a variety of potential business cases such as green leaves for protein extraction, natural extraction of steviol glycosides from stevia, sugar refinery to produce platform chemicals and the optimal use of aquatic plant biomass to process water waste streams in the circular economy. This brochure gives the reader a glance into the exciting journey that the companies together with Wageningen University & Research experienced during the four year Public Private Partnership on small-scale biorefinery.

Topsector
Agri&
Food
TKI Agri&Food

Small-scale
Biorefining

2

Does size matter? Success factors for small-scale biorefineries

Design Rules

The public-private partnership "PPS Kleinschalige Bioraffinage" has explored several biorefinery business cases to identify success and fail factors related to the size at which the processes are run. Economies of scale are a major obstacle to the implementation of small-scale processes. This is illustrated, for example, with scale factors for the equipment and manpower. However, if we look at the entire chain, there are clear situations where small size offers advantages. This will be further explored in this chapter by means of example cases, both from the literature or on the basis of information available within the "PPS Kleinschalige Bioraffinage".

Design rules for small scale biorefinery were generated within the project, because small scale processes need to be designed in a different way than their large-scale equivalents. Many cases were analysed to get to the design rules. The cases started with evaluation of unit operations, which mostly have an economy of scale, followed by process and system analysis. Examples of small scale processes studied were the local biogas units at van der Valk (Swillgasser), small scale chloride factories for AkzoNobel, and agricultural machinery such as the combine harvester. Also other systems were identified that suffer from diseconomy of scale, such as large airports. These examples led to the development of a number of design rules.

These design rules can be summarized as follows:

- Limit equipment investment costs, in particular heat exchange.
- In contrast to drying, dehydration is an option at small scale.
- Establish the right combination of small scale (pre) processing and additional centralized processing.
- Use local (preferably on site) residues for the generation of heat and energy.
- Produce for a local or domestic market.
- Use the difference between selling and purchase value within your own process.
- Work with minimal man-hours and provide automation and central support.
- Use modular, transportable, units when the process can be applied at several locations.

Small scale biorefinery examples

Small scale sugar processing

Wageningen Food & Biobased Research has developed a new technology to isolate water soluble components such as sugars, amino acids and organic acids from aqueous streams. Part of this research was done within this PPS. The new technology is based on anti-solvent crystallization and can be applied to various raw materials. This technology differs from the conventional anti-solvent crystallisation approaches in the chemical and pharmaceutical industry, as this technology allows the solvent with the higher boiling point to be removed from the liquid. This is also the reason why this technology is particularly interesting for biomass containing streams as many biomass components are water-soluble.
It is expected that this technology will be performed at different scales. A regional, smaller scale approach is particularly interesting when looking at logistics, reuse of heat, energy and raw materials. More specific the arising benefits include:

- Water, fibres and other valuable minerals remain on site for soil fertility, or can be recycled over short distances.
- Production requires a relatively small investment.
- Local employment.
- Additional value creation.

The new technology can be used for separation and purification of components such as a variety of sugars, amino acids and organic acids from more complex mixtures. The technology has been demonstrated at lab scale for direct sucrose crystallisation from raw beet juice

Benefits from grass and maize biorefinery integration in a chain

Global agricultural production, where specific crops are grown in specific areas, leads to transportation of these crops to other areas in the world. Large amounts of soy needed as feed are thus translocated from South America to Europe causing disruption of the global nutrient balance. Biorefinery can be an alternative method to increase the amount of protein rich feed available in Europe, and especially The Netherlands. Local biorefinery of grass and maize is technically feasible and can be applied to make regional produced protein available for livestock. This concept was applied in a new chain design that next to the two biorefinery units also included grass, maize, pig and dairy farms, and was complemented with a biogas fermenter. The new chain was designed based on protein production and use, maintaining animal production. It was then optimized for economics,
P-recycling and land use.

The intensively farmed region of ‘De Achterhoek’ in the Netherlands was used as an example for the quantitative modelling and optimization of different scenarios. ‘De Achterhoek’ exists of 80,000 hectare arable land, of which 50,000 hectare is grass land and the other 30,000 hectares are cultivated with maize and other feed crops. The land area of 'De Achterhoek' is not large enough to be self-sufficient in feed production for the number of animals present. ‘De Achterhoek’ contains 4,300 agricultural companies. One-third of the employment in these agricultural companies is in dairy industry, and 12% in the pig industry. Typical for the existing agriculture is the high import of protein feed products and artificial fertilizer and the intensive livestock production, resulting in a local manure problem.

The model shows how we can minimize the import of protein feed products, while keeping the same economic profit in the modelled region. The main challenge in the implementation of the model is the combination of different farmers and entrepreneurs. Although the technology is available and the entire chain is profitable, profits are not evenly distributed over all individual steps. Good cooperation and good agreements throughout the chain are essential for successful small scale implementation.

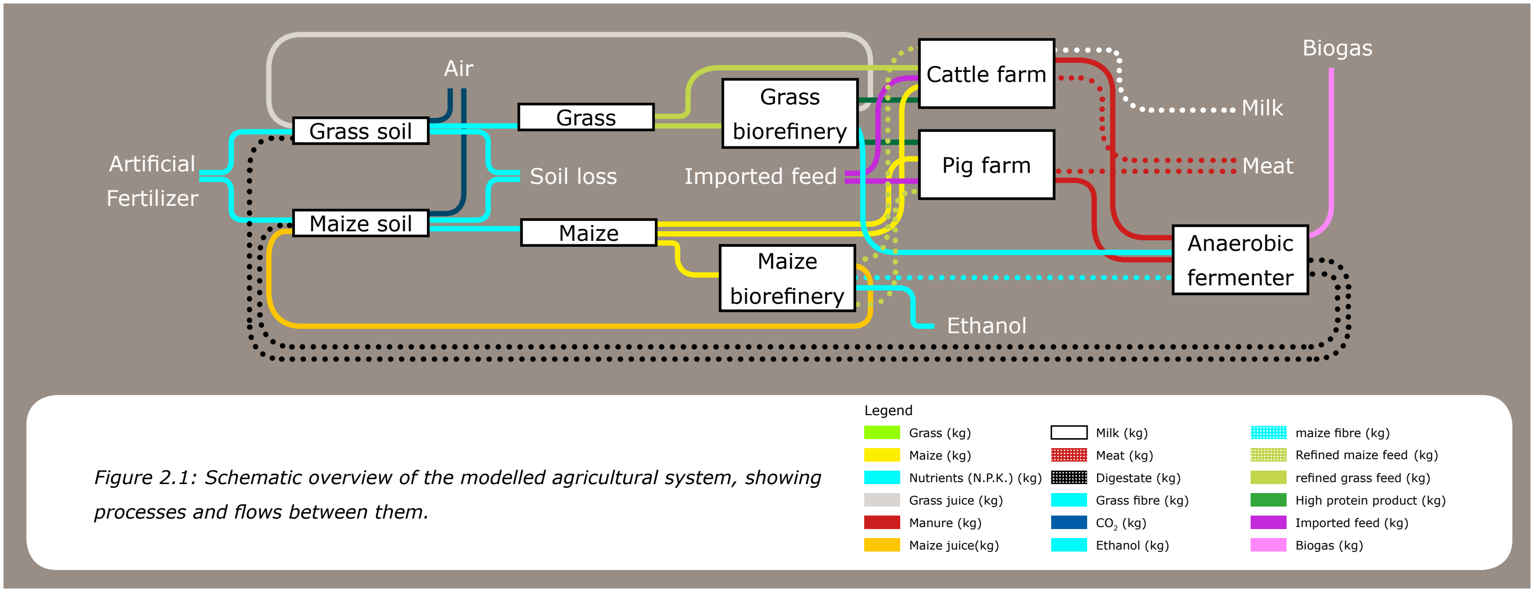


Figure 2.1: Schematic overview of the modelled agricultural system, showing processes and flows between them.

Challenges, future research and participation

We continue our work on the technological development and improvement of small scale processes. Often this is through commercial bilateral research or within subsidized (PPS) programs. One example is the continuing development of the small scale anti-solvent technology that is currently also tested for application on other liquid (side)streams beside raw sugar juice. Other important examples are on partial (local) fractionation of biomass for e.g. protein or pectin. All of these topics are under investigation and interested parties will be able to join. The chain approach is also being developed, but often within more fundamental programs that support sustainability goals. However, also here we are looking for interested parties to develop new concepts and ideas.

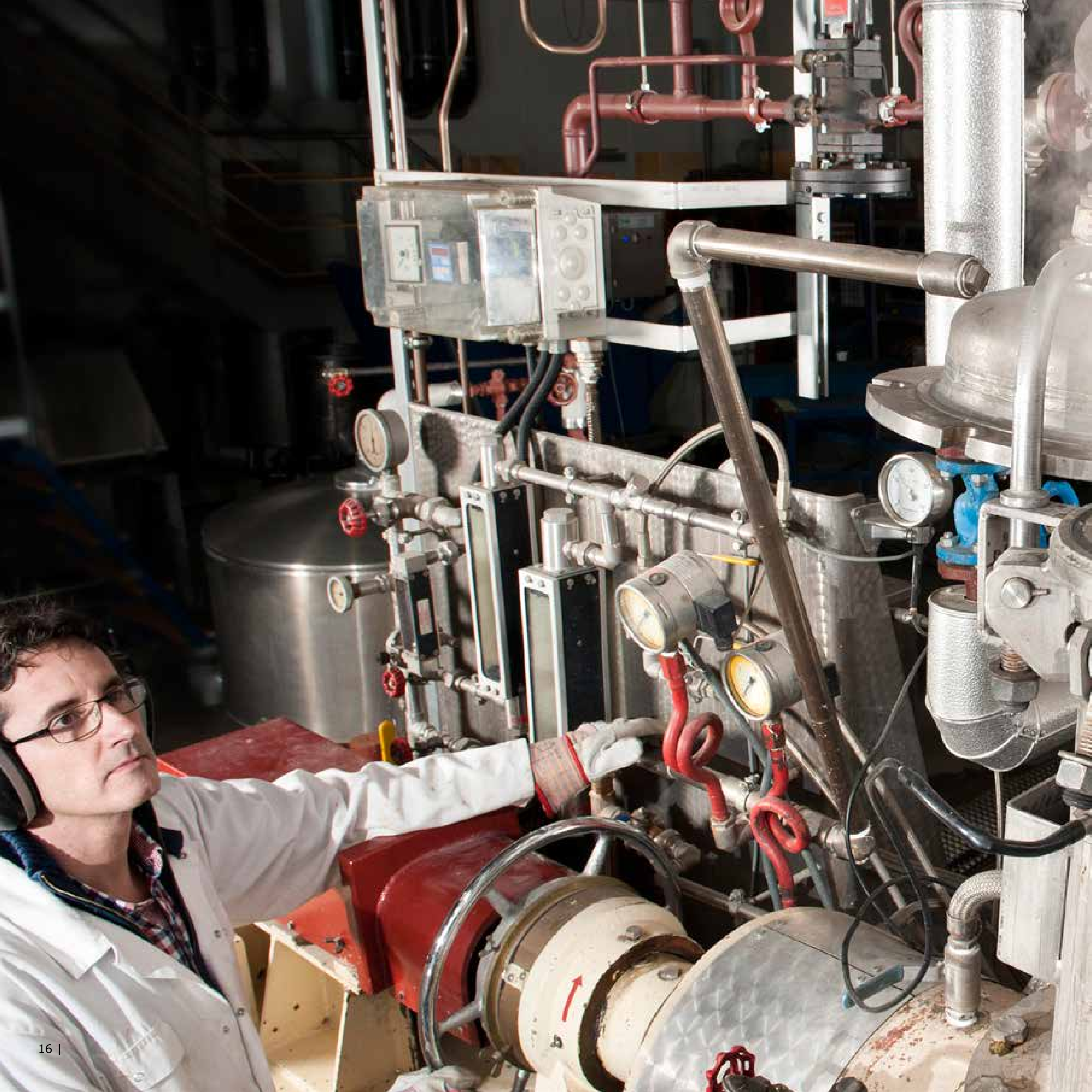
Literature

Bruins, M.E.; Verbaanderd, J.A.; van Willigenburg, L.G.; Sanders, J.P.M. Integratie van landbouwsectoren d.m.v. kleinschalige clustering van bioraffinage: Rapportage in opdracht van het Biorenewables Business Platform 2012

Reports

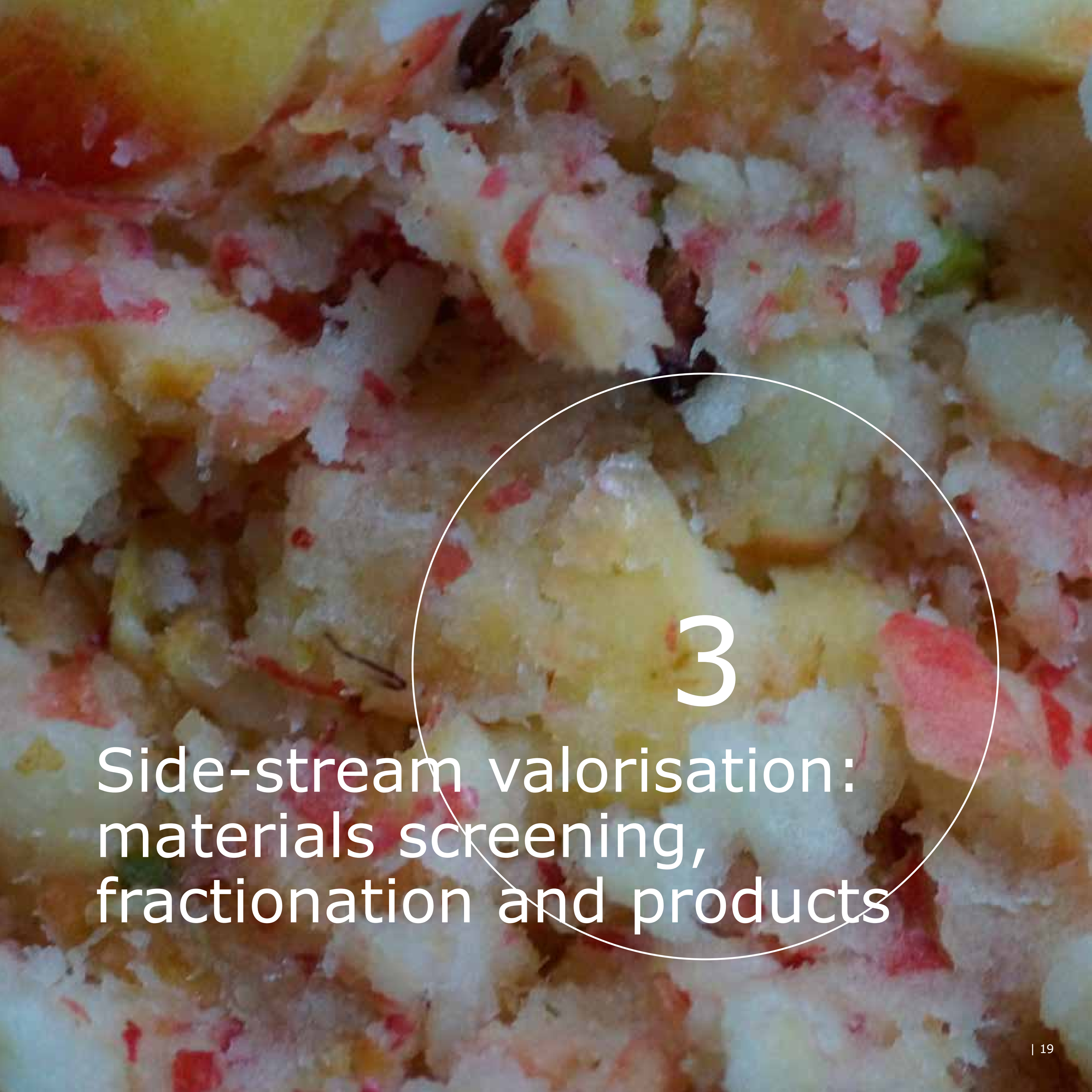
Bruins, M.E.; Meesters, K.P.H.; Scott, E.L.; Kolfschoten, R.C.; Haer, T. Succesfactoren kleine schaal: tussentijdse rapportage 2013

Bruins, M.E.; Togtema, A.; Meesters, K.P.H. Succesfactoren kleinschalige bioraffinage: tussentijdse rapportage 2014
Bruins, M.E.; Teekens, A.M.; Keijsers, E.R.P.; Meesters, K.P.H. Succesfactoren kleinschalige bioraffinage: tussentijdse rapportage 2015




Steef Lips


MIEDEMA



3

Side-stream valorisation: materials screening, fractionation and products

Author: Jan Broeze en Wolter Elbersen

Introduction

Agro-food side streams are generated in a supply-driven form: composition, quality and (seasonal) variations are largely determined by the company's main products. Most commonly straightforward solutions, like application for feed or bio-energy, are chosen.There is large potential for valorisation with higher value than nutritional feed value or energy value. However, high-value opportunities are unexploited due to insufficient knowledge of molecular complexity, processing options, logistic opportunities, market potential and economic value. The question is how to identify and assess the potential of such alternative options.

Within the Public-Private Partnership (PPP) Small-Scale Biorefinery project we have assessed potential alternative valorisation options for a number of agro-food processing side streams. Specifically we focussed on apple press pulp as a typical example of a side stream that – although it contains very interesting bio-molecules – for practical reasons is valorised at a basic level: bio-energy.
Based on experiences in that search and assessment process, combined with learnings from similar analyses on other side streams, a stepwise methodology was developed for quick-scan of potential valorisations of any agro or food-processing side stream.

Analysis of alternative valorisation of apple press pulp

Apple press pulp is by far the largest by-product of apple juice extraction. The Dutch company Flevosap presses apples and pears according to a traditional process (which results in their typical juice type). 1/3 of the fruit ends in the press pulp. Current and historical valorisation types for this side steam are bio-energy and feed.
Over 1,000 tons of press pulp is generated annually. Seasonal variations are limited (meaning that fresh pulp will be available throughout the year).

In a brainstorm workshop by participants from the industry and various experts we have identified and discussed a long-list of improvement options, varying from:

- increasing juice extraction yield through enzymatic treatment (which would affect juice properties/quality).
- isolation of valuable bio-molecules, like polyphenols, specific vitamins, dietary fibres, proteins, etc.
- fermentation: bioethanol, apple vinegar or other organic acids for food or non-food industries.
- supplying fresh or processed (and dried) press pulp to food producing industries.

The latter option was selected by the entrepreneur. The entrepreneur and project team defined a number of scenarios for that improvement option:

I. supplying fresh press pulp to bakery, with challenges:

- how to separate seeds, peels and other 'impurities' from the pulp?
- how to preserve the fresh pulp?
- market interest.

II. drying the pulp to a powder, aiming for bulk market (like pectin industry); challenges:

- market price.
- energy & equipment costs.
- interest from pectin industries.

III. drying the pulp to a powder, finding added value market; challenges:

- removing seeds, peels and other impurities.
- entering this market; how to reduce risks of the investment.

The further analysis learned that the combination of option II and III gives best perspectives: drying the material makes it available for pectin as well as for direct food ingredient.

Pectin is generally produced in large centralized factories; the economic efficiency largely profits from economics of scale. Locally producing the pectin at the scale of a medium-size juice factory is not economical. However, pulp drying at the juice factory and selling the dried material to a centralized pectin industry is more promising. Nearest pectin industries are located in Germany.
Apple pulp is used in various bakery products, with function of filler, (mild) flavouring and fibre source.

Drying is a common step in the pectin production chain. After drying, the physical characteristics of seeds, peels and other impurities significantly differ from the characteristics of the pulp material. Hence, for the added-value applications, a relatively straightforward physical or mechanical separation process is considered effective for removing these unwanted compounds.

The added-value markets can be served with the dried, purified pulp. Hence, after investing for the pectin valorisation, the added-value markets can be reached with relatively limited additional investments.

Chain development model:
First start with drying the pulp, and sell it to a pectin producer. Next step is exploring added-value markets for dried apple pulp. Starting to serve that market will require relatively small investments in the purification process.

Process of identifying and assessing valorisation options for side streams

The method for finding and analysing (alternative) options for by-products and side stream as applied for apple pulp has been generalized to a step-wise methodology. This methodology is schematized in Figure 3.1.

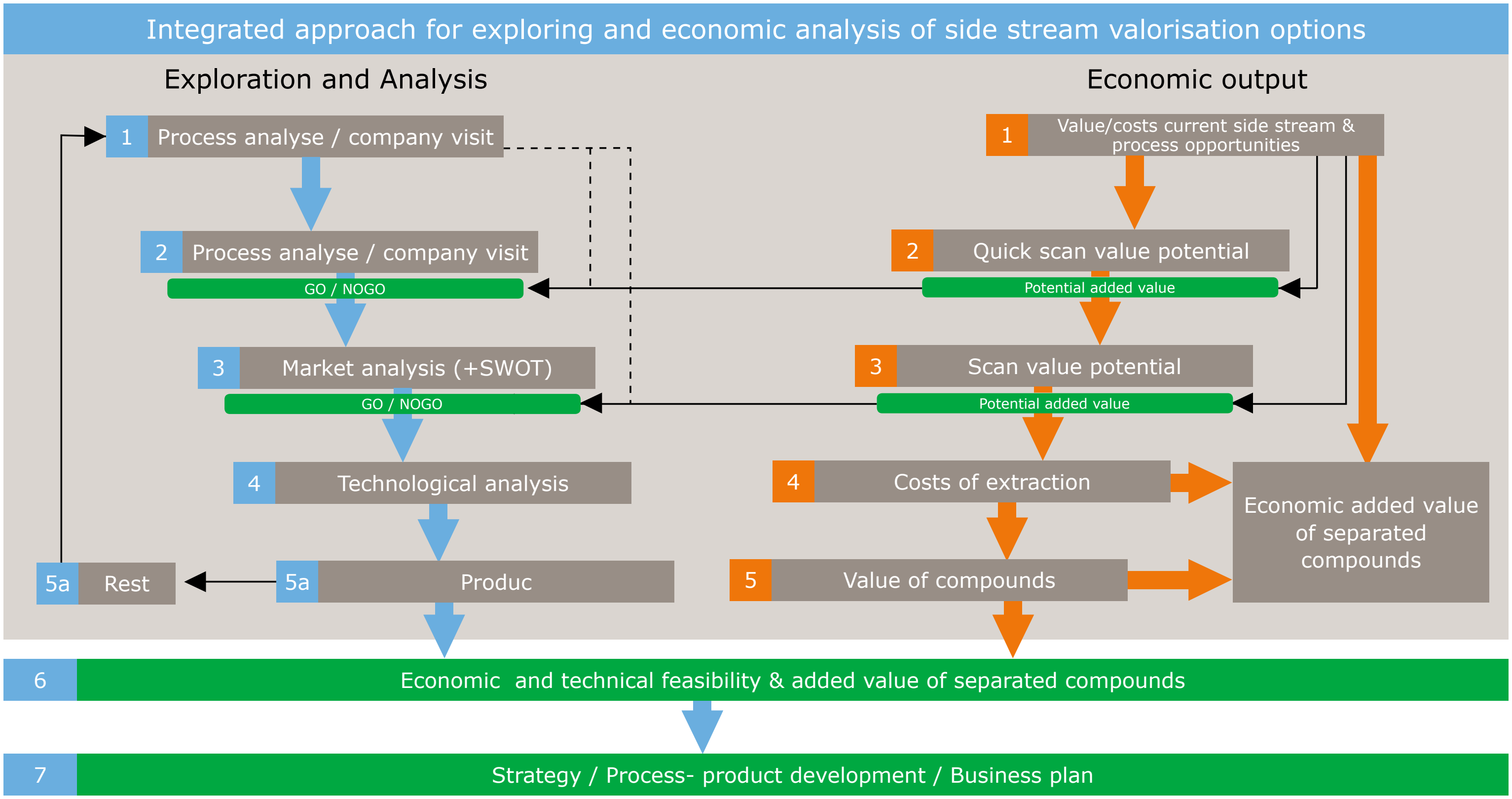


Figure 3.1. Step-wise methodology for finding options for alternative valorisation of by-products and side streams.

Roel Vermeulen, Flevosap
"I want a base solution with a secure market, and would like to explore added-value market from that situation"

Some highlights of the methodology:

- With a small number of relatively simple initial steps (1 & 2, process analysis, using available data and brainstorming) a long-list of ideas can be generated. Through involving experts with diverse knowledge background, a broad source of experience is activated.
- Based on the long-list of ideas, the entrepreneur(s) involved formulate their priorities (based on arguments supplied by the experts; these may vary from economic arguments to current state of development of required technology, legal restrictions, etc.).
- In following steps, selected ideas can be further elaborated, up to technological analysis and business case analysis.

In the form of a short project – requiring relatively limited resources – industries can profit from the knowledge of diverse scientists (first steps in the process). When promising ideas pop up, a follow-up project may be formulated (either in co-operation with scientific support or a practical project).

Jeroen Tideman, Bioclear:
"Combining creativity of technological scientists with practical business analysist further enhances the potential of identifying new business opportunities in the domains of side-streams and biobased developments."

Experience from other scans

As part of various projects, the above methodology has been applied to other practical cases:

- insects.
- meat processing side streams.
- fish by-catch.
- monk fruit extraction side streams.
- specific potato processing side streams.
- and others.

One of the brainstorms resulted in direct measures by the industries, without need of further analysis. Some others gave perspectives on major innovations, but these did not fit in the strategy of the company. For these, also the valorisation scan was finished after step 2.
The remaining cases resulted in further technological and economic analysis, with perspectives on new products and added-value business development.

New value chains in the circular economy - valorising waste water and side streams with aquatic biomass

4

Aerial photo of ACRRES site in Lelystad, the Netherlands.

Authors: H. Elissen, W. van Dijk, S. Huurman, R. van der Weide

Background

Globally, there are expected shortages in protein production, (as is for example demonstrated by rising fish meal prices) and limitations in concentrated phosphate and non-renewable energy reserves. In addition, fertile soil area is decreasing and climate problems are increasing. Therefore, companies, governments and research institutes cooperate in taking steps to overcome these challenges. Recovery of nutrients and production of sustainable sources of energy are crucial in the circular economy, as well as the production of new biomass varieties. Several agri, horticultural and processing industries locally produce waste water and side streams containing nutrients and organic matter or flue gases and residual heat. Often treatment and disposal of these streams lead to costs and loss of nutrients and energy. Therefore, recovery on site is an attractive option: for example by producing aquatic biomass on side streams containing nutrients and organic matter. Several new crops such as microalgae, macroalgae, aquatic higher plants, aquatic and terrestrial invertebrates (insects and worms) have received growing scientific attention (van der Weide et al, 2016), because of potential high biomass yields for protein production on small surface areas and their content of speciality chemicals, oils and fibres. Microalgae and aquatic plants are suitable for recovery and valorisation of nutrients and organic matter from waste water and side streams. Process integration with biogas production (anaerobic digester) adds to the availability of CO_2 and residual heat for optimized aquatic biomass production (van der Weide et al, 2014). In Lelystad, Wageningen and at other sites, several pilot set-ups have been constructed and evaluated during the last years.

Goal

The objective of this project was to evaluate whether aquatic biomass products, for example animal feed (ingredients), oils and plant or soil additives, can be produced economically and sustainably by using local waste streams from bioenergy production or other aquatic side streams from processing industries.

Aquatic waste streams can be used to produce biomass

An inventory has been performed into the availability (amount and characteristics) of aquatic waste streams and a selection has been tested as input stream for the production of algae. Selected aquatic waste streams were effluents from a digester, waste streams from a brewery and potato processing industry, cleaning water from automatic milking robots, waste water from an air stripper in a livestock housing system and waste water from a fruit cleaning operation. Most of these streams could be used for the production of algae although some of the streams require addition of extra nutrients or pre-treatment to prevent light interference by solids or bacterial growth. Side streams of an anaerobic co-digester were chosen for further testing and collection of data on the production of microalgae and aquatic plants. The application of nutrients from liquid fraction biogas slurry for algae and aquatic plants production and their refinery was investigated.

Research with addition of liquid fraction biogas slurry from the Wageningen University & Research pilot site in Lelystad (0.2 m^3 added on a total pond volume of 100 m^3) showed that the culture got more coloured and the harvested biomass was contaminated with the organic matter that was present in the added biogas slurry. However, the algae growth seemed not to be negatively affected. The use of the nitrogen from an air stripper drying the biogas slurry and the other minerals after diffusion from the separated thick fraction, proved to solve the problem with the solids. The liquid fraction biogas slurry after screw press filtration could be used for aquatic plant production without further separation. However, for microalgae further separation of the solids from liquid waste streams can improve the value and application possibilities of the product.

Production and value of aquatic biomass

Microalgae

Research is conducted to improve the production and energy use efficiency of microalgae, finally aiming at better economics of production. Innovative algae productions systems have been further developed by participating algae producers. One of the

Figure 4.1 Water hyacinth production on liquid fraction biogas slurry in pond.

participating companies has developed a relatively cheap production system based on plastic bags, for which initial tests showed a productivity of 40 MT dry algae/ha/year. Another company has further increased efficiency of LED lighting for algae culturing and improved LED lighted basins with volumes up to 20 m^3, which enables year-round algae production on a small surface area. Production possibilities in open ponds and closed systems have been investigated and improved, as well as harvesting, refining and utilization of the produced biomass.

The annual biomass productions of microalgae in an outdoor race way pond were 4, 7, 6 and 8 metric tons (MT) dry matter per ha in 2013, 2014, 2015 and 2016 respectively (van Dijk et al, 2016). The lower production in 2013 was due to the fact that in the first half year the algae were harvested with a sedimentation system that appeared not to result in high yields. In the other years algae yields were realised with a centrifugation system. Model calculations predicted an annual production of about 17 MT dry matter per ha. The yield gap can be due to periods with low or no production after crashes or suboptimal CO_2 availability and harvest efficiency. In periods with stable production the harvested biomass yield corresponded with annual production levels of 11-17 MT dry matter per ha.

In 2014 and 2015 alternative harvest systems were tested: dissolved air flotation (DAF) and sedimentation in combination with flocculants. For the DAF unit the best results were obtained for the feed grade flocculant BC floc. Harvest efficiency was > 90% for a flocculant dose of 1-4 g/m^3 algae water. By using a feed grade flocculant application options of the harvested biomass are increased. Furthermore, the effluent of the DAF unit sometimes caused unwanted algae flocculation in the race way pond. Algae sedimentation in combination with an increased pH level (10-11) and the addition of a flocculant also resulted in a harvest efficiency > 90%.

The race way ponds were continuously vertically mixed using perforated air sparging tubes at the bottom of the pond. This sparging system also functioned as transport system for injected flue gas which was used as a carbon source for algae growth and as a pH regulator. Measurements indicated a relatively low CO_2 recovery with the used flue gas addition method, furthermore the dissolved CO_2 concentration in the algae culture was fluctuating. Without affecting algae growth a more stable dissolved CO_2 concentration and a 65-80% lower energy demand was realized by limiting air sparging to the period when CO_2 was required. To improve the energy footprint for CO_2 transfer from flue gas a gas scrubber is tested and compared to the high energy consuming blower driven sparger system.

The contribution of the energy demand for harvesting to the total energy demand of the algae system is about 55% for harvest by centrifuges and 25% for harvest by a DAF unit. When air sparging is restricted to periods with CO_2 supply and harvest efficiency is 90%, the energy demand of the total system is decreased with 20 and 35 % when harvesting was done by centrifuge and DAF unit, respectively.

A report was written on the high potential of microalgae to be used as animal feed (additive) (Spruijt et al, 2016a). A method for low temperature drying of algae has been developed and the resulting dried biomass is currently tested in vitro tests that indicate whether algae can counteract animal diseases as for example *Escherichia coli* infections. In general, the value of algae biomass based on both nutrition as well as health effects on animals is at least a factor ten higher than their value as protein (Spruijt, 2016a).

Based on literature research a report has been written on applications of microalgae in agriculture (Spruijt et al, 2016b). Different algae species are involved (many of them cyano-bacteria) and promising applications are for example as crop protection agent, growth regulator and/or soil additive. Selling prices of microalgae for these applications are hard to define since algae dosage data in the compound products are often missing.

Figure 4.2 Outdoor algae raceway pond.

Aquatic plants

Basins for aquatic plants like duckweed, cattail and hornwort but also (usually terrestrially produced) soybean plants have been constructed to quantify growth efficiency on liquid fraction biogas slurry and residual heat. In a 250 m^2 race way pond water hyacinths are successfully produced on liquid fraction biogas slurry and residual heat.
Not yet optimised production of water hyacinth was 24-32 MT DM/ha/year and productivities of cattail and hornwort were higher than in natural ecosystems (Table 4.1). It was possible to grow soybean plants in an aquatic system with comparable yields to terrestrial systems.

Figure 4.3 Harvested algae biomass.

Table 4.1 Production and protein yields for several aquatic plants and algae produced in Lelystad at Wageningen University & Research (ACRRES) in 2016

Species	Production MT DM/ha/year	Protein % DM (values from literature and own analyses)	Protein yield MT/ha/year
Duckweed	12-16	16-45	1.9-7.2
Water hyacinth	24-32	12-25	2.9-8.0
Hornwort	10	20	2.0
Cattail	32	10-14	3.2-4.5
Algae	8	50	4.0

The productivity of duckweed in open ponds was 12-16 MT DM/ha/yr. One of the participating companies has developed a system for duckweed production similar to that for microalgae.

However, at first a strong business case has to be built for high value and other products from duckweed. Aquatic plants (or refined fractions) can be valorised as animal feed, fuel source, fibres, co-digestion substrate, source of VFAs, compost and organic fertilizer, antioxidants, colour and plant disease control agents. In addition, they can be used for cleaning or metal uptake from waste water streams.

Figure 4.4 Cross-section of water hyacinth spongy stalk.

Challenges, future research and participation

Our ambition is to produce valuable products on side streams and to investigate new or improved options for a circular economy. Based on the experiences in this project, the main challenge is to increase economic viability by:

- decreasing production and processing costs and energy use for cultivating, harvesting and processing these novel crops.
- further optimizing manure and digestate separation machinery, harvest- and biorefinery machinery, cultivation systems, tools, processes and waste stream use efficiency.
- investigating new aqueous waste streams and interesting aquatic biomass varieties.
- generating data to prove additional value as feed additive, crop protection or stimulation agent or organic crop fertilizer and to prove product safety (legislation).
- generating more knowledge on (scaling up) production (e.g. on waste streams) on pilot and demonstration scale level.
- working on economic and societal acceptance, evaluation and communication.

Taco Neeb (AF&F)

- *The unique location and infrastructure of Wageningen University & Research enables practical cooperation with us for testing and further innovating our algae production system.*
- *The researchers have a flexible and proactive attitude within our joint projects.*
- *The scientific basis for our results provided by Wageningen University & Research and their innovative input are very valuable to us.*

Literature

- Valorising side streams in circular animal feed additives: Opportunities and challenges. Weide, R.Y. van der; Elissen, H.J.H. ; Dijk, W. van; Huurman, Sander ; Krimpen, M.M. van (2016) Proceedings European Biomass Conference and Exhibition- 5 p.
- Algae production pilot open ponds Lelystad : Results 2013 - 2015. Dijk, W. van; Weide, R.Y. van der; Gennep, Coen van (2016) Lelystad : ACRRES - Wageningen UR, (PPO rapport 583) - 63 p.
- Algae cultivation using digestate as nutrient source: opportunities and challenges. Weide, R.Y. van der; Schipperus, R. ; Dijk, W. van (2014) Proceedings European Biomass Conference and Exhibition.
- Opportunities for algae as ingredient in animal diets. Spruijt, J, Weide, R.Y. van der; Krimpen, M (2016a) Lelystad : ACRRES - Wageningen UR, (PPO rapport 712) - 46 p. http://acrres.nl/ppo-712-opportunities-for-micro-algae-as-ingredient-in-animal-diets.
- Kansen voor toepassing van microalgen in landbouwgewassen. Spruijt, J. ; Weide, R.Y. van der (2016b) Lelystad : ACRRES - Wageningen UR, (Rapport / PPO-AGV 691) - 62 p.
- Aquatische biomassa, het verwaarden van waterige reststromen op lokaal niveau : Technisch deelrapport 1: Verkenning van de mogelijkheden van reststromen en aquatische biomassa. Huurman, Sander ; Weide, R.Y. van der (2015) Lelystad : Wageningen UR/PPO-agv, (Rapport / PPO-AGV 650) - 54 p.

Figure 4.5 Cultivation of different algae strains under artificial (LED) lights.

Figure 4.6 Several submerged, emerged, terrestrial and aquatic plants growing on liquid fraction biogas slurry and residual heat in basins.

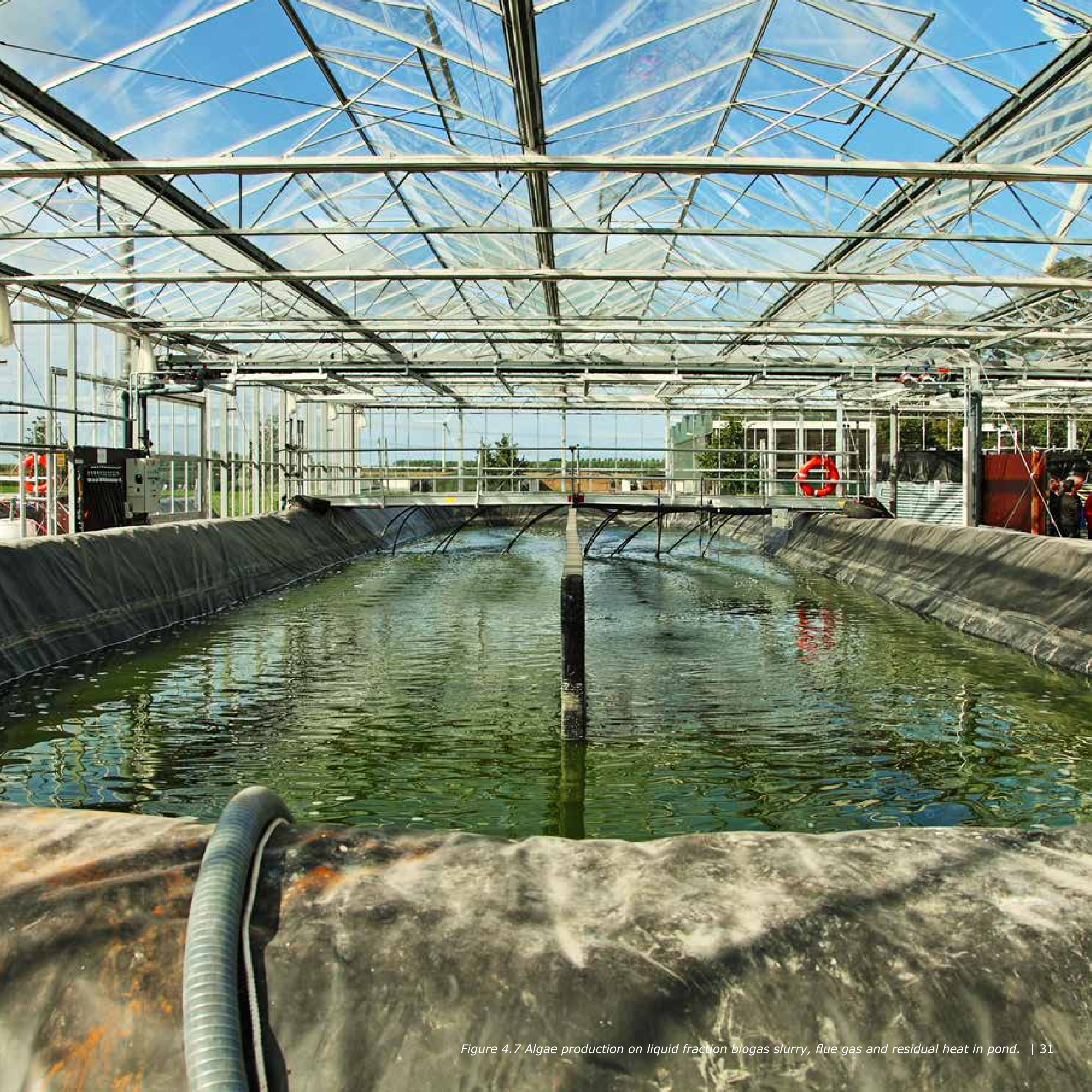
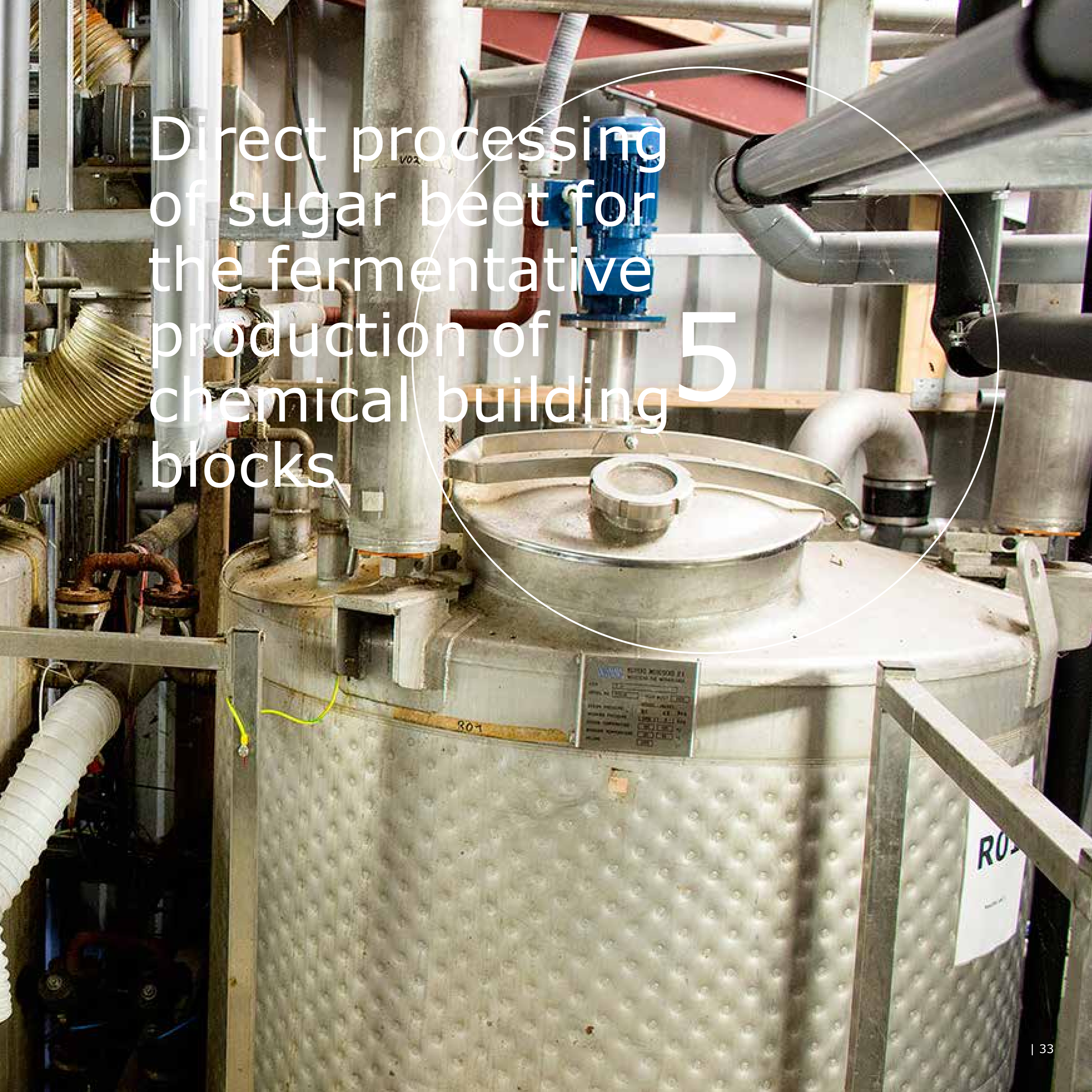


Figure 4.7 Algae production on liquid fraction biogas slurry, flue gas and residual heat in pond.



Direct processing of sugar beet for the fermentative production of chemical building blocks

5

Authors: Maarten Kootstra en Anna Lopez Contreras

Background

Sugar beet is an important crop in the Netherlands and the EU, as it is the main raw material for the production of sugar. In addition to the current centralised processing facilities for sugar beets, the capacity for sugar beet processing may be expanded with small decentralised processing plants.

Sugar production costs in North-West Europe are among the lowest worldwide, and they are the lowest for sugar production from sugar beet in the Netherlands. In addition to the traditional processing of sugar beet to crystalline sugar, there is increased interest for sugar beet as a raw material for the production of chemical building blocks, mainly by fermentation, to be used in the chemical industry. In 2017, the quota on sugar beet production is lifted, and the EU production of sugar beet is expected to increase, as described in a report by Deloitte [1].

A process design for directly processing sugar beet that is proposed by DSD is the BetaProcess, in which sugar beets are pretreated by vacuum extrusion. The idea is that the sugar beet cells are opened up during vacuum extrusion, hereby facilitating the uptake and conversion of the sugar present by microorganisms to fermentation products such as ethanol. At the pilot plant facility of Wageningen University & Research in Lelystad, this process is integrated in the already present pilot scale ethanol fermentation plant, so that tests can be performed to determine the effect of BetaProcess on the speed and yield of the ethanol fermentation by yeast of the treated ground sugar beet.

Furthermore, the effect of BetaProcess is tested in the fermentative production of acetone, butanol and ethanol. These ABE fermentations are carried out at the fermentation facilities of Wageningen University & Research (Food & Biobased Research).

Partners

- DSD (Dutch Sustainable Development): DSD Betaprocess BV is responsible for marketing and sale of Betaprocess technology.
- KH Engineering: development of model to establish viability of business case.
- Wageningen Research, Application Centre for Renewable RESources (ACRRES): Pilot scale research on BetaProcess application in direct fermentation of sugar beet to ethanol.
- Wageningen Research; Food & Biobased Research (FBR); Lab scale research on direct fermentation of sugar beet fermentation to Acetone/Butanol/Ethanol (ABE fermentation).

Goal

The goal of the sugar beet to chemical project activities was to develop, test and demonstrate the use of the BetaProcess technology and the direct processing concept. This goal was targeted at ethanol production on a 1.5 m3 scale level while the production of acetone, butanol and ethanol via the ABE fermentation was tested at a 1 L scale level in the laboratory. The ethanol production system resulted in process data that could be used as parameter input for a model designed to establish the viability of the business case.

Process integration

At Wageningen University & Research in Lelystad, the BetaProcess equipment is integrated in the already present pilot scale ethanol production facility containing two 1500 L fermenters (Figures 5.2 and 5.3).

In figure 5.1, the entire array of processes is depicted:

1. The sugar beets are cultivated and harvested.
2. Sugar beet cleaning to minimise inclusion of soil in the process.
3. Sugar beets crushing to obtain a slurry.
4. In the BetaProcess, the crushed beets are heated up and then pumped into a vacuum tank.
5. The treated sugar beet is fermented.
6. Distillation results in a distillate rich in ethanol and a residual stream that may for example be digested to produce biogas and digestate.

7. Digestate can be used as fertiliser in sugar beet cultivation.

Achieved results

Ethanol production

Pilot scale production called for adjustments to:

- beet cleaning.
- beet crushing.
- stirring in 1500 L fermenters.
- minimising growth of unwanted microorganisms in fermentation.
- inline monitoring of ethanol fermentation by gas volume measurement.

A standardised test protocol has been developed for the complete process, from beet cleaning to fermentation. Using this test protocol and sugars beets from (up to 7 months) storage, a series of 700 kg runs has been carried out, in which it was found that:

- final sugar-to-ethanol yields of ~85% have been reached, based on gas production measurements.
- formation of glycerol was observed.
- limited formation of lactic acid and succinic was observed.
- formation of only traces of methanol, butanol, and acetic acid was observed. (It is assumed that 5 % to 10 % of all sugar is used for yeast propagation.)
- the fermentation took 30 to 36 hours to complete.
- peak production of ethanol took place within 10 hours from the start.
- 90 % of all ethanol production occurred within the first 24 hours.

Although the visual appearance of the ground sugar beet does seem to be changed in the BetaProcess treatment, a clear effect on the speed or final yield of the subsequent pilot scale ethanol fermentation remains to be shown. A new series of runs is currently underway, using newly harvested beets.
The ground sugar beet does seem to liquefy during fermentation, but distillation of the fermented material requires a different set up than currently present at the Acrres ethanol pilot plant, as the fermented material clogs the currently present distillation column.
Another observation is that gas produced during ethanol fermentation needs to be able to readily escape from the fermenting material in order to prevent rising of the content of the fermenter. This is important for minimising production costs related to needed fermenter volume per amount of produced ethanol.

KH Engineering has developed a model in which data from the pilot plant tests are used for the business case of producing ethanol from sugar beet through direct processing.

ABE fermentation

Tests were performed in closed, anaerobic flasks of 118 mL with 50 mL of culture medium followed by pH-controlled fermentations in 0.5 L bioreactors with 150 mL medium to determine the ABE yield on BetaProcess treated sugar beet.
ABE is produced to high solvent titres of more than 15 g/L by efficient conversion of sucrose from Betaprocessed sugar beets (400 g of fresh weight/L) by the bacterium Clostridium acetobutylicum. The microflora of the sugar beet was overgrown by C. acetobutylicum. Besides sucrose, sugar beet contains other carbohydrates, such as cellulose, arabinan, and pectin. Valorisation of these compounds by the solventogenic Clostridia is an important step toward the economy feasibility of the ABE fermentation using sugar beet as substrate. But as they are polymeric, an extra process step is necessary to convert them to fermentable sugars that can be converted to ABE in the subsequent fermentation. Part of the cellulose was converted to glucose by treatment with commercial cellulase.

> *"The testing of the direct processin concept using Betaprocess on the Wageningen University & Research in Lelystad has supported the development of the technology to a large extent. We are now at the point were we are developing a demoplant in which we will continue the co-operation."*

Opportunities for further development

Ethanol production

- A distillation set up is needed capable of processing the thick fermented sugar beet mass.
- Apart from fermentation speed and yield, the BetaProcess treatment may have effects on the crushed sugar beet that play a role during direct fermentation; for example on needed intensity of stirring, on liquefaction, and on the ease of CO2 escaping from the fermenting material in order to reduce the occurrence of rising. These mentioned examples all influence production costs.

ABE fermentation

- ABE fermentations need to be optimized for ABE yield and productivity by adjustment of operating conditions.
- In addition to sucrose, currently non-fermentable polymeric components of sugar beet such as cellulose, arabinan, and pectin need to be converted to fermentable sugars to be used for ABE fermentation, in order to increase overall economic feasibility.

> *Hans van Klink & Cees van Loon (DSD):*
> *"Working with Wageningen University & Research in Lelystad has proven to be real collaboration: to each their own expertise, provide clarity, and move forward. The practical-minded researchers have helped us to reach green solutions."*

Figure 5.1 Processing diagram Betaprocess.

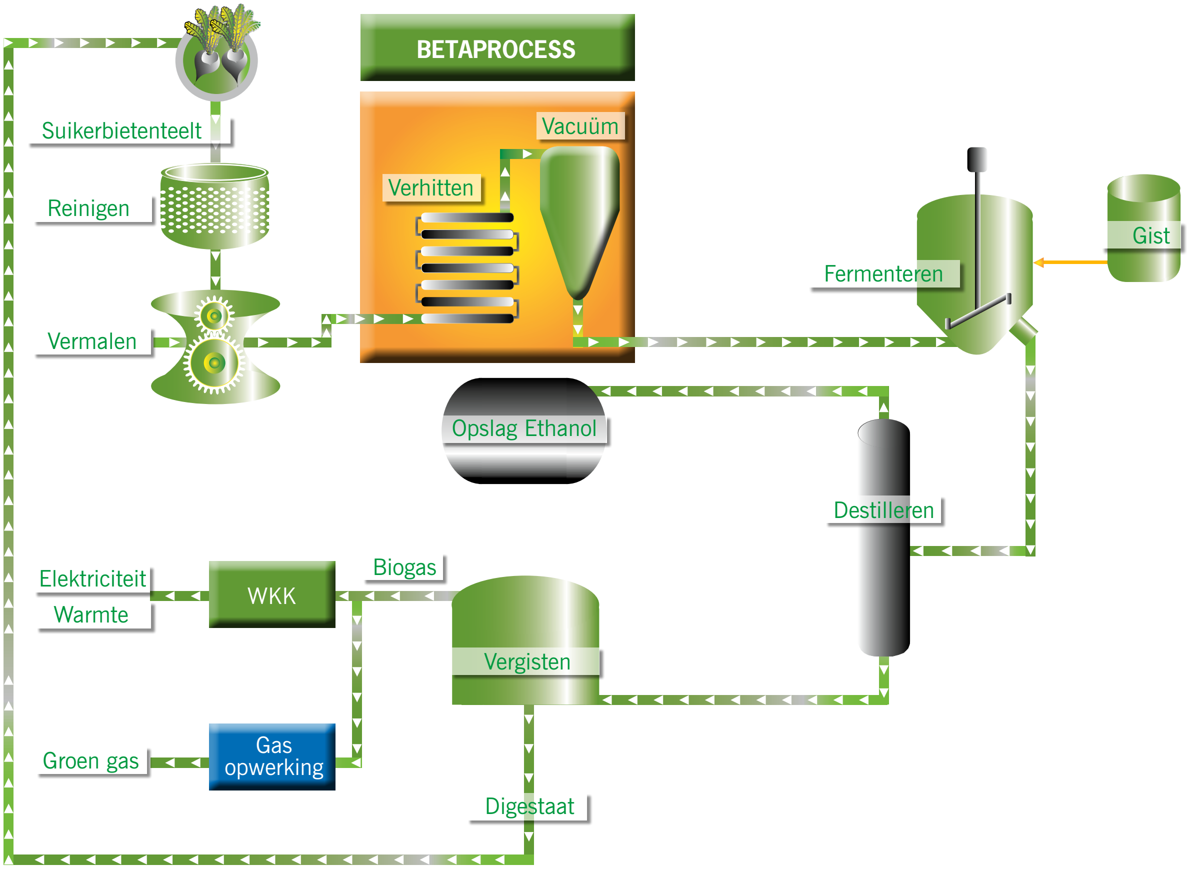


Figure 5.2 Sugar beet cleaning in progress on the Lelystad pilot facility.


www.betaprocess.eu

Figure 5.3 Betaprogress pilot in the Lelystad.

Refinery of green leaves to produce protein products

6

Author: E. Keijsers

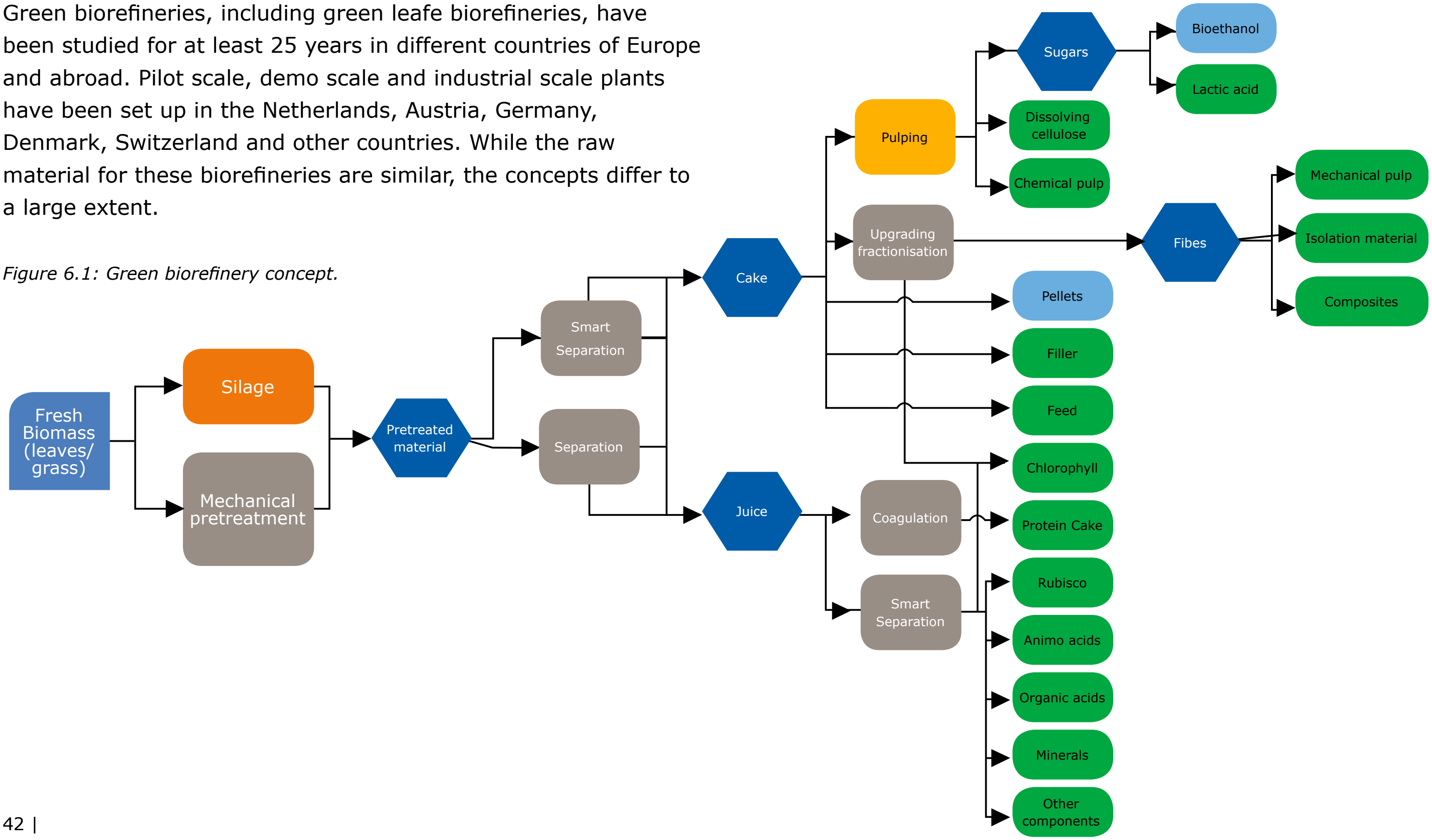
In this research project the possibilities for a small scale biorefinery based on green leaves have been studied. The research focussed on sugar beet leaves and spinach as raw material. During the four years of research a large number of small and medium sized enterprises have contributed to this work package and jointly directed the research topics. In the first two years the project focussed on the development of an economically viable biorefinery based on sugar beet leaves to produce a protein rich feed product, including the side-effect on soil organic matter and nutrient availability. In the latter years the project focussed more on developing the production of a protein product from sugar beet leaves and spinach with a higher added value, to overcome the poor economic feasibility of the biorefineries producing feed and energy as main products.

Green Biorefineries

Green biorefineries, including green leafe biorefineries, have been studied for at least 25 years in different countries of Europe and abroad. Pilot scale, demo scale and industrial scale plants have been set up in the Netherlands, Austria, Germany, Denmark, Switzerland and other countries. While the raw material for these biorefineries are similar, the concepts differ to a large extent.

Two different types can be distinguished based on the treatment of the raw material directly after harvest. The first type ensilages the raw material to be able to produce year-round. The second type processes as fresh as possible after harvest resulting in seasonal production. The processing season is lengthened by diversification of the raw materials (eg. grass, followed by consecutively sugar beet leaves and green manures). Biorefinery concepts based on silage mainly produce a relative clean fibre product and a peptide/amino acid product to be used as chemical or feed. Alternative products for these concepts include energy and heat from anaerobic digestion and chemicals like lactic acid and sugars. Biorefinery concepts based on fresh materials mainly produce a coagulated protein product and a fibrous press cake to be used respectively as pig or chicken feed and cow feed. Alternative products for these concepts are also energy and heat from anaerobic digestion, fertiliser (phosphate and minerals) and fibres for technical applications with relatively low quality. In figure 6.1 a general overview of green biorefinery concepts is given.

Figure 6.1: Green biorefinery concept.



Green leaf biorefinery

The green leaves selected in this research limit the type of products that can be produced. In contrast with grasses the selected raw materials (sugar beet leaves and spinach) do not contain a fibre fraction with significant fibre strength to use as raw material for e.g. composites, paper or building materials. Biorefinery concepts based on silage, although still having the benefit of year round production, thereby lose their main other benefit: the production of a relatively clean fibre product. The main benefit of a biorefinery concept based on fresh raw material - the possibility to produce high value protein products - was decisive for the choice of the different green leaf biorefinery processes studied in this research.

Economic evaluation of a sugar beet leaf biorefinery

In the first year of this project the economic viability of three different biorefinery concepts was studied (Wolf, 2013).

The three concepts were selected based on a set of criteria:

- The process had to be small scale and decentral in line with the goal of the overall small scale biorefinery project.
- The process had to be low tech and low cost because of the low value of the proposed end products
- The process had to consist of near to market or market ready technology, focussing on a market ready biorefinery in a few years time.
- The process had to take into account that sugar beet leaves are a secondary product. The process should not interfere with the sugar beet harvest or the quality of the harvested sugar beets.

Based on these criteria the three selected concepts were:

- Production of coagulated protein from the juice obtained after pressing of the sugar beet leaves
- Production of a protein rich press cake obtained after consecutive heating and pressing of the sugar beet leaves
- Production of heat and energy by anaerobic digestion after ensilage

All concepts were tested on labscale to obtain data for the mass balances used in the economical evaluation. Besides pressing by using a screw press, the vacuum explosion process (Beta-process) from DSD was used. The economic viability of all three processes was calculated to be very poor and major increases of yield and selectivity of the selected biorefinery processes were identified to be necessary to obtain a profitable business concept.

The effect of a sugar beet biorefinery on soil fertility and Nitrogen loss

Besides the benefits of using sugar beet leaves as raw material for the biobased and circular economy, the effect of removal of sugar beet leaves from the field was considered (Dijk, 2013). For the three biorefinery concepts mentioned above the effect on soil fertility and nitrogen loss was calculated in reference to the current situation, where the leaves are left on the field after the sugar beet harvest. The different concepts result in different amounts of minerals and phosphate that are returned to the field. All concepts result in a loss of effective organic matter in the field and a lower nitrogen availability. The amount of phosphate and potassium is also reduced. On the positive side, the removal of sugar beet leaves will result in a lower emission of the greenhouse gas nitrous oxide during winter time. It was concluded that the effect of a sugar beet biorefinery concept that returns most of the side products directly back to the field was the preferred choice based on soil fertility.

Pilot scale production of Rubisco protein from sugar beet leaves

Based on the economic evaluation of the three “low costs, low value products” biorefinery concepts and the focus of the industrial partners, large scale pilot trials were performed in the second year to produce a high value white protein product: Rubisco. In doing so, the idea of a low cost concept was exchanged for a concept focussing on Rubisco as main product and several low value side products. The trials were based on a process patented by TNO. They were performed at the facilities of Bodec in Helmond, partly using separation equipment shipped from the biobased products innovation plant in Wageningen. Basically the process consists of a series of separation processes using a pressing stage and a selective chemically aided coagulation stage, followed by several centrifugal and filtration stages. Although the results of the pilot trials were less optimal because of the lack of lab scale experience with the process, the insights gained in the process were very valuable. Results showed that intensive further development of all major processing stages is necessary to obtain maximum yield and selectivity of the process.

Development of processes on labscale to produce white protein from green leaves

The results of the pilot trials shifted the focus of the industrial partners to the development of a more simple process to obtain white protein from green leaves. As a raw material spinach instead of sugar beet leaves was chosen. This choice was partly based on the idea that the use of white protein from spinach would be more easy to introduce into the food market than white protein from sugar beet leaves, and partly on the less rigid cell wall structure of spinach. The latter would enable a less rigorous cell opening process. Different processes were studied on labscale producing white and green proteins. Tests to produce protein from spinach using the Betaprocess technology in combination with selected chemicals, were reported in Kootstra (2016). On labscale a relatively simple process consisting of a cell disruption stage, a coagulation stage and a dialysis stage was developed resulting in a white protein product from spinach. Alternative routes, including among others the addition of chemicals during coagulation, an activated carbon separation stage and extensive washing stages, resulted in less optimal products. However the yield of the white protein product was small (about 8-10% of the original protein content of the spinach). This small yield, together with the results and insights of the pilot scale trials of the second year gave rise to the assumption that the amount of white protein present in spinach and sugar beet leaves is actually small. Most white protein seems somehow bonded with coloured substances.

Figure 6.2: Press pulp from green leaves.

Development of processes on lab scale to produce green protein with improved taste

The combination of the knowledge obtained from the economic evaluation of the low cost biorefinery, the large scale pilot trials and the lab scale development of a process to obtain white protein, resulted in a focus shift in the fourth year. On lab scale a process was developed to obtain a green protein product from spinach without the normally obtained bitter taste (D1.5). This small scale biorefinery concept focusses on the production of a higher added value protein product with a relatively high yield (compared to the white protein option). Again the process was initially developed for spinach instead of sugar beet leaves to simplify market introduction of the Food product. After establishing a long list of possible reasons for the development of bitter taste during protein separation, two main focus parameters were selected and studied: the effect of processing time and temperature during the total process. On labscale the optimisation of the processing parameters resulted in products with improved smell and taste.

> *Paulus Kosters (GreenProtein):*
> *"The PPS project has given us access to a wide array of knowledge within WUR and colleagues in participating companies. The work carried out has increased our insights into our own quest."*

Conclusions

Green biorefineries based on grasses are currently booming in the Netherlands and abroad. Based on fresh grass, pilot scale (mobile) plants are in operation in the Netherlands (Grassa) and other countries (Denmark). Based on silage, industrial scale plants are running in the Netherlands (Newfoss) and e.g. Germany (Biowert). All grass biorefineries are partly driven by the fibre product, for feed, paper or insulation. The green biorefineries studied in this project are based on leaves with fibre fractions of insufficient quality to serve as valuable product. The economic viability of the biorefinery concepts therefore strongly depend on the quality and yield of the protein products and the amount of energy and heat obtained by anaerobic digestion. As a “low costs, low value products” biorefinery concept was not economic, the focus was shifted to biorefinery concepts producing high value food quality protein products. The processes developed and partly optimised on lab scale will have to be further studied before a small scale leaf biorefinery will be technical and economic viable. The obtained insights into the different processes and concepts have brought the spinach and sugar beet leaves biorefinery a step closer.
For information on the results of the pilot scale trial and the developed processes to obtain white and green protein, contact the author.

Reports

Dijk, W. van; Geel, W.C.A. van; Voort, M.P.J. van der, 2013. Effect van verschillende varianten van raffinage van bietenblad op bodemvruchtbaarheid en stikstofverliezen (Effects of different refinery solutions of sugar beet leaves on soil fertility and nitrogen losses). http://edepot.wur.nl/378460

Wolf, P.L. de; Visser, C.L.M. de; Keijsers, E.R.P.; Meesters, K.P.H.; Heesakkers, J.W.M.; Aerts, M, 2013. Kleinschalige raffinage van bietenblad : eerste verkenning van de mogelijkheden (Small scale biorefinery of sugar beet leaves: exploration of possible routes). http://edepot.wur.nl/313485

Kootstra, A.M.J.; Huurman, S., 2016. Protein extraction from spinach juice using vacuum explosion and their separation by active carbon, heat, and CaCl2. Wageningen UR, PPO/ACRRES (Rapport / PPO-AGV 687) - 21 p.

Acidified water extraction and purification of steviol glycosides from fresh Stevia

7

Figure 7.1 Stevia plant from greenhouse cultivation.

Author: A.M.J. Kootstra

Background

Stevia rebaudiana is a source of natural high potency low-calorie sweeteners in its leaves: steviol glycosides. Stevioside and rebaudioside A extracted from Stevia leaves have been widely used for years as a sweetener in a wide variety of foods in Japan, South Korea, China, South-East Asia and South America. Since the approval of Stevia sweeteners in the US by the FDA in 2008, and by the European Union in 2011, application of steviol glycoside has risen -mainly for soft drinks- and industrial interest has risen accordingly [1,2].

For the extraction and purification of the steviol glycosides from the plant material, several possibilities exist. A commonly used extraction method consists of extracting dried and powdered leaves with hot water, followed by a purification process that may include clarification by filtration or centrifugation, chelating agents, adsorption columns, etcetera [2,3]. For purification purposes, ultra- and nanofiltration are also suggested, including a centrifugation step for primary clarification of the extract, in a study using dried and powdered Stevia leaves [3].

To reduce process costs related to drying, it may be preferable to process fresh Stevia, possibly at relatively small scale –for instance close to the area of cultivation. The company NewFoss proposes to extract fresh Stevia plant material in water at room temperature –also saving energy in the extraction phase. In order to facilitate the extraction of steviol glycosides through the cell wall, the water is acidified in order to promote cell wall permeability. The acidification is achieved by microorganisms present on the plant material that produce organic acids.

Goal

To develop and evaluate the acidified water extraction of steviol glycosides from fresh Stevia plants, including purification. The research is performed with practical application in mind, which means that industrially available technology and materials are used, and mostly at bench/pilot scale. A factory design is to be made, taking into account water recycle.

Achieved results

Water at room temperature is added to fresh Stevia plant material. Some saccharose is added to aid the microorganisms that are naturally present on the plant in the production of organic acids and the mixture is left to acidify. After a certain time, the aqueous fraction -the primary extract- is separated off to be purified.
In 2014, the focus was on the evaluation of the acidic water extraction by comparing three consecutive Stevia harvests from the same plot in Romania. Purification was performed by ultra- (UF) and nanofiltration (NF). Results showed that 80 to 90 wt% of all steviol glycosides present in the plant ended up in the extract, and in the same relative concentration as in the plant. The purity of the final product was lower than desired, with 15 to 20 wt% of the dry matter consisting of steviol glycosides. The results indicated no differences in extraction efficacy between the three harvests. The dry matter content of the plants did increase with harvest time, mainly due to thickening of the stems, and as was to be expected, this led to a somewhat decreased average glycoside level in the plant. A strikingly large variability of glycoside levels was found in the plant material (Figure 7.2).

In 2015, the focus was on improving the clarification of the primary extract (Figure 7.3), in order to reduce the load on the ultrafiltration, for which a smaller pore size of the UF membrane was chosen to increase the purity of the filtrate. Although the improved clarification resulted in a much clearer liquid entering the UF step, the resulting filtrate only contained ~10 wt% glycosides in the dry matter, which was similar to the original extract. Clearly, the improved clarification and smaller UF pore size did not improve the purification.

In 2016, using Stevia plants cultivated in a greenhouse on site (Figure 7.1), the focus is on evaluating the purification potential of the acidified water extract, using industry standard purification technology such as clarification, adsorption resin (Figure 7.4) , and ion exchange resins. A comparison is made with hot water extraction of dried Stevia. Preliminary results show very nice potential for the acidified water extraction, regarding purity of the final product. A factory design is developed by NewFoss.

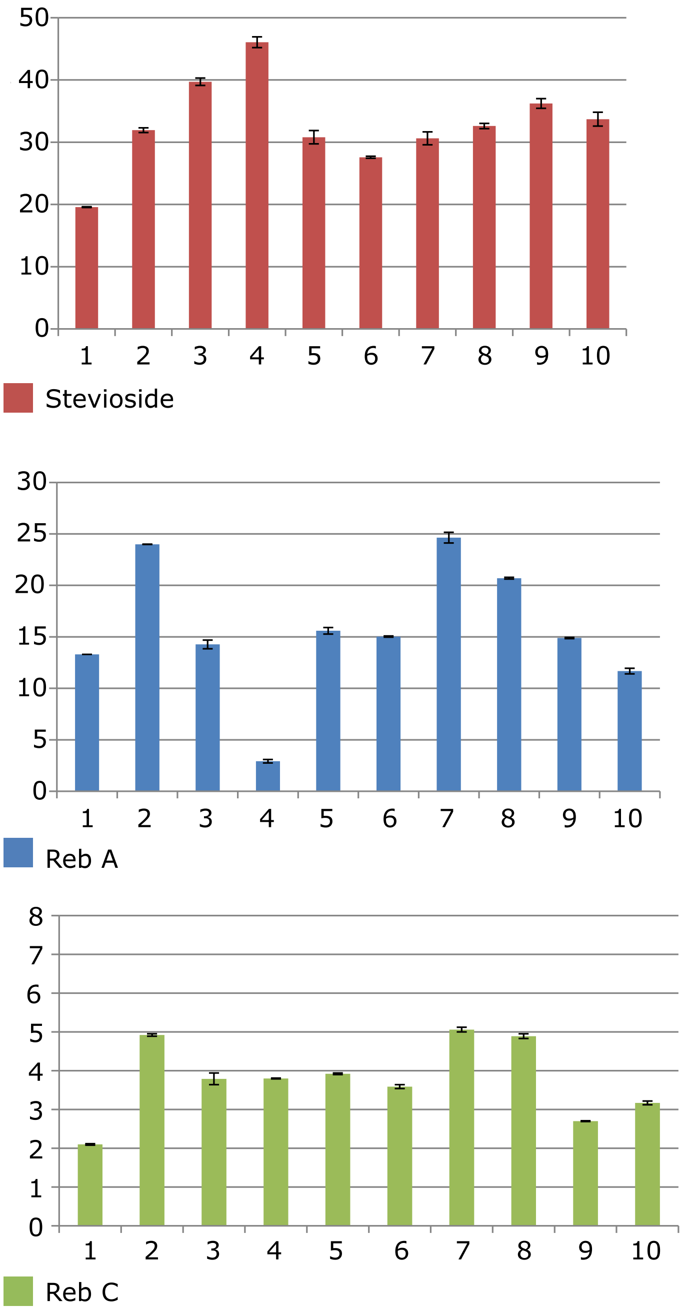


Figure 7.2. Steviol glycosides in first 2014 harvest of Stevia (mg/g dry matter). Average values of two samples, error bar = standard deviation

Figure 7.3. Clarification of primary extract by filtration.

Opportunities

The acidified water extraction process of steviol glycosides can be developed to a larger scale. Managing further scale up would be interesting, and also the optimisation of the purification by, for example, combining membrane filtration with application of adsorption, improving purification by introducing a decolouration step, or possibly separating glycosides during purification. Another point of focus may be the separation of the acidification and extraction, as is proposed in the original NewFoss process, which would mean that acidified water can be used multiple times, and remove the need for adding saccharose. A third point of focus could be on the factory design. For example, would it be preferable to first filtrate and concentrate an extract in a decentral set up, with further purification at a central site, or to do the extraction and entire purification on one central site?

> *Ivo Kretzers & Rob Kwinten (NewFoss):*
> *”Thanks to a strong collaboration and clear communication, the combined efforts of NewFoss and Wageningen University & Research have led to a very positive end result.*

Literature

Stoyanova, S., et al., The food additives inulin and stevioside counteract oxidative stress. International Journal of Food Sciences and Nutrition, 2011. 62(3): p. 207-214.

González, C., et al., Main properties of steviol glycosides and their potential in the food industry: a review. Fruits, 2014. 69(02): p. 127-141.

Chhaya, et al., Clarifications of stevia extract using cross flow ultrafiltration and concentration by nanofiltration. Separation and Purification Technology, 2012. 89(0): p. 125-134.

Reports

Kootstra, M. Extraction of steviol glycosides from fresh Stevia using acidified water; clarification by ultrafiltration and concentration by nanofiltration. 2015. Wageningen UR, PPO/ Acrres (Report / PPO-AGV 632)

Kootstra, AMJ, et al. Extraction of steviol glycosides from fresh Stevia using acidified water; clarification followed by ultrafiltration and nanofiltration. 2016. Wageningen UR, PPO/ Acrres (Report / PPO-AGV 686)

Kootstra, AMJ and S. Huurman. Extraction of steviol glycosides from fresh Stevia using acidified water; comparison to hot water extraction, including purification. 2016. Wageningen UR, Wageningen Plan Research/Acrres. In preparation.

Figuur 7.4. Elution of steviol glycosides from adsorption column.


B3
B1

8

Fatty acid and PHA production from residues

Authors: Hans Mooibroek and Christiaan Bolck

Background

Despite the financial crisis in Europe the bioplastics market is growing steadily, by about 20 percent per year. PHAs are a textbook example of biodegradable plastics made from biomass and microorganisms. For this family of polymers an increase in the world production is expected, which already rose to 400 kilotons in 2013 with a price of € 1.50 - € 3.70 / kg (Chanprateep, 2010). A more recent estimate of the current value for many different types of PHAs is between 3.80 - € 4.50 / kg (Ravenstijn, 2014). An important advantage of PHAs is that micro-organisms store them as a reserve substance and both can produce them as well as break them down in almost any environment (composting tray, ground and sea). Features of the simplest type PHA; Polyhydroxybutyrate (PHB), are comparable to, inter alia, polypropylene and polyethylene. There are numerous application possibilities for the other, about 150 types of PHAs: consumer, agricultural and horticultural products, catering, packaging materials, coatings and rubbers, and moreover high quality pharmaceutical and medical products. However, at this time the cost for production of PHAs by the conventional fermentation route is still too high. This is caused by the use of expensive components in growth media for the bacteria, high investment costs for fermentation facilities, and due to high costs for the extraction (downstream processing). But there are smart routes possible to reduce the cost price. For example, by choosing for the production of PHAs from various waste streams, such as waste water (sludge) coming from sewage treatment plants (WWTP), and / or industrial waste water, for example, from the paper, food processing or chemical industry. Also exudation coming from fresh heterogeneous biomass such as municipal solid waste (MSW) or other biomass such as fresh exudation from beet leaves and even manure can potentially be used as feedstock. And that seems to fit well into existing processes and systems. Thus, a sewage treatment plant is an already working system in which biological conversion processes occur, including logistics and permits.

History

For decades work has been devoted to the production and development of different types of PHAs and their specific industrial applications. For the production, use was made of micro-organisms that store the PHA in the cell as a reserve of carbon and energy. The cultivation of these bacteria took place in fermenters in which the growth conditions can be adjusted perfectly and maintained.
The desired types of PHA's (especially medium chain-length, mcl-PHAs with more than 5 C-atoms, containing functionalized C-chains, or short chain-length, scl-PHA's with 3-4 C-atoms) can be obtained on the basis of the administration of specific fatty acids. These fatty acids can be incorporated by the PHA-producing bacterium in order to be converted by means of the so-called β-oxidation in the acyl-CoA-hydroxyalkanoates. The net effect is that of the administered fatty acid is an acetyl-CoA molecule and an acyl-CoA molecule are formed, which are two carbon atoms shorter than the fatty acid included. This CoA-functionalized fatty acid may, in turn, are then used in the polymerization to mcl-PHA.

PHAs have many advantages. Microorganisms can produce PHAs and also degrade them again; they are not produced from the mineral oil and are biodegradable in the composting container, the bottom and the sea. Properties of a much investigated type

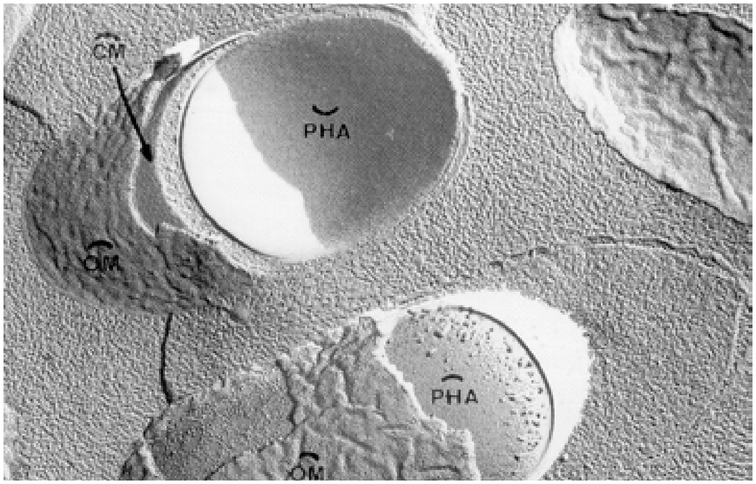


Figure 8.1: Pseudomonas oleovorans containing PHA.

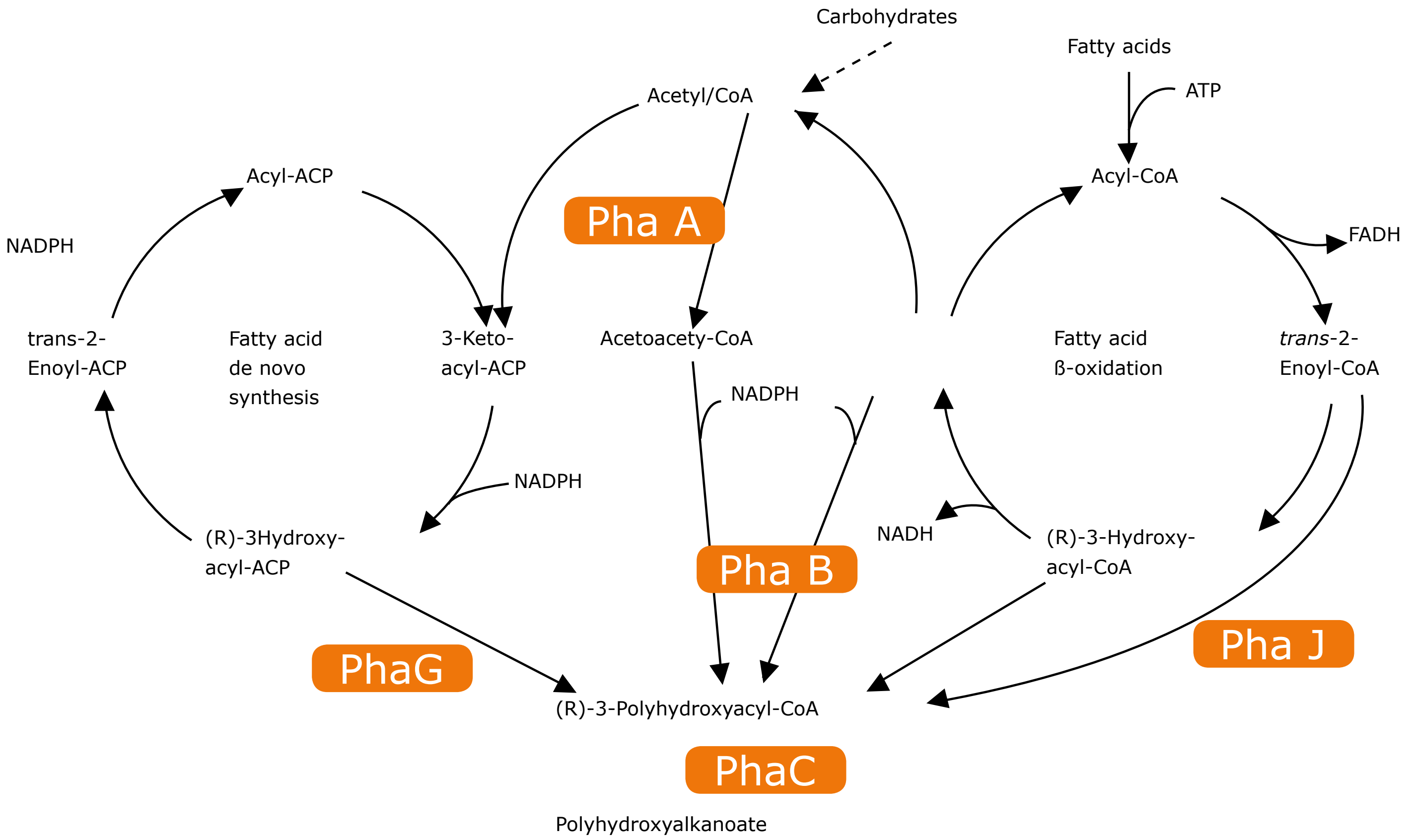


Figure 8.2: Biosynthesis of PHAs (Doddema, 2014).

scl-PHA polyhydroxybutyrate / hydroxyvalerate (PHB / HV), are comparable to, inter alia, polypropylene and polyethylene. In addition, other types of PHAs, mcl-PHAs, the applicability in paints, coatings, rubber, and demonstrated in blends with other polymers. The applications of PHA are in the market segments of consumer products, pharmaceutical and medical products, agriculture and horticulture, catering and packaging materials. An example of a high-quality application, the application in pharmaceutical and medical products (e.g. tissue engineering, controlled release, surgical sutures, wound dressings, orthopedic use and as pericardial substitute). However, there are also disadvantages. It is still difficult, even under well-controlled conditions in a fermenter, in the end always to obtain the appropriate PHA composition and quality. The production price is also still too high, caused by expensive components of the growth medium for the bacteria, the high investment costs for fermentation facilities, the extraction and downstream processing.

A prerequisite is making the production and downstream processing stable. If the quality is stable, this knowledge can also be applied to improve the quality of a material based on PHA. The production costs of conventional PHA production will be approximately 40% based on the raw materials. These raw materials include a carbon source (glucose, starch or vegetable oil), and nutrients. The cost of the carbon source amount to approximately 70% of the aforementioned 40%. For this reason, PHA production from industrial waste streams, and (primarily) sewage sludge may be of interest because this sludge costs very little and after processing, can be used as an alternative

substrate for the PHA-producing bacteria. It is expected that PHA from waste and sewage sludge first is useful in applications such as biodegradable plastics for agriculture and horticulture.
In recent years much attention has been devoted to the production of the PHA-rich biomass. Various microbial production systems have been investigated to reduce the cost. Netherlands have a prominent position in this area. The common challenge seems to be the processing of the PHA-rich biomass into a marketable products with a sound business case. One of the bottlenecks is the extraction. The current method is admittedly pretty selective but also very expensive and not very environmentally friendly. Partly because of cost of PHA bioplastics for use as higher than the reference process: the production of biogas. Search for more efficient extraction of the PHA-rich biomass and purification of the PHA are necessary for the success of the planned business cases. In addition, research is needed into the technical and economic feasibility of various business cases and the role of companies herein. The conversion of low-value waste streams into high quality products such as bioplastics based on PHAs is one of the most promising routes for valorization relatively wet biomass on a small scale.

In addition, microorganisms produce PHAs and also can degrade it; they are not produced from the mineral oil and are biodegradable in the composting container, the bottom and the sea. Within this optimum PPS chains are defined for the production and application of PHA, the bringing together of the stakeholders and development of different types of PHAs and their specific industrial applications.

Approach

The project aimed at generating critical indicators related to end use, conversion process, features PHA rich biomass, PHA production process and raw materials. The approach was to work from the end of applications by means of a system of backward integration in 5 tasks as shown in the following diagram (Figure 8.3).

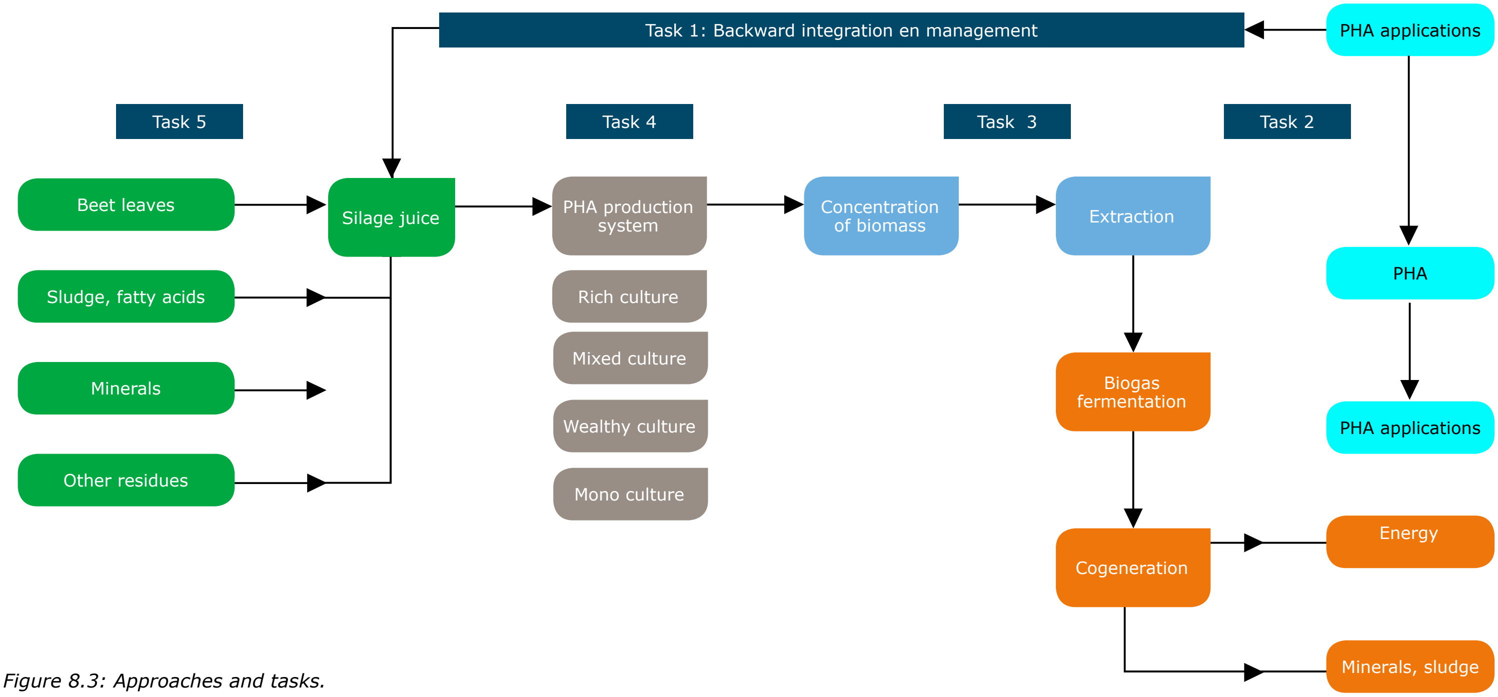


Figure 8.3: Approaches and tasks.

Figure 8.4: Water treatment plant Bath.

Based on key figures, generated from public literature, analysis of samples from partner companies and specific tests, partners will build promising PHA value chains.

The work included five tasks as follows:

9.1 *Management + backward integration.*
The entire business case has been investigated and in particular the desired PHA properties and the requirements of the end user have been back-translated into activities upstream in the supply chain. Also the project looked at the technological and economic feasibility of the chain and the role of companies herein.

9.2 *Applications.*
All activities in this task were focused on the development of practical applications of PHAs such as thermoplastic processable material (plastics), coating and rubber from the intermediate products from task 3. Product developments have employed reference products including PHAs already commercially and pilot-scale produced by other parties.

9.3 *Extraction.*
In this task, methods were developed for isolating PHAs from the bacterial biomass. Different matters such as purity, water content, type (s), molecular weight, melting behavior etc. were considered

9.4 *Production systems.*
Optimization of production conditions (PHA) under lab-, pilot- and real life conditions. In this part the project also examined whether the addition of extra additives could increase the PHA productivity and / or quality. Also the project looked at selected bacterial species (and) suitable for mixed, rich and possibly pure cultures (incl. Wealthy cultures) and tested their under laboratory conditions (through mutagenesis). Also, determining the abundance of individual species under lab, pilot and real life conditions, coupled with the (PHA) productivity fitted within this task.

9.5 *Raw Materials / silage juice / fatty acids.*
This task concerned all activities related to the definition and provision of heterogeneous biomass residues for the benefit of fatty acid production. These included the use of microbial production technologies, type and purity fatty acid (s), fatty acid source such as sludge, organic waste, ensiled beet leaves or other residues.

Perspective

In addition to a cheaper source of short chain PHAs from agricultural residues and sewage treatment plants, which often yield short-chain PHAs, such as PHV and PHB-co-HV and which can be processed into bioplastics, the project also considered the production of mcl-PHAs. Here, longer fatty acids were offered to the bacteria which should create a much wider range of different molecular forms that are less crystal line by their longer chains. These can then be processed in other applications such as coatings, hot melts, PHA-rubber. In addition, research has also been conducted on the application of PHAs in different blends, for example, with 1-10% of poly-lactic acid (PLA), which thereby gets entirely different, desirable properties. The slightly higher price of PHAs is then less important.

What has been achieved

In this research, cooperation with a number of companies and organisations was realised: Rodenburg RB biobased Institute (applications of PHAs), Moon Research & Development (applications), Feyecon D & I (supercritical extraction), KNN (applications, recycling), BIONND (production), Opure (production), Brabantse Delta Water and Water Board De Dommel (WWTP production both in collaboration with Veolia / AnoxKaldnes). With all partners WFBR has organized interviews in which the specific requirements for the intended uses were back-translated into appropriate type(s) of PHAs (or blends) required. Striking examples are the applications of Rodenburg (plant pots) and Moon (hot melts, glues). Making use of the press juice from grass silage Wageningen University & Research has developed an inexpensive growth medium, on which the PHA-producing Pseudomonas putida was found to grow well. The process conditions still need be optimized for (more) efficient PHA production. This work also formed the basis for a new development, socalled "Wealthy cultures", which employes monocultures in open systems that are able to make the preferred types of mcl-PHAs. These are not yet or hardly commercially available. Different types of PHAs were produced by BIONND (from) potato foliage, by Opure and the Water Boards in cooperation with Veolia / AnoxKaldnes (Phario project). Feyecon and Wageningen University & Research continue their research into more efficient and cheaper extraction methods using supercritical CO2 extraction and bead mill extraction, respectively. The continuous bead mill extractor is now operational for testing of larger samples PHA containing bacterial biomass. A Techno-Economic Analysis of processes modified within this project (e.g. use of various waste streams, extraction method, scale) as compared to the existing commercial processes (Choi & Lee, 1997) is part of the Deliverable report.

Literature

Chanprateep J. Current trends in biodegradable polyhydroxyalkanoates. BIOSCI. BIOENG., 110 (2010) 621-632

Choi J-i, Lee SY. Process analysis and economic evaluation for Poly(3-hydroxybutyrate) production by fermentation. Bioprocess Engineering 17 (1997) 335-342

Doddema H. PHA from green leaves. Presentation First TKI-PHA Progress Meeting, December 2014. Wageningen

Ravenstijn J (2014): PHA... Is it here to stay? Presentation. http://www.kcpk.nl/algemeen/bijeenkomsten/presentaties/20140508-jan-ravenstijn-pha-is-it-here-to-stay

Colofon

Uitgave
Wageningen University & Research

Research groups involved are:

ACRRES
Chris de Visser
T | +31 320 291692

FBR Biobased Innovation Plant
René van Ree
T |+31 317 480710

Final editing
Chris de Visser
René van Ree

Pictures
Wageningen University & Research

Design
Caroline Verhoeven-Poelhekke
Styling & Design

Print
vanLieremedia

Edition
300 copies

www.wur.nl

Companies involved	*website*
ABC Kroos	*abc-kroos.nl*
ACConsult	
Algaecom	*www.algaecom.nl*
Algae Food & Fuel	*www.algaefoodfuel.com*
Attero	*www.attero.nl*
Bioclear	*www.bioclear.nl*
BIONND	
Bodec Food Tech	www.bodec.eu
Byosis	www.byosis.com
CSVCOVAS	www.bieten.nl
Den Ouden Groen Recycling	*www.en.denoudengroep.com*
DSD	*www.dsdbv.com*
Dumea	*www.dumea.nl*
Ecofys	*www.ecofys.com*
FeyeCon Development & Implementation	*www.feyecon.com*
Flevosap	*www.flevosap.nl*
GreenProtein BV	*www.greenproteinbv.com*
Innovatiecentrum Greenport Venlo	*www.innovatiecentrumvenlo.nl*
Imperial Ventures	
Kelstein	*www.kelstein.nl*
KH Engineering	*www.kh-engineering.nl*
Koers	
KNN Advies	*www.knnadvies.nl*
KplusV Advies	*www.kplusv.nl*
Maan Research & Development	*www.maanrd.nl*
Melkveehouderij Geenen	*www.dezorgboerderij.com*
NewFoss	*www.newfoss.com*
Nuplex Resins	*www.nuplex.com*
Opure	*www.opure.nl*
Rodenburg RB Biobased Institute	*www.biopolymers.nl*
ROVA Duurzaam	*www.rova.nl*
Sensus	*www.cichorei.nl*
Spring Biobased Products	
Waterschap Brabantse Delta	*www.brabantsedelta.nl*
Waterschap De Dommel	*www.dommel.nl*


HIJSTRADITIE | SCHIEDAM | 2500 KG 1993
EMAG
ingeo