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In cooperation with

Max Rubner-Institute
Institute of Safety and Quality of Cereal
and the
University of Hohenheim
Institute of Food Science and Biotechnology

10th European Bioethanol and Bioconversion Technology Meeting

April 08th – 09th 2014
Detmold, Germany

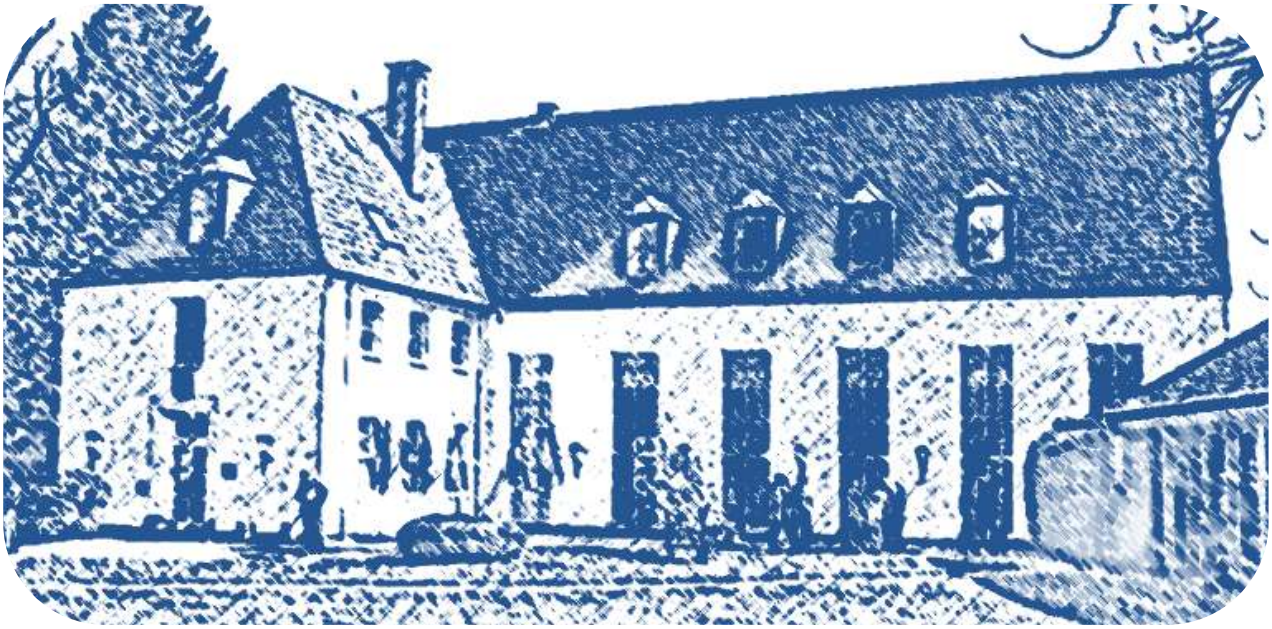
Program

Evening Program

Exhibition

Participants

Summaries



Tuesday, April 08th 2014

08^{30} **Opening Remarks** by the President of the Association of Cereal Research, **Götz Kröner**, Ibbenbüren (Germany)

1. Plenary Lecture

1.1. **Gloria Gaupmann**, München (Germany)
Das biobasierte Industriekonsortium (BBI)

2. Second Generation

2.1. **Jan Lindstedt**, Örnsköldsvik (Sweden)
From tree to tank, ethanol development in Sweden

2.2. **Michael Studer**, Zollikofen (Switzerland)
Consolidated bioprocessing of lignocellulose to ethanol in a multispecies biofilm membrane reactor

10^{15} Coffee Break

2.3. **Jan Lindstedt**, Örnsköldsvik (Sweden)
Transformation from Ethanol Pilot to Biorefinery Demo Plant

2.4. **Peter Punt**, HE Zeist (The Netherlands)
Identification of Inhibitors in Hydrolysates of Lignocellulosic Biomass

2.5. **Helge Holm Larsen**, (Denmark)
FirstSugar™:a cost-efficient and de-risked route to 2G sugar

3. Technology

3.1. **Gerhard Kerns**, Leipzig (Germany)
The SSF-process for ethanol production on pulp from wheat straw

12^{45} - 14^{00} Lunch Break

3.2. **Jean Népomuscène Ntihuga**, Stuttgart (Germany)
Newly Designed Continuous Bio-Ethanol Fermentation Systems using Blenke-Cascade and Plate-Heat-Exchanger

3.3 **Hannes Richter**, Hermsdorf (Germany)
Process optimization of bioethanol synthesis by membrane technology

3.4. **Timo Broeker**, Lemgo (Germany)
How to extend a distillery into a lignocellulose biorefinery – material- & energy flow management for bioethanol, biogas und HTC biochar

15^{30} Coffee Break

4. Enzymes

4.1. **Bart Koops**, AE Leiden (The Netherlands)
Optimizing ethanol yields by improved enzyme dosing strategies in grain liquefaction and fermentation

4.2. **Arjen van Tuijl**, AE Leiden (The Netherlands)
Maximizing ethanol yields by action of a novel Trehalase

To be continued on page before last

Evening Program

Monday, April 07th 2014

19^{30} **Welcome Evening** at the **Convention Hall**, Detmold, Schuetzenberg 10

Tuesday, April 08th 2014

19^{30} **Social gathering** at the restaurant “Teutonenhof”, Holzhausen-Externsteine
transfer by bus.

Buffet

Slice of beef with rosemary and a taste of garlic

***

Ham of pig escalloped with anise, gravy

***

Cauliflower and broccoli, baconed beans

***

Marjoram potatoes, potato wedges

***

Salad Buffet

***

Red fruit jelly with custard and Mousse au chocolat

Participants tickets

The Tickets for this evening, including Food and Beverages, are available at the convention office for 35 Euro. Please make your reservation until 4pm, if possible.

Bus transfer

A bus transfer is organized for you.

19.05 h	**Bus stop 1**	**Train station Detmold** (Elisabethhotel)
19.15 h	**Bus stop 2**	**Sparda Bank - Willi-Brandt-Platz/Paulinenstrasse** (For the Hotels Lippischer Hof, Detmolder Hof and Best Western Residenz, Altstadt Hotel)

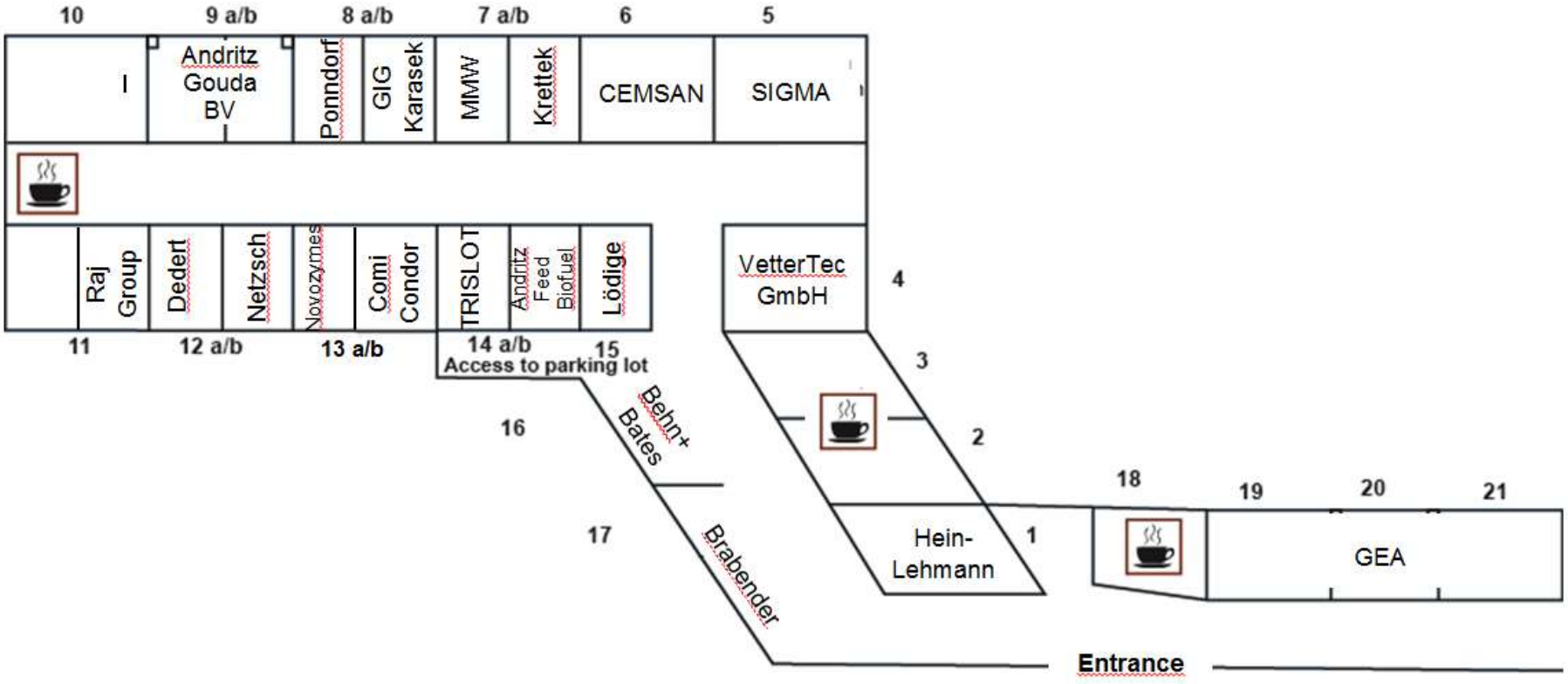
Departure: from 22^{00}

Thank you!

Exhibition Hall Association of Cereal Research

Stand allocation

10th European Bioethanol and Bioconversion Technology Meeting and 65th Starch Convention from April 8th – 10th 2014



Exhibition

Exhibition

Andritz Feed & Biofuel Div., Cumming, GA 30040 (USA)

Andritz Gouda BV, PD Waddinxveen (Netherland)

Behn & Bates Maschinenfabrik GmbH & Co. KG, Münster (Germany)

Brabender GmbH & Co.KG, Duisburg (Germany)

Cemsan A.S., Arifiye, Sakarya (Turkey)

Comi Condor SPA, Santa Christina E Bissone (Italy)

GEA Westfalia Separator Group GmbH, Oelde (Germany)

Gebr. Lödige Maschinenbau GmbH, Paderborn (Germany)

GIG Karasek GmbH, Gloggnitz (Austria)

HEIN, LEHMANN GmbH, Krefeld (Germany)

Krettek Filtrationstechnik GmbH, Viersen (Germany)

MMW Technologie GmbH, Lutherstadt Wittenberg (Germany)

Netzsch Pumpen & Systeme GmbH, Waldkraiburg (Germany)

Novozymes Deutschland GmbH, Bad Kreuznach (Germany)

Ponndorf Anlagenbau GmbH, Kassel (Germany)

RAJ PROCESS EQUIPMENTS & SYSTEMS PVT. LTD.,
Akurdi, Pune (India)

Sigma Process Technologies, Atasehir/Istanbul (Turkey)

Trislot N.V., Waregem (Belgium)

VetterTec GmbH, Kassel (Germany)

W. Kunz dryTec AG Swiss Combi / Dedert Corporation,
Homewood (Schweiz)

Participants

Effective April 03rd 2014

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Wolf, Klaus

Summaries

1. Plenary Lecture

1.1. **Gloria Gaupmann**, München (Germany)
Das biobasierte Industriekonsortium (BBI)

The summary couldn't be finished in time.

A doctoral graduate from the University of Bonn, Gloria Gaupmann holds a PhD in European political science. Prior to joining Clariant's Group Biotechnology in 2013 as Public & Regulatory Affairs Manager, Gloria was Deputy Secretary General at the European Renewable Ethanol Association (ePURE) since 2010. From 2007 to 2010 Gloria held various positions in the European Bioethanol Fuel Association (eBIO), where she specialized in energy policy and environmental affairs. She has previous professional experience in the Centre of European Integration Studies (University of Bonn, Germany) and in the European Parliament, where she worked for a German MEP focusing on European transportation issues.

Clariant is an internationally active specialty chemical company, based in Muttenz (Switzerland). The main focus of Clariant's Group Biotechnology is on bio-catalysis and bio-refining. The company develops energy-efficient processes for the manufacture of biomass-based chemicals and fuels. Clariant has developed the sunliquid process which uses enzymatic hydrolysis followed by fermentation to produce cellulosic ethanol from agricultural residues.

2. Second Generation

2.1. **Jan Lindstedt**, Örnsköldsvik (Sweden)
From tree to tank, ethanol development in Sweden

Commercial production of ethanol and other biofuels based on lignocellulose feedstock is close to realisation. The technology is verified in small industrial Demo Plant and is commercially interesting as a Biofuel but not as cheap as fossil fuels. To motivate and justify new investments we must develop the market, with high goals that require low blending, but also high blending in the gasoline and diesel engines. Use in the transport sector and production is closely connected from tree to tank.

The presentation will give an overview of some of the experiences, challenges and opportunities facing both development and commercialisation of Cellulose Ethanol technology but also some words on market introduction of ethanol in Sweden. General aspects are covered as well as the specific approach and experience of SEKAB E-Technology. With the NER300 project (New Entrants Reserve) in Poland as an example, the speech will give a summary of some of the actual challenges (June 2014) and opportunities facing the commercialisation of Cellulose Ethanol.

Jan has been involved in the development of technology and introduction of Ethanol in Sweden during the last 20 years partly as Vice President at SEKAB E-Technology with responsibility to scale up and commercialize the cellulose to ethanol technology. With an education as Master of Chemical science at Chalmers University of Technology Jan started as production engineer and site manager in pulp and chemical production in Sweden for 20 years.
The presenter has been involved in the Swedish Ethanol development during the last 25 years with focus on cellulose to ethanol development. The story of how the Swedish scientists and stakeholders gathered to build the Ethanol Pilot will be presented. Focus will be on lessons learned, some figures, data and actual status of implication of the Lignocellulose to Ethanol technology.
SEKAB the Swedish CE-technology developer, got the decision from the European Commission in late December 2012, that it was awarded 30,9 MEUR for a project they initiated in Poland. The lesson learned so far from feasibility studies starting in Sweden up to the Polish NER300 awarded project, as well as actual situation in the project will be covered, including some of the barriers for commercialisation.
The presentation will also share some of the experience, from introduction of ethanol fuelled heavy vehicles, beginning in 1986 and flexi fuel cars in 1994, to increased low blending and blend pump infrastructure development 2004 to now. Personal and general experience will be highlighted in successes as well as setbacks. What could others learn from Sweden's long ethanol history?

2.2. **Michael Studer** and **Simone Brethauer**, Zollikofen (Switzerland),
Robert Shahab, Zurich (Switzerland),
Consolidated bioprocessing of lignocellulose to ethanol in a multispecies biofilm membrane reactor

The biochemical conversion of recalcitrant lignocellulosic biomass to desired chemicals requires complex process schemes that need to be simplified in order to improve economics. To this end, we developed a process, which allows the consolidated bioprocessing of lignocellulose to ethanol or organic acids in a single multi-species biofilm membrane (MBM) reactor. This process features coexistent aerobic and anaerobic conditions necessary for the simultaneous fungal cellulolytic enzyme production and fermentation of the hydrolysis-derived sugars, respectively.
After demonstrating the successful production of ethanol from pretreated wheat straw in 30 mL flat sheet membrane batch reactors by the combined action of T. reesei, S. cerevisiae and S. stipites (70% yield) we scaled up the process to 500 mL. The improved reactor design allowing for state of the art process control features a tubular silicone membrane submerged in the fermentation slurry. We then set up a continuous MBM process by connecting two of such stirred tank reactors in series in order to characterize the system stability, optimize fermentation conditions and establish achievable yields.
Furthermore, the product range of the MBM process was extended by changing the product forming microorganism. In conjunction with T. reesei, e.g. Lactobacillus delbrückii produced lactic acid based on microcrystalline cellulose. These examples show the potential of the MBM-process to be applied as a universal synthesis platform for desired bio-chemicals based on lignocellulosic biomass.

Dr. Michael Studer is currently professor of chemical engineering at Bern University of Applied Sciences (HAFL-BFH, Zollikofen). Prior to that, he was a project leader at ETH Zurich and a Post-Doc at UC Riverside

working for the BioEnergy Science Center. He holds a Diploma as a Process engineer and a Doctorate from ETH Zurich.

2.3. **Jan Lindstedt**, Örnsköldsvik (Sweden)
Transformation from Ethanol Pilot to Biorefinery Demo Plant

As commercial production of ethanol and other biofuels based on lignocellulose feedstock is close to realisation, the possibilities for other applications opens up, in the development units that have been used for the biofuels development. The chemical sector is close related to the biofuels and a natural next step. In the Ethanol Pilot plant in Örnsköldsvik in Sweden this has been a process for some years.

The presentation will give some of the experiences, challenges and opportunities facing the change of the plant from biofuels to biochemicals in a Biorefinery Demo Plant. Both technical challenges and change of organisation and stakeholders will be covered.

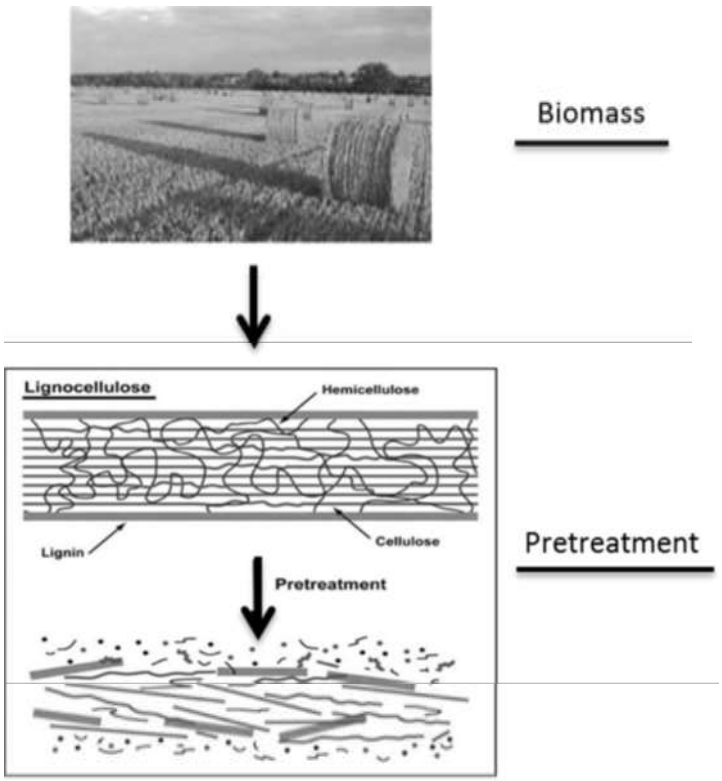
Jan has been involved in the development of technology and introduction of Ethanol in Sweden during the last 20 years partly as Vice President at SEKAB E-Technology with responsibility to scale up and commercialize the cellulose to ethanol technology. With an education as Master of Chemical science at Chalmers University of Technology Jan started as production engineer and site manager in pulp and chemical production in Sweden for 20 years.
SEKAB the Swedish CE-technology developer has been responsible for the Ethanol Pilot since it was build and started in 2004. But as the technology became ready for commersialisation, time became available in the plant to verify other processes. SEKAB invited other companies and organisations to take part and introduce new ideas'. Today the largest Swedish Institute SP has taken over the management responsibility in close cooperation with SEKAB that host the Demo plant in their premises.
The cellulose to sugars and chemicals development has started all over the world. Some experience how to convert the Ethanol Pilot to a Biorefinery Demo Plant in Örnsköldsvik will be presented. Focus will be on lessons learned to open up for other applications and actual status of the Lignocellulose to Chemicals technology in Sweden.

2.4. **Peter Punt**, HE Zeist (The Netherlands)
Identification of Inhibitors in Hydrolysates of Lignocellulosic Biomass

Lignocellulosic biomass is the future feedstock for the production of biofuel and bio-based chemicals. The pretreatment-hydrolysis product of biomass, so-called hydrolysate, contains not only fermentable sugars, but also compounds that inhibit its fermentability by microbes.
To reduce the toxicity of hydrolysates as fermentation media, knowledge of the identity of inhibitors and their dynamics in hydrolysates need to be obtained. In the past decade, various studies have applied targeted metabolomics approaches to examine the composition of biomass hydrolysates. In these studies, analytical methods like HPLC, RP-HPLC, CE, GC-MS and LC-MS/MS were used to detect and quantify small carboxylic acids, furans and phenols. Through applying targeted metabolomics approaches, inhibitors were identified in hydrolysates and their dynamics in fermentation processes were monitored. However, to reveal the overall composition of different hydrolysates and to investigate its influence on hydrolysate fermentation performance, a non-targeted metabolomics study needs to beconducted.

A second topic of our studies refers to another aspect of feedstock hydrolysis. Production of second generation feedstock hydrolysates used for bioethanol fermentation processes largely relies on the development of suitable cocktails of fungal (hemi)cellulolytic enzymes. The major constituent of these enzyme cocktails are cellulases and hemicellulases, such as xylanases. Given the relatively complex structure of the carbohydrate fraction of feedstock substrate, for complete hydrolysis a suite of enzyme activities is required. Moreover, a wide variety of feedstocks from diverse agricultural and forestry residues, and several different chemical pretreatment methods are used. This leads to variable composition of the material used for a feedstock source, thus requiring different enzymes for its hydrolysis. In our research we have addressed two approaches to get further insight in improving feedstock enzyme cocktails.

First we have developed a new method to analyse enzymatic hydrolysates using a combination of HPAEC and MS, allowing the identification of a wide array of sugar and sugar-acid oligomers remaining from incomplete hydrolysis. Interestingly, from this analysis it was clear that not only hydrolytic activities play an important role in the quality of the resulting hydrolysates but also novel transglycosylation reactions. Secondly, genome mining approaches were followed to identify novel enzymatic activities contributing to hydrolyses of feedstock substrates. Clear differences in the enzymatic repertoire of different fungal species were observed. Novel genes encoding hydrolytic and alternative substrate modifying activities were discovered from hitherto unexplored fungal genomes.



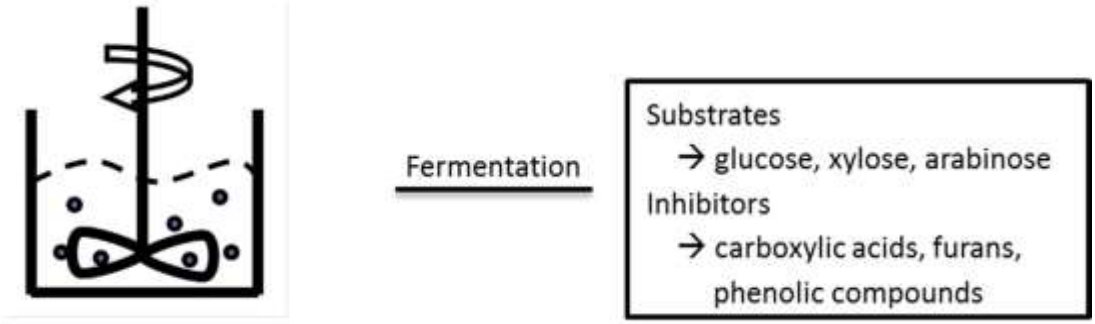


Figure 1 Schematic workflow for the preparation of lignocellulosic biomass hydrolysates and their use in microbial fermentation.

Peter Punt is appointed by the Lorentz - van Iterson Fonds TNO extra-ordinary professor Industrial Biotechnology at Leiden University (Institute for Biology; IBL).
Peter Punt is senior scientist in molecular genetics at TNO Microbiology, Zeist, Netherlands. He obtained his M.Sc. in Biology from Leiden University in 1984 and his Ph.D. in Molecular Genetics from University of Amsterdam in 1992. Since 1992 he is working at TNO as project leader in many projects for international biotechnology industries. Besides his position as project manager, he has supervised several Ph.D. students and many undergraduate students in molecular genetics and gene regulation of filamentous fungi. He has been involved in collaborative projects within the framework of the Wageningen Centre for Food Sciences, the Kluyver Centre for Genomics of Industrial Fermentation and the European EUROFUNGbase consortium and is currently Principal Investigator at the Netherlands Metabolomics Centre. He is a member of the Dutch Society for Microbiology, the Dutch Biotechnology Association and the American Society for Microbiology Since 2010 he is section editor for BMC Biotechnology and Associate Editor for Eukaryotic Cell. He (co-)authored more than 100 peer-reviewed papers, 10 patent applications and several book chapters.

2.5. **Helge Holm Larsen**, (Denmark)
FirstSugar™: a cost-efficient and de-risked route to 2G sugar

The lecture introduces the concept of FirstSugar™ which is the extraction of hemi-cellulosic sugar in connection with the manufacture of fuel pellets, in particular wood pellets. The manufacture of wood pellets is well known and growing at a CAGR of about 20%, primarily driven by conversion of coal fired power stations in Europe and increasingly in the ROW.

Wood pellets are well suited for this purpose, because they can be transported efficiently over significant distances, stored and fired in existing coal fired power stations with a minimum of conversion. On the other hand, the wood pellets also introduce more ash and slag formation and a lower calorific density than coal.

In the FirstSugar™ concept, the BioGasol proprietary Carbofrac® technology is applied in the wood pellet plant to treat the wood and separate the soluble hemicellulose fraction from the insoluble cellulose & lignin fraction. This permits a non-enzymatic extraction of 2G hemi-cellulosic sugars for biochemical and is leaves the insoluble fraction of the biomass for continued wood pellet production.

Advantages of this concept includes:

- Low cost sugar platforms for biochemicals

- De-risking from costly and unproven enzyme technology otherwise applied for 2G sugars
- De-sensitized sugar cost structure compared to the typical price volatility of the sugar industry
- Wood pellets with higher calorific density
- Wood pellets with less ash and slag formers
- Stronger and more weather resistant wood pellets

The lecture describes the technical layout of the concept and seeks to quantify the advantages. The lecture will also provide some information on the wood pellet market, the application of the 2G hemi-cellulosic sugars manufactured and the enabling BioGasol technology, the Carbofrac®, which has a hemi-cellulosic sugar yield of about 90%, essential for the feasibility of the FirstSugar™ concept.

Joined BioGasol with more than 20 years of international sales and business development experience within the energy conversion and cleantech sectors, including the Topsoe group and in the start-up company TEGnology. Obtained a M.Sc. in Chemical Engineering from Technical University of Copenhagen and a HD-U in Foreign Trade from Copenhagen Business School.

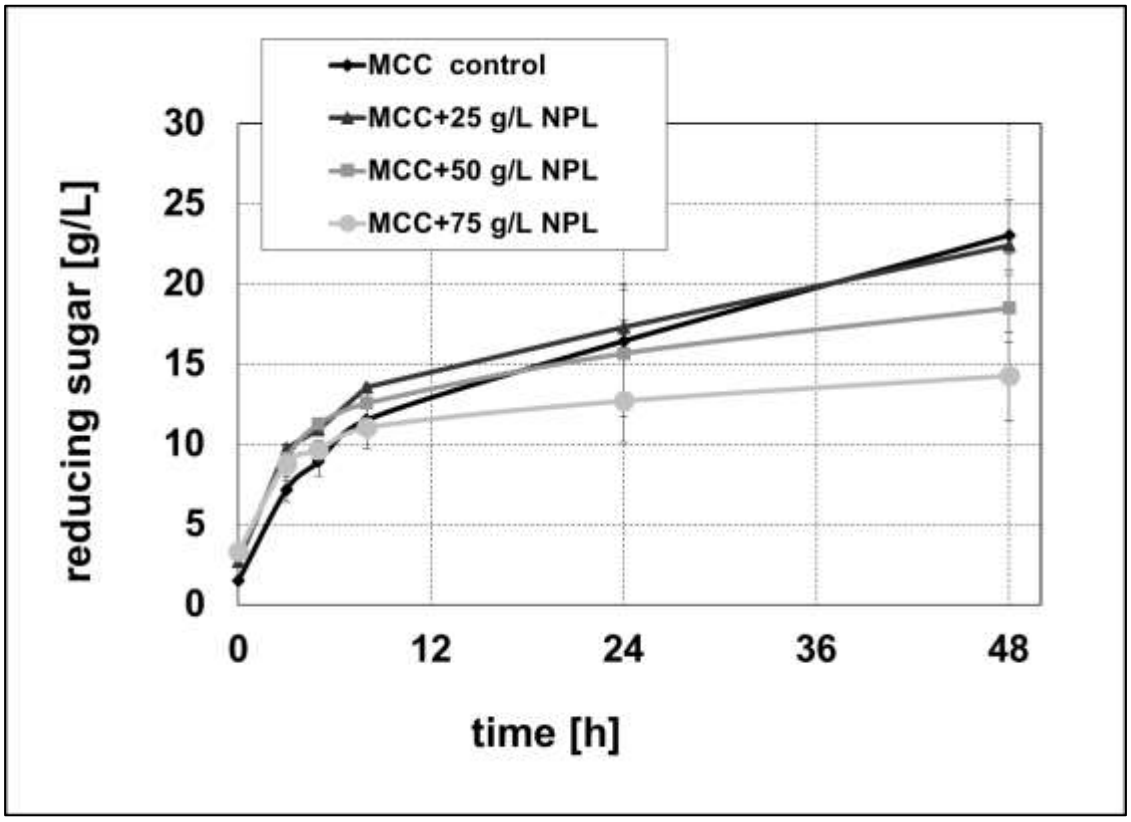
3. Technology

3.1. **Gerhard Kerns**, Leipzig (Germany)
The SSF-process for ethanol production on pulp from wheat straw

In the process known as the second-generation process, the pre-treatment of lignocellulose is to be optimized from an economic point of view. In the case of the material utilization of the pulp and lignin fraction, the pre-treatment process has to be optimized with respect to the properties of the separated pulp and lignin. The advantage of the SSF process of simultaneous saccharification and fermentation of cellulose to ethanol is mainly found in the reduction of the process steps.
The conditions for an economical SSF process are (i) stability of the cellulase complex in the SSF process during the entire fermentation period, (ii) less inhibition of the cellulase complex by ethanol and by-products of the lignocellulose-pre-treatment such as lignin and (iii) the optimal supply of the required amount of pulp in the fermentation process. These conditions have been investigated in an SSF process with a model yeast strain.

Results
Pre-treatment of wheat straw:
Three different pre-treatment processes have been investigated with regard to their suitability for material utilization of pulp and lignin. The pre-treatment process known as Natural Pulping (NP) with formic acid/hydrogen peroxide and an alkaline pre-treatment process have been optimized up to pilot scale. Hydrothermal treatment (autohydrolysis) has been developed on a laboratory scale. The resulting pulp and lignin were analyzed using different methods. Based on these data, the different pre-treatment processes are compared with respect to their suitability for the use of pulp for the ethanol production and lignin for the material utilization.
The NP pre-treatment appears to be favored. Pulp from NP process has a lower intrinsic viscosity and generates more ethanol in the SSF process than pulp from alkaline pulping. The disadvantage of NP is a higher lignin content in the pulp. The hydrothermal treatment is favored for the recovery of hemicellulose, especially xylose oligosaccharides (XOS).

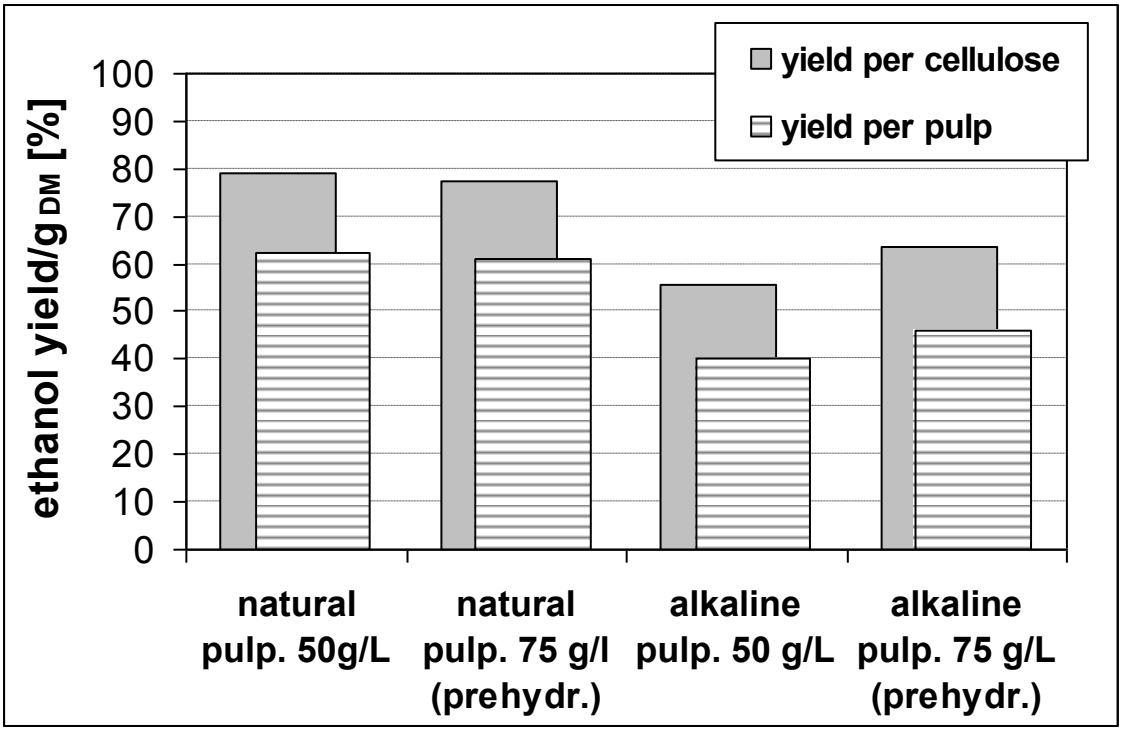
Penicillium verruculosum cellulase-complex:
The cellulase complex for use in the SSF-process has been produced with the production strain Penicillium verruculosum-M28-10b up to 400-L-scale. In terms of the hydrolysis of microcrystalline cellulose and pulp from pre-treated wheat straw, the Penicillium enzyme complex showed better yields than the Trichoderma enzymes, including mixtures from ROAL Oy with added ß glucosidase. This is mainly caused by increased ß-glucosidase content in the P. verruculosum-enzyme complex. The P. verruculosum-cellulase complex is not inhibited by NP lignin up to 25 g/L. For these reasons, the P. verruculosum-enzyme complex is favored for the second generation of ethanol production.



Saccharification of microcrystalline cellulose (MCC) in presence of NP-lignin
50 g/L MCC; *P. verruculosum-cellulase*: 32.6 FPU/g MCC; hydrolysis at 35°C, 90 rpm.

SSF-process with pulp from wheat straw:
Investigations on the SSF process for the production of ethanol were carried out from lab-scale up to 220-L scale using a model yeast strain. The strain was supplied by Zuckerforschung Tulln Ges.m.b.H. (ZFT) as lyophilized culture. The ethanol production in the SSF process was compared between pulp from NP and alkaline-pre-treatment. Higher ethanol production could be achieved with the pulp of the NP digestion, despite the higher lignin content of about 10% in the pulp.
A high pulp content (>10% w/v) is required to obtain more than 4% ethanol. Such contents resulted in a very high viscosity. Problems therefore arose with the classical Continuous stirred-tank reactor (CSTR). To solve this problem, studies both on the pre-hydrolysis of pulp and the feeding in fed-batch technique were carried out. The SSF fermentation on a large scale was carried out in a 220-L reactor with a total of 100 g/L pulp. Pre-hydrolysis of 50 g/L of the pulp was carried out with the P. verruculosum cellulase at 50°C in the reactor; the remaining pulp was added after the pre-hydrolysis together with the yeast. The yield (ETOH) in terms of cellulose was about 70% after 24 hours.

By increasing the pulp concentration to 50% (v/w) in a Lab-solid state reactor, the ethanol yield increased to 13% (v/w) in the SSF process with the P. verruculosum cellulase-complex, but after a fermentation time of more than four days.



Comparison of ethanol yield in SSF-process between pulp from NP- and alkaline pulping
P. verruc-cellulase: 50 FPU/g pulp (dry matter); 35°C, pH 5, 72 h, 5 g/L yeast (ZFT); 160 rpm, 3-L-bioreactor

Discussion and evaluation of the results achieved
In terms of the commercial exploitation to the results, we see good opportunities for the production of ethanol in the SSF process with pulp from wheat straw, but further investigations are necessary.
The provision of the necessary amount of pulp for > 10% ethanol in the SSF process must be optimized. The possibilities for this are partial pre-hydrolysis of the pulp, feeding of pulp in fed-batch-technique or a solid-state-fermenter.
The P. verruculosum-enzyme complex is favored for the SSF-process in the "second generation". For economical use, the P. verruculosum production strain must be improved, in particular to eliminate the carbon catabolite repression. The yeast needs to be optimized for the SSF process.

Diploma as Chemist at Leipzig University. Ph.D. 1976 to fermentation processes in water-in-oil-emulsions at the Akademie der Wissenschaften (GDR). 1997 Co-founder of the Saxon Institute for Applied Biotechnology. Development of fungal strains and process techniques for cellulase production up to industrial scale.

3.2. **Jean Népomuscène Ntihuga**, **Thomas Senn, Peter Gschwind** and **Reinhard Kohlus**, Stuttgart (Germany)
Newly Designed Continuous Bio-Ethanol Fermentation Systems using Blenke-Cascade and Plate-Heat-Exchanger

The perspective of oil depletion, the concerns of energy security and global warming are the main drivers of biofuel promotion. The development of reliable, renewable energy and high-efficiency bioreactors systems are important goals in the present bioprocess research.

We aimed to develop high efficiency continuous bio-ethanol fermentation systems. The ethanol fermentation using S. cerevisiae was conducted in two different continuously operating bioreactor systems. We investigated the Blenke cascade system whose fermenter is a cylindrical bioreactor having inserts causing toroidal vortices, thus enhancing mixing. The other investigated system is plate heat exchanger whose fermenter is a rectangular bioreactor having corrugated plates to improve plug flow characteristics and an effective mass transport. The constructed Blenke cascade system included the gas stripping, ethanol condensation units and designed enhanced sedimentation rate settler for yeast separation. The plate heat exchanger system included gas stripping and temperature control units. The overall performance of the Blenke cascade system was studied using the best parameters (filtered mash, double saccharification principle (DSP), batch start-up strategy), with different yeast-recycle models and an enhanced sedimentation rate settler connected in series with a small conventional gravitational settling settler for yeast cells separation. The overall performance of plate heat exchanger system was studied using unfiltered mash, double saccharification principle (DSP) and zero start-up strategy. Energy and environmental effects of wheat-based fuel, produced continuously by a Blenke cascade system, were assessed by life cycle assessment (LCA) using GaBi software. Data for the LCA model included primary data, which was obtained from the Blenke cascade system experiments; and secondary data, which came from literature and Lean database.
Using Blenke cascade system, high volumetric ethanol productivity (Qp = 20.43 g/L h) and yield Ey = 98% were achieved. The energy values needed (cradle-to gate) range from 6 – 24 MJ/kg ethanol and energy efficiency (energy ratio) from 1.15 – 4 MJ/MJ fossils. Continuous fermentation, yeast recycling and sedimentation were contamination-free processes. At room temperature, the average ethanol productivity was Qp = 3.07 g/L h and Qp = 2.31 g/Lh for cascade and plate exchanger configuration, respectively. The analysis of operational power costs indicates relevant differences between the two bioreactors for laboratory scale; however, systems with different pumps and viscosities have been compared.
In conclusion, plate heat exchanger bioreactor configuration could be suitable for continuous bio-ethanol fermentation using solid mashes in large scale (unfiltered mash). However, the results suggest that, in laboratory and pilot plant scale, the Blenke cascade could be more suitable economically. The use of a continuous fermentation system based on Blenke cascade and plate heat exchanger bioreactors is a promising technology that increases wheat based bio-ethanol's productivity and energy benefits.

Dr. Jean Népo Ntihuga finished his doctorate in natural sciences, fermentation technology at Hohenheim University, Germany and master in chemical engineering at VIT University, India. He has published several papers in several international journals. His research interests include biofuel processing technology, fermentation process development, eco-balances and environmental sustainability.

3.3 **Jan-Thomas Kühnert** and **Hannes Richter**, Hermsdorf (Germany)
Process optimization of bioethanol synthesis by membrane technology

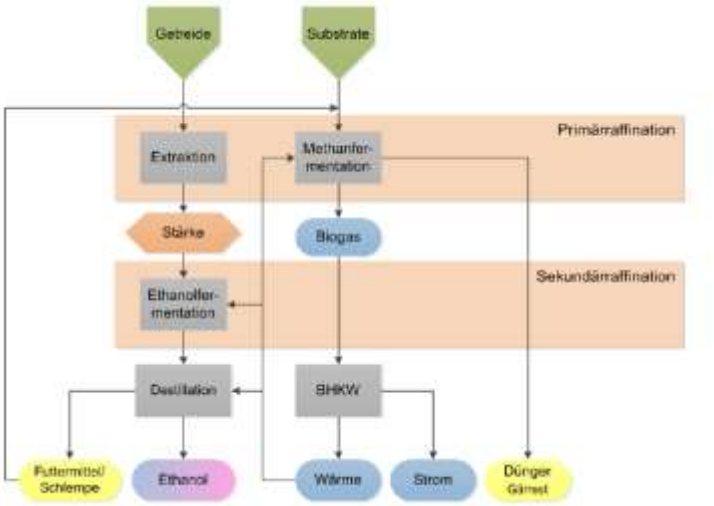
The summary couldn't be finished in time.

3.4. **Timo Broeker**, Lemgo (Germany)
How to extend a distillery into a lignocellulose biorefinery – material- & energy flow management for bioethanol, biogas und HTC biochar

In order to lower the hurdle for Lignocellulose-Bioethanol, the group at the University of Applied Sciences HS Ostwestfalen-Lippe investigates a combined concept for pre- & posttreatment of lignocellulose substrates at small scale distillery.

Germany has a traditional structure of decentral small and mid-scale distilleries, whose existence is currently threaded. A perspective could be the utilization of unused or inefficiently used substrates. Therefore it's necessary, to apply an extension module for the pretreatment of lignocellulose with a quick ROI. It is further claimed, that this module is operated with waste heat from posttreatment ways and that material- & energy flow is coupled in the sense of a biorefinery.

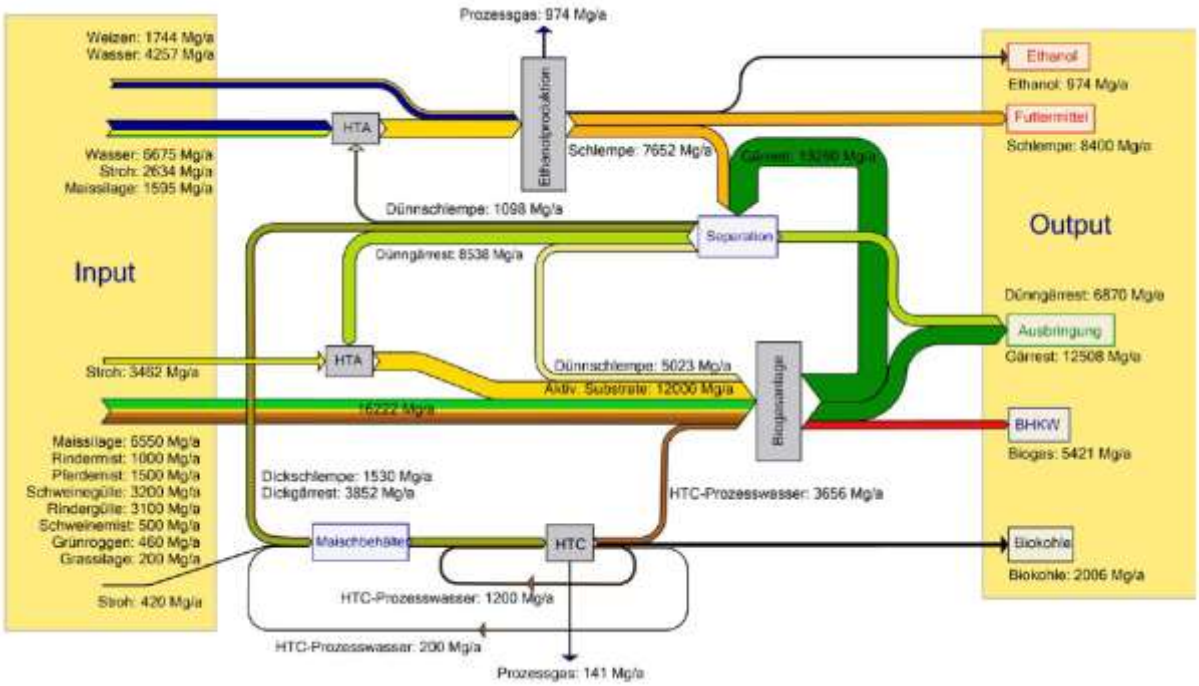
After taking a look at biorefinery concepts, like they are discussed for example in the ROADMAP BIOREFINERY (which was published by the government in 2012), a conceptionell extension for a not only secondary but also a tertiary refining way added. Hydrothermal biochar (HTC) can be produced using the same extension module, which is applied for the pretreatment.



To proof feasibility of the concept, a well examined, mid-scale agricultural plant with biogas digesters is used as a model to the project.

By using a mobile container-sized continues pilot reactor, the research group is able to activate the lignocellulose substrates towards enzyme accessibility and sugar release by pressure and steam explosion around 160° C in hydrothermal conditions (HTA). After the bioethanol is obtained, the bagasse is separated into fluid and solid phase. While the fluid phase goes into the biogas path, the solid is processed through the reactor in a second hydrothermal step at high pressure and temperatures to produce biochar (HTC).

The concept considers energy flow, especially heat management, within the plant. In order to run all processes at high efficiency, the yield of lots of different, available substrates will be investigates. A matrix will suggest the right proportion of input for



every stream.

The concept analysis shows, that an extension of generator can be accomplished while using less renewables at the same time. Better efficiency and the utilization of more agricultural wastes is one reason. The biochar is an important factor to power the process with the lacking amount of steam. Better biogas t and more electricity output results also from the increased biogas potential after the pretreatment.

Timo Broeker, born in 1976, studied Biotechnology at the University of Applied Sciences HS Ostwestfalen-Lippe and works in the field of bioethanol research since 2009. He leads a working group bioethanol and integrated biorefinery concepts.

4. Enzymes

4.1. **Bart Koops**, AE Leiden (The Netherlands)
Optimizing ethanol yields by improved enzyme dosing strategies in grain liquefaction and fermentation

The production of fuel ethanol from grains in Europe has been a growing market since early 90's. The initial focus of many producers has been to optimize the process based on cost. As a result, additions like enzymes were decreased as far as possible which does lead to reduction in final yield, although this is often difficult to determine accurately. In this presentation DuPont will present the benefits that arise from optimizing the process on yield rather than cost and show that this optimization will be very beneficial for profitability and sustainability.
DuPont has developed a method that can be used to qualify the quality of the liquefaction process. This method is a very useful tool to monitor and optimize the ethanol production process. In this presentation DuPont will show that the improvement of the liquefaction quality leads to improved fermentation performance. Examples will

show the reduction in residual starch and increase ethanol yield in an optimized process. For such optimizations, no plant adjustments are needed, other than the application of innovative enzyme solutions.
The benefits from the different enzyme solutions are shown to be improved sustainability and increased profit through increased ethanol yield. Any plant interested in optimizing their process is welcomed to contact DuPont to collaboratively optimize their ethanol production process.

Bart Koops is Director EMEA Biorefineries Applications at DuPont Industrial Biosciences. Bart studied biochemistry at the University of Utrecht in The Netherlands and obtained his PhD in enzymology at the same university in the Department of Enzymology and Protein Engineering in 1999.
Bart worked as Scientist at the R&D facility of Royal Numico in the Netherlands, and at Yakult as interim Science Manager and moved to the industrial biotechnology division Genencor within Danisco A/S in 2005.
After being responsible for technical sales for 6 years Bart moved to his current role in 2011 as EMEA Director Biorefineries Applications. He is leading the team in developing new products and processes for the grain processing industry and providing support for customers.

4.2. **Arjen van Tuijl, Tom Kleinhout, Gerhard Konieczny-Janda** and **Bart Koops**, AE Leiden (The Netherlands)
Maximizing ethanol yields by action of a novel Trehalase

During fermentation of glucose to ethanol, yeast accumulates and excretes significant amounts of non-fermentable trehalose. Trehalose is a storage carbohydrate and accumulates as the cell enters the stationary growth phase. It is also linked to different forms of stress like osmotic stress, temperature shocks or dehydration.

By using Dionex HPLC technique, trehalose has been identified as the most abundant DP2 sugar in end-of-fermentation samples from different feedstocks and plants. This trehalose ends up in the DDGS material, and can be considered as a loss in ethanol yield.

We present the novel product OPTIMASH™ TREHALASE. This product can be added in fermentation to hydrolyse extracellular trehalose into fermentable glucose. This results in an increased ethanol yield of up to 1%. In multiple fermentations we have demonstrated a clear decrease of trehalose in end-of-fermentation samples that were treated with OPTIMASH™ TREHALASE. This is accompanied by an increase in ethanol in the range of 0.1 %v/v - 0.2 %v/v. The absolute levels of ethanol may be small, but they are a very significant benefit on a yearly basis. In order to measure these relative small differences on lab-scale, experiments need to be repeated several times. Also on plant scale such a small difference can be measured, provided that enough data points are collected for a proper statistical analysis on the ethanol output.

DuPont is happy to assist producers to apply OPTIMASH™ TREHALASE and improve plant performance.

Arjen van Tuijl joined DuPont Industrial Biosciences in 2007, and has been working in the Grain Applications team since then. His job consists of product development and technical support, both for the fuel ethanol market as well as carbohydrate processing market. He holds a BASc in

Biotechnology and has worked at two universities (Delft and Amsterdam) before joining DuPont.

5. Fermentation

5.1. **Nicolas Dohn**, Berlin (Germany)

Technical Challenges in the Fermentative Production of Butanol and its Economic Implications

A potential alternative to fossil butanol could be butanol produced from renewable feedstocks. The majority of all available n-butanol and iso-butanol is produced via the oxo process or hydroformylation based on propylene, butylenes or syngas. Whereas n-butanol is currently mainly utilized in the lacquers segment and other solvent applications, iso-butanol is used as a direct solvent in coatings, adhesives and pharmaceuticals.[1] The current global demand for this alcohols is approximately 3 Mio t/a of n-butanol and 0,5 Mio t/a of iso-butanol, making both a commodity in the chemical market[1] with prices for n-butanol in Europe varying around 1.110 €/t (no VAT). This corresponds to 0,90 €/L or 0,034 €/MJ heating value. With market prices for Diesel of 0,021 €/MJ (no VAT) there is currently no utilization of fossil butanol for fuel or heating purposes.

Although the fermentative conversion of renewable feedstock to n-butanol is known on an industrial basis since 100 years, its role today is limited to niche applications. The so-called ABE-fermentation produces n-butanol (no iso-butanol) and the co-products acetone and ethanol in a ratio of roughly 6:3:1. As the utilized Clostridiaceae bacteria have the ability to ferment hexose and pentose sugars simultaneously, lignocellulosic substrates are generally qualified as a feedstock, however, facing implementation obstacles mainly due to complex feedstock preparation. Whereas the sugar degradation products furfural and hydroxymethylfurfural have an adverse effect on most yeast fermentations, they stimulate the fermentation rate of C. beijerinckii in a range of 500 mg/L[2]. Main inhibitors for C. beijerinckii are e.g. ferulic acid, ρ-coumaric acid and syringaldehyde[3]. One of the main obstacles towards commercialization is the product inhibition by n-butanol, effecting the fermentation rate negatively and inhibiting further production above 20 g/L of butanol. To overcome this constraint, butanol fermentation requires constant product recovery by in-situ removal (e.g. by gas stripping).

In order to elaborate to what extent butanol based on lignocellulosic feedstocks can be an economic option, a study utilizing wheat straw was undertaken, assuming a slightly advantageous scenario which incorporates batch fermentation laboratory results from Qureshi[4]. For up-scaling into a commercial process predominantly proven technologies were considered. The feedstock preparation is based on pretreatment with sulfuric acid and subsequent neutralization. The fermentation simultaneously enzymatically hydrolyzes and ferments the prepared substrate with the help of C. beijerinckii (Simultaneous Saccharification and Fermentation, SSF). For in-situ product removal, constant gas stripping by fermentation gases, subsequent condensation and product separation by distillation is examined. Finally, a mechanical vapor-compression evaporation is contemplated for water recycling, so that residuals are thermally used for the generation of steam and power.

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[1] Koumpouras, G., Oxo Alcohols, PERP 2011-2, Nexant, 2012

[2] Qureshi, N. et al., Butanol productivity enhancers in wheat straw hydrolyzate: employing potential of enhanced reaction rate, 33rd Symposium of Biotechnology for Fuels and Chemicals, Seattle, 2011

[3] Ezeji, T.C. et al., Butanol production from agricultural residues. Impact of degradation products on Clostridium beijerinckii growth and butanol fermentation. Biothechnology and Bioengineering, Biothechnology and Bioengineering, 2007, 97, p. 1460-1469

[4] Qureshi, N. et al., Butanol production from wheat straw by simultaneous saccharification and fermentation using Clostridium beijerinckii: part I – batch fermentation, Biomass and Bioenergy, 2007, 32., p. 168-175

Within the basic scenario the production cost of one liter of n-butanol calculate to 1,21 €/L or 0,045 €/MJ, representing a spread of 0,31 €/L to the market price of fossil n-butanol of 0,90 €/L. This cost already include a bonus for the sale of acetone and ethanol. Based on a capacity of 100,000 t/a of ABE-products, the fixed capital investment and total capital investment add to 252 Mio € and 280 Mio € respectively. The cost for wheat straw (0,28 €/L) and for transport (0,14 €/L) account to roughly 35% of the production cost, based on a straw price of 58 €/t plus 38 €/t average cost for transportation. The highest single position is related to the cost of enzymes with 0,29 €/L or 24% of the overall cost. The calculatory cost for internally produced electric power account for 0,13 €/L or 11% of the overall cost. Main power consumers are the blowers for the gas strippers (62 %), the electric compressor of the water purification (23%) and the coolers for the condensation of stripping gases (7%). The cost for annuity, maintenance and chemicals are 0,21, 0,09 and 0,06 €/L respectively and account for roughly one quarter of the production cost. A sensitivity analysis shows that the theoretical optimum plant capacity of 340.000 t/a with cost of 1,13 €/L is outside of a feasible or realizable size. In summary, the major technical levers for a potential cost reduction are the demands of enzymes, sulfuric acid and electric energy.

A so-called future scenario, was developed in order to evaluate under which circumstances an optimized scenario under very positive conditions could make the production economically favorable. In comparison to the basic scenario, the future scenario considers non-proven technologies which are currently in an early deployment phase. In this scenario the major above mentioned levers are addressed. It is assumed that enzyme recycling via immobilization is technically feasible. For this recycling 40% less enzyme consumption, but additional cost of 10% for the preparation of enzymes were assumed. Furthermore, in-situ product recovery with pervaporation is assumed to lead to a decrease in energy consumption of around 37 %, compared to gas stripping, but additional CAPEX of the changed fermenter design of 15 %. An increased biomass/water ratio by one third to 20 % decreases the CAPEX, but requires detoxification of the substrate due to an elevated level of released inhibitors. The removal of the inhibitors is possible by overliming, but causes a sugar loss of estimated 2 % and additional cost of 20 % for chemicals for this process stage. Nevertheless, through the detoxification further substrates such as rye and corn straw can be utilized, which without detoxification are not suitable[5]. Therefore, the average straw availability for the example of sustainably available straw in Germany increases from 11,5 to 16,1 t/km² and leads to a reduction of transportation distance of around 15 km down to 91,3km.

The production cost of this future scenario calculate to 0,99 €/L of butanol or 0,044 €/MJ with total investment cost of 269 Mio € (-4% to basis scenario). Despite of all improvements there is no economic production and a spread of 0,09 €/L to the market price that still needs to be covered. The results of a sensitivity analysis show that economic production could potentially be realized by assuming an optimized positioning of the plant with 20% higher wheat straw availability or an average transport distance of 83 km. Further leverage points are the cost reduction for the straw provision to around 85 €/t (including transport) or a decrease of total investment cost by 18% to around 221 Mio €. Moreover, an increase of the plant capacity to 380.000 t/a would lead to an economic operation, which is, however, out of a feasible range. Nevertheless, doubling the capacity ceteris paribus to 200.000 t/a already leads to production cost of 0,92 €/L close to economic production.

In summary the fermentative production of n-butanol out of lignocellulosic feedstocks today does not represent a favorable option for investors. However, it is potentially feasible under optimized technological conditions, applying a combination of above

[5] Qureshi, N. et al., Production of butanol (a biofuel) from agricultural residues: part II – use of corn stover and switchgrass hydrolysates, Biomass and Bioenergy, 2010, 4/34, p. 559-565

improvements and optimized localization with low feedstock provision cost. Further research should be directed to overcome the main implementation barriers in feedstock preparation, e.g. enhanced steam-explosion and enzyme reuse, as well as in-situ product recovery, e.g. liquid-liquid extraction. Furthermore, an integration into existing supply chains, e.g. selected substrates from food processing industry, should be considered as well as the realization of co-product benefits, e.g. a material instead of energetic utilization of lignin[6], for chemical industry.

Nicolas Dohn graduated in 2007 from the University of Karlsruhe (TH) as Industrial Engineer. In 2008 he joined Ferrostaal AG as Manager Technology for biofuels and then subsequent Executive Assistant. In 2013 he completed his doctoral thesis about the utilization of n-butanol in the domestic heating market at the Technische Universität Berlin. Since 2013 he works for Air Liquide as Business Development Manager.

5.2. **David Dietz**, **Hendrik Wetzel** and **Maren Wandrey**, Potsdam (Germany)
Analysis of Biomass composition

Biomass components, especially proteins, cellulose, starch, and hemicellulose represent besides lignin interesting and promising biopolymers for industrial processes. Native and pretreated wood and agrarian raw materials as well as isolated fractions were evaluated with regard to composition of containing biopolymers and associated substances.

Analytical methods for total hydrolysis of contained polysaccharides and an analytical protocol for the quantification of the single components have been developed and applied on the basis of internationally accepted methods of NREL, ASTM and TAPPI at Fraunhofer IAP. A combination of gravimetric and chromatographic analysis, especially the HPAE-PAD (high performance anion exchange chromatography with pulsed amperometric detection) allowed to quantify starch components after enzymatic hydrolysis or the portions of ash, protein, extractives and lignin, cellulose and hemicellulose after acid hydrolysis.
To assess information on specific proteins, ELISA and enzyme activity assays are applied. For a more general conclusion several chromatographic methods are used e.g. size exclusion, ion exchange and hydrophobic interaction.

examples
Enzymatic hydrolysis of starch was controlled by the method HPAE-PAD. Starch degradation products were described by determination of saccharide profiling. The influence of several factors as enzyme dosage and pH on the enzymatic degradation of gluten in wheat flour was analyzed by size exclusion chromatography.
Gentle and sustainable biotechnological processes which separate biopolymers from lignocellulose were developed with wheat straw. Both raw material and isolated polysaccharide or conversion products as well as lignin fractions were investigated according to their composition. Wood materials e.g. poplar hybrids, were predominantly differentiated by the portion of hemicellulose and lignin.

summary
The results of the composition of various biomass products show that the used methods are suitable for the investigation of fermentation processes. Especially in the case of

[6] Haase, M., Entwicklung eines Energie- und Stoffstrommodells zur ökonomischen und ökologischen Bewertung der Herstellung chemischer Grundstoffe aus Lignocellulose, KIT Scientific Publishing, 2012

complex substrates as lignocellulosic waste streams this analysis is crucial to identify inhibitors, characterize consumption and production behavior and finally specify a reproducible production process.

David Dietz studied biotechnology at the University Münster focusing on molecular biology and analytics. In his PhD he investigated fermentation processes of microbial co-cultures for production of organic acids and alcohols. At the Fraunhofer Institute for Applied Polymer Research IAP he is now working on biotechnological production of polymer precursors, biopolymers and polymer-modifying enzymes to valorize lignocellulosic waste streams.

5.3. **Stijn Mertens, Tim Snoek, Martina Pica Nicolino, Jan Steensels** and **Kevin J. Verstrepen**, Leuven (Belgium)
Generating superior industrial yeasts through high-throughput robotized genome shuffling

The bioethanol industry is constantly striving for improved production efficiency. A widely adopted strategy is the use of very high gravity (VHG) fermentation procedures. However, the use of VHG fermentation is limited by the natural stress resistance of yeast cells.
In order to generate strains with improved industrial performance, we exploit the huge genetic and phenotypic diversity among different yeasts combined with novel robotized genome shuffling and breeding approaches. In brief, we assembled a collection of more than 500 industrial and feral Saccharomyces yeasts. Each of these strains was genotyped and screened for more than 100 industrially relevant properties including fermentation capacity, ethanol resistance, temperature tolerance, flavor production profile, flocculation, hybridization properties, etc. Together, this large dataset allows us to select yeasts with particular properties to accommodate specific fermentation processes (for instance high ethanol tolerance for bio-ethanol production, or high aroma production suitable for making fermented beverages). Moreover, our large collection of genetically and phenotypically characterized yeasts also allows us to select specific parental strains to generate novel hybrid yeasts that show further improvement of specific properties. This strategy yielded hundreds of new superior yeasts, several of which are already used commercially.
Specifically for the improvement of ethanol tolerance and production, we explored novel genome shuffling strategies. Genome shuffling has emerged as a powerful approach to rapidly enhance complex traits; yet current efforts have not exploited the rich genetic diversity of Saccharomyces yeasts, since they relied on mutagenesis or the use of a limited number of different strains. Here, we developed novel large-scale genome shuffling strategies to create hybrids incorporating the genetic material of multiple and genetically diverse strains. Eight genetic divergent heterothallic parental strains were selected after a thorough screening of more than 300 Saccharomyces cerevisiae strains for ethanol accumulation, genetic relatedness and their hybridization potential. In a first, targeted robot-based genome shuffling approach, we create F3 hybrids that incorporate the genetic material of the initial strains. In order to achieve this, we mated these eight parental strains in all pair-wise combinations, and tested the ethanol tolerance of the resulting F1 hybrids. The best performing hybrids, some outperforming both respective parental strains, were selected to create F2 hybrids, from which the best performing hybrid were selected for creating F3 hybrids. In a second approach, using random genome shuffling of spores derived from the same parental strains, we created a heterogeneous F1 population and subjected it to multiple rounds of genome shuffling using different selection strategies. We obtained F3 hybrids that showed improved ethanol survival capacity or growth capacity in the presence of ethanol. Next, we tested the fermentation performance of hybrids from all approaches in lab-scale fermentations using YP+35% (w/v) glucose as a substrate. Interestingly, some hybrids reached higher final ethanol levels than the parental strains, and yielding a gain of up to 1 volume%

ethanol compared to today's most commonly used industrial strains. The excellent fermentation performance of one specific hybrid was validated on a 10L-scale, making this strain a potential candidate for industrial purposes.

I received a BSc in Bioscience Engineering from the University of Leuven, Belgium in 2011 and a MSc in Bioscience Engineering, major "Cell and Gene technology", minor "industrial Microbiology" from the same university in 2013. I did my master thesis in the VIB laboratory for Systems Biology led by prof. Kevin Verstrepen in 2012-2013 for which I was awarded the annual MSc thesis prize of the Royal Society of Brewing Schools. I later on joined the lab of prof. Verstrepen as a PhD student.

5.4. **Johan Thevelein**, **María R. Foulquié-Moreno, Françoise Dumortier, Jean-Paul Meijnen, Stijn De Graeve, Thiago Pais, Georg Hubmann, Yudi Yang, Mekonnen Demeke, Annelies Goovaerts** and **YingYing Li**, Leuven (Belgium)
Polygenic analysis of complex traits for development of superior yeast strains for first- and second-generation bioethanol production

Most traits of industrial importance in yeast are polygenic traits, i.e. traits determined by multiple genes acting together. Screening of S. cerevisiae strain collections has revealed a wide diversity for such traits, with certain natural strains often being far superior for a desirable trait compared to industrial yeast strains. However, the genetic analysis of polygenic traits has been an important challenge for many years. We have developed pooled-segregant whole-genome sequence analysis to map all QTLs (Quantitative Trait Loci) determining a complex trait in such superior yeast traits relative to an industrial strain of interest. Reciprocal hemizygosity analysis and allele exchange are then used to identify and confirm the causative genes in the QTLs. The superior alleles found in this way are transferred into industrial yeast strains for first and second-generation bioethanol production in order to improve their performance. We have applied this technology platform to several yeast traits of prime importance in first- and second-generation industrial bioethanol production: ethanol tolerance of cell proliferation and maximal ethanol accumulation capacity, thermotolerance, reduced glycerol/enhanced ethanol production, acetic acid tolerance, xylose fermentation rate, osmotolerance, etc. For all these traits we have identified multiple causative alleles. In addition, we have developed the Ethanol Red industrial yeast strain for first-generation bioethanol production into a strain for second-generation bioethanol production, combining high xylose fermentation capacity and high inhibitor tolerance. This was done with a combination of strain engineering approaches: metabolic engineering, mutagenesis, genome shuffling, evolutionary engineering and breeding. The superior alleles for traits of industrial importance that we identified with our technology platform are being used to systematically improve the performance of this cellulosic ethanol strain as well as industrial strains for first-generation bioethanol production with corn, wheat and sugar cane as substrates.

Johan Thevelein is professor and head of the KU Leuven Laboratory of Molecular Cell Biology and director of the Department of Molecular Microbiology at the VIB (Flanders Institute of Biotechnology) in Leuven, Belgium. His main research interests concern the mechanisms involved in yeast nutrient sensing and the polygenic analysis of complex traits for the development of superior industrial yeast strains.

5. Biobased Chemicals

6.1. **Jeroen den Hollander**, AX Delft (The Netherlands)
Biosuccinium™ production by Reverdia; Product quality and cost price as key drivers for process development

Bio-based succinic acid is a versatile renewable raw material in the production of a number of polymers and chemicals. In June 2010, Royal DSM N.V., the global Life Sciences and Materials Sciences company headquartered in the Netherlands, and Roquette Frères, the global starch and starch-derivatives company headquartered in France, announced the signing of a joint venture (JV) agreement, named Reverdia, for the production, commercialization and market development of Biosuccinium™, sustainable succinic acid.

Biosuccinium™ is the first non-fossil feedstock derived chemical building block that allows customers in the chemical industry to choose a bio-based alternative with a lower eco-footprint for a broad range of applications, from packaging to footwear. The process was developed striving for best environmental footprint, high quality and best economics.

By fermenting our yeast at low pH with special low pH yeast technology a significant lower carbon footprint is achieved than a neutral pH bacterial process. Besides various operational advantages low pH technology was specially chosen for the virtually negligable salt production during product recovery. Thereby the carbon dioxide footprint is much lower than for processes with neutral fermentations.

The use of succinic acid in polymer applications requires a high purity while the acceptance level of the impurity depends on the nature of the impurity and the application. Impurities originating from non-fossil feedstock derived succinic acid typically differ from petrochemical derived succinic acid. A process for succinic acid was successfully developed in close collaboration with potential customers and application specialists to meet the required purity demands.

Next to the quality requirement, for future economic success of its major applications it is important to realize the lowest possible cost price for succinic acid. We deliberately chose for a fermentation process at low pH, thereby directly producing the acid which results in the optimal cost of goods.

The impact of key process design choices for the production of Biosuccinium™ , such as choice of production host on life cycle assessment, cost of goods and product quality will be discussed in the presentation. In 2011 Reverdia announced the construction of a commercial-scale plant for the production of Biosuccinium™ and in December 2012 Reverdia has begun operations.

Jeroen den Hollander has a degree in Chemical Engineering from Delft University of Technology in The Netherlands. In 2001 he obtained his PhD in the field of Bioseparation technology. After international consultancy assignments in the field of antibiotic process development and pharmaceutical process development, he joined DSM in 2006. For DSM he worked on process development, technology transfer and production support for various business units.

6.2. **Arno van de Kant**, AD Delft (The Netherlands)

New open access pilot facility: From biomass to end product on pilot scale

Situated at the Biotech Campus Delft, the Netherlands, the Bioprocess Pilot Facility B.V. (BPF) is a unique open access facility where companies and knowledge institutions can develop new sustainable production processes by converting bio-based residues into useful chemicals or fuels and production processes for Food and Pharma.
The facility has been specifically designed to enable the transition from laboratory to industrial scale. The facility has a modular setup. BPF allows users to construct complex operations by linking the separate process modules: Pretreatment, Hydrolysis, Fermentation and/or Downstream Processing.
About 30 people, mainly experienced process operators.
Investing about 37 Million Euro in expanding the facilities with pretreatment and food grade capabilities and also building a modern state-of-the-art control unit from which all the pilot plants can be controlled.
Based at the Biotech campus Delft all infrastructure is present to perform chemical/biotechnology upscaling processes.
Biobased production today: Examples of products/product range
BPF is a service provider with very flexible facilities to help our customers to upscale their process. We have experience with many different chemicals, food and pharma ingredients of which many cannot be disclosed because of confidentiality. Known examples are ethanol, FDCA and 7-ADCA.
Because of its high quality standards, the BPF can also be used to produce kg-quantities of material for pre-marketing and application tests at customers and/or preclinical trials (for Food or Pharma applications). The BPF has a long standing historical track record in bioprocess piloting with an experienced crew.
Research and innovation activities
BPF is well equipped to be able to upscale the process of the technology holder, and use our experience to improve the process.
We have 4 different pilot plants which can interact being:

- Pretreatment (biomass, also lignocellulosic) on benchscale and pilot scale.
- Fermentation from 10l up to 8m3. (ATEX, GMO)
- Downstream processing, chemical processing (ATEX)
- Food grade pilot plant.

All product streams can be connected, to mimic a downscale of a commercial plant. The upscaling of the lab process can than be proven on pilot scale with a good prediction of the process on commercial scale.
Future ambitions
BPF wants to be the provider of scale up for biobased products that are or have been developed on labscale in EU projects or within companies to overcome the valley of death, by providing access to equipment, knowledge and experience of the BPF.
Chemicals like ethanol, lactic acid, succinic acid, ethanol, FDCA, enzymes.

Working in the Industrial Biotechnologie field more then 20 years, Arno is an experienced business development director, now working for the Bioprocess pilot facility in Delft. He worked for several companies in marketing, sales and business development functies , as there are; New Brunswick Scientific, Akzo Nobel, NIZO food research, bioMerieux, and TNO. He also worked as CEO of ARKI and MicroDish, a startup company, discovering and detecting novel organisms. He was also active in several European networks, like member of the industrial biotech councel of EuropaBio and the Key Enabling Technology working group of the European Union for Industrial

Biotechnology. Now dedicated to help companies to upscale their processes from biomass to products in the open acces pilot facilities in Delft.

Wednesday, April 09th 2014

08^{30} Beginning of the lectures

5. Fermentation

5.1. **Nicolas Dohn**, Berlin (Germany)
Technical Challenges in the Fermentative Production of Butanol and its Economic Implications

5.2. **David Dietz**, **Hendrik Wetzel** and **Maren Wandrey**, Potsdam (Germany)
Analysis of Biomass composition

5.3. **Stijn Mertens**, Leuven (Belgium)
Large-scale systematic approach to select and create novel yeast strains with superior fermentation characteristics

10^{00} Coffee Break

5.4. **Johan Thevelein**, Leuven (Belgium)
Polygenic analysis of complex traits for development of superior yeast strains for first- and second-generation bioethanol production

6. Biobased Chemicals

6.1. **Jeroen den Hollander**, AX Delft (The Netherlands)
Biosuccinium™ production by Reverdia; Product quality and cost price as key drivers for process development

6.2. **Arno van de Kant**, AD Delft (The Netherlands)
New open access pilot facility: From biomass to end product on pilot scale

12^{00} Closing remarks
by the Chairman of the Starch Experts Group of the Association of Cereal Research, **Willi Witt**, Oelde (Germany)

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