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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

11th European Bioethanol and Bioconversion Technology Meeting

April 14th – 15th 2015
Detmold, Germany

Program

Evening Program

Exhibition

Participants

Summaries

Tuesday, April 14th 2015

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

1. Biochemicals

1.1. **Christian Abel,** Ettlingen (Germany)
Biochemicals – roots towards commercialisation

2. Technology

2.1. **Prashant Madhusudan Bapat** and **Nicholas Giffen**, Copenhagen (Denmark)
Novozymes Golden Batch: Finding gold by analyzing process data

10^{15} Coffee Break

2.2. **Ioannis Papapetridis,** Delft (The Netherlands)
Carbon dioxide fixation by Calvin-Cycle enzymes improves ethanol yield in yeast

3. Analytics

3.1. **Patrick E. Williams** and **Ye Chen**, Franklinton (United States)
Enhanced Residual Starch Determination Using NIR and Advanced Enzyme Technology

4. First Generation

4.1. **Maria Braune**, **Elias Grasemann, Arne Gröngröft, Marcel Klemm, Katja Oehmichen** and **Konstantin Zech**, Leipzig (Germany)
The production of biofuels in Germany – State-of-the-art and optimization approaches

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

4.2. **Muriel Dewilde**, Gent (Belgium)
Bio Base Europe Pilot Plant – Turning grams into tons

5. Second Generation

5.1. **Pieternel Claasen**, **Truus de Vrije, Miriam Budde, Patrick van Doeveren, Andrea Alberini** and **Koen Meesters,** Wageningen (The Netherlands)
Biochemical hydrogen production from 2nd generation biomass

15^{30} Coffee Break

5.2. **Mirjam Kabel**, WG Wageningen
(The Netherlands)
Basics in enzymatic (hemi-)cellulose biomass conversion and analysis.

5.3. **Prashant Madhusudan Bapat**, **Nicholas Giffen** and **Long Nguyen**, Copenhagen (Denmark)
Use of a Unique Cellulase to Tap New Starch Pools for Ethanol Production

5.4. **Michael Buck**, Stuttgart (Germany)
Effect of gluten on enzymatic hydrolysis of hemp and miscanthus

5.5. **Padma Priya Ravi**, Stuttgart (Germany)
Influence of energy input and stirrer geometry on hydrolysis and subsequent C5-fermentation in lab scale

To be continued on page before last

Lunch

This year we have reorganized our Lunch. Please sign in at the convention office. You will get a receipt and a special bracelet you have to wear during lunch.

The menue:

Tuesday, April 14th 2015
Curried sausages

Mini-Sandwiches

Spit of tomatoes and mozarella cheese

Chicken on the spit with ASIA-Dip

Puff pastry filled with mushrooms or cheese

for the costs of 10,-€ including beverages

Wednesday, April 15th 2015
Lasagne "Italian Style"

Bruschetta with tomatoes

Mixed green salad

for the costs of 10,-€ including beverages

Beverages:
Mineral water

Coca-Cola

Orange juice

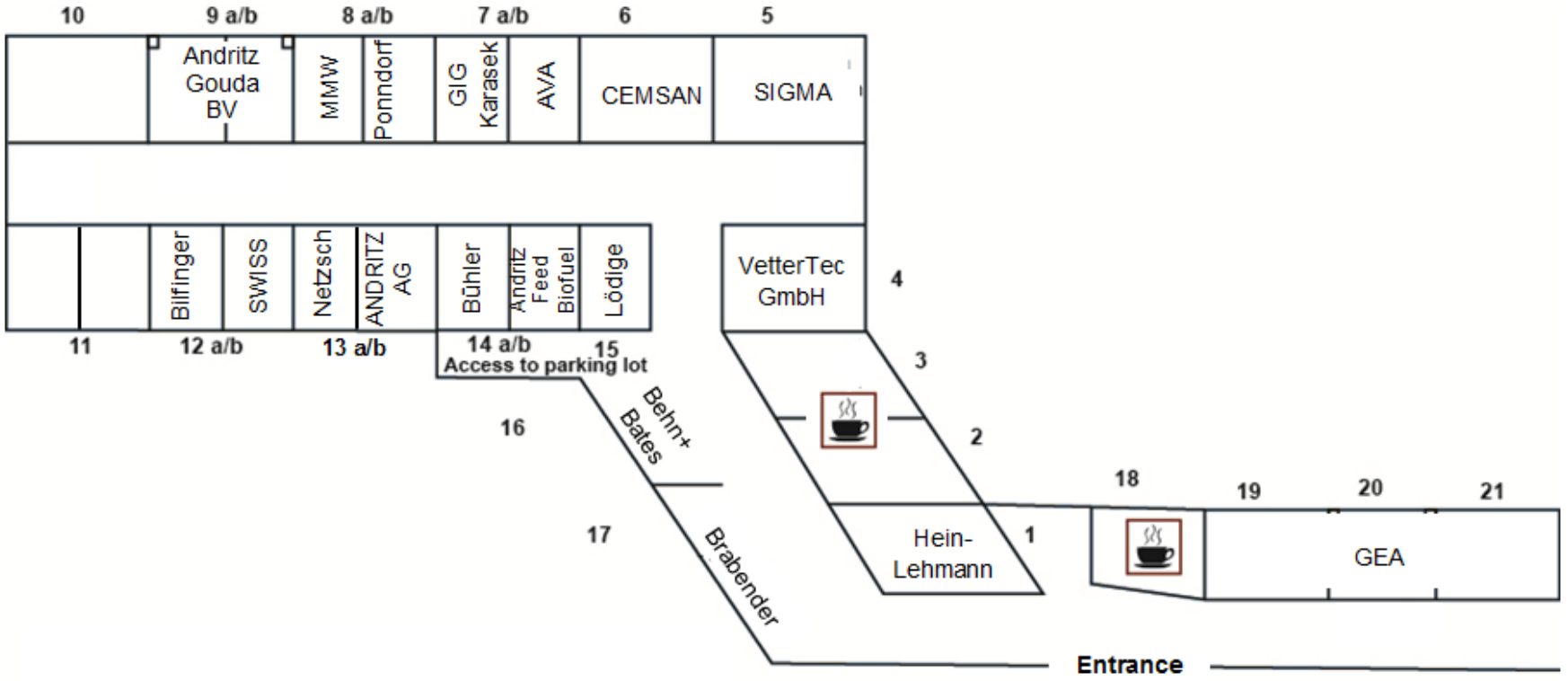
Apple Spritzer

Bon appétit
and interesting conversations!

Exhibition Hall Association of Cereal Research

Stand allocation

11th Bioethanol and Bioconversion Technology Meeting and 66th Starch Convention from April 14th – 16th 2015



Exhibition

Exhibition

Andritz Feed & Biofuel Div., Muncy (USA)

Andritz Gouda BV, PD Waddinxveen (Netherland)

ANDRITZ AG, Pumps, Graz (Österreich)

AVA- Huep GmbH & Co. KG, Herrsching (Germany)

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

Bilfinger Water Technologies GmbH, Aarbergen (Germany)

Brabender GmbH & Co.KG, Duisburg (Germany)

Bühler GmbH, Braunschweig (Germany)

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

GEA Westfalia Separator Group GmbH, Oelde (Germany)

GIG Karasek GmbH, Gloggnitz-Stuppach (Austria)

HEIN, LEHMANN GmbH, Krefeld (Germany)

Krettek Filtrationstechnik GmbH, Viersen (Germany)

Gebr. Lödige Maschinenbau GmbH, Paderborn (Germany)

MMW Technologie GmbH, Lutherstadt Wittenberg (Germany)

NETZSCH Pumpen & Systeme GmbH, Waldkraiburg (Germany)

Ponndorf Anlagenbau GmbH, Kassel (Germany)

Sigma Process Technologies, Atasehir/Istanbul (Turkey)

VetterTec GmbH, Kassel (Germany)

W. Kunz dryTec AG, SWISS COMBI, Dintikon (Schweiz)

Evening Program

Monday, April 13th 2015

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

Tuesday, April 14th 2015

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

Buffet

Pork steaks marinated in basil

***

spicy spare ribs of pork

***

Turkey steaks marinated

***

Sausages

***

Cheese potatoes

***

Vegetable selection,

***

roasted potatoes

***

Salad buffet with seasonal salads

***

Fresh fruit salad with Marascino

***

Pear Helene with vanilla ice cream and hot chocolate sauce

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!

Participants

Effective April 08th 2015, 1 p.m.

Abdelrahim, Ahmed	AlMonairy for Corn Products, Cairo (Egypt)
Abel, Christian	GEA Wiegand GmbH, Ettlingen
Abeln, Dieter	Behn & Bates Maschinenfabrik GmbH & Co. KG, Münster
Acildi, Eren	CEMSAN DIS TIC. A.S., Arifiye/Sakarya (Turkey)
Althoff, Friedrich, Dr.	Kröner-Stärke, Hermann Kröner GmbH, Ibbenbüren
Andreev, Nikolay	All Russia Research Institute for Starch Products, Moscow (Russia)
Bapat, Prashant Madhusudan	Novozymes A/S, Copenhagen N (Denmark)
Beißner, Gerd	HEIN, LEHMANN GmbH, Krefeld
Bergsma, Martien	DuPont Industrial Biosciences, Leiden (The Netherlands)
Bergthaller, Wolfgang, Prof. Dr.	Lage
Bessner, Frank	AVA-Huep GmbH & Co. KG, Herrsching
Bischof, Ralf	HEIN, LEHMANN GmbH, Krefeld
Bley, Jens	MMW Technologie GmbH, Lutherstadt Wittenberg
Boles, Eckhard, Prof. Dr.	Institut für Molekulare Biowissenschaften, Frankfurt/M.
Boschma, Peter	GEA Hovex B.V., Veendam (The Netherlands)
Bosshard, René	W. Kunz dryTec AG, Swiss Combi, Dintikon (Switzerland)
Böttcher, Jürgen	CropEnergies AG, Zeitz
Braune, Maria	DBFZ Deutsches Biomasseforschungszentrum gemeinnützige GmbH, Leipzig
Brinkmann, Franz	Gebr. Lödige Maschinenbau GmbH, Paderborn
Broeker, Timo	Hochschule Ostwestfalen-Lippe,Lemgo
Brunt, Kommer, Dr.	Rotating Disc bv, HV Haren (The Netherlands)
Buck, Moritz	ANDRITZ AG, Pumps, Graz (Austria)
Buck, Michael	Universität Hohenheim, Gärungstechnologie, Stuttgart
Bücker, Manfred	GEA Westfalia Separator Group GmbH, Oelde
Burgess, Julie	Dedert Corporation, Homewood (USA)
Ciftci, Umut	Sigma Process Technologies, Atasehir/Istanbul (Turkey)
Claasen, Pieternel, Dr.	Wageningen UR Food & Biobased Research, WG Wageningen (The Netherlands)
Creutz, Stefan	Detmolder Institut für Getreide- und Fettanalytik (DIGeFa) GmbH, Detmold
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Estermann, S.	ANDRITZ Gouda B.V., Waddinxveen (The Netherlands)
Fleck, Dirk-Michael	Bühler AG, Uzwil (Switzerland)

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Fromanger, Romain	Leaf Technologies, Marq en Baroeul Cedex (France)
Gatidis, Athanassios	GEA Wiegand GmbH, Ettlingen
Genugten, van, Bernard	AB Enzymes GmbH, Darmstadt
Granner, Josef, Dipl.-Ing.	Agrana Stärke GmbH, Gmünd (Austria)
Groenestijn, van, Johan, Dr.	TNO Innovation for life, Zeist (The Netherlands)
Grüll, Dietmar, Dr.	Agrana Stärke GmbH, Gmünd (Austria)
Haase, Jana, Dipl.oec.troph	Detmolder Institut für Getreide- und Fettanalytik (DIGeFa) GmbH, Detmold
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Kennet, Paul	VetterTec Ltd., Kent (Great Britain)
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Langfelder, Kim	AB Enzymes GmbH, Darmstadt
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Ouzounis, Christos	HEIN, LEHMANN GmbH, Krefeld
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Schnelle, Alexander	Bühler GmbH, Braunschweig
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Zillmann, André	Bilfinger Water Technologies, Aarbergen

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Bonte, Anja	Münzing, Klaus, Dr.
Brühl, Ludger, Dr.	Sciurba, Elisabeth, Dr.
Fehling, Eberhard, Dr.	Scheibner, Andreas
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Grundmann, Vanessa	Stabenau, Gisbert
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Hübert, Julia	Unbehend, Günter, Dipl.-Ing.
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Kersting, Hans-Josef, Dr.	Weber, Lydia, Dipl.oec.troph.
Langenkämper, Georg, Dr.	Wiege, Berthold, Dr.
Lindhauer, Meinolf, Prof. Dr.	Wolf, Klaus

Summaries

1. Biochemicals

1.1. **Christian Abel,** Ettlingen (Germany)
Biochemicals – roots towards commercialisation

Chemicals produced from renewable resources are a promising trend with huge future potential. However, to become competitive against the omnipresent petrochemicals many decisive aspects have to be considered to design a feasible process. The most important aspects are

- Target product and byproducts
- Product markets and applications
- Raw material selection
- Process design

To implement a new process successfully it is therefore not sufficient to develop a new fermentation method for the production of a new kind of biochemical. The development has to follow a stringent feasibility model already in an early phase.

Product selection:
From the variety of possible products market aspects are as important as fermentation performance. During implementation and scale up it is very likely that a “green premium” is necessary to be profitable. The product has to be selected according to this requirement. At present it appears that only products targeting at end-customer markets are able to justify a “green premium”.

Raw material selection:
Raw material is the most important cost driver, therefore the decision is of great importance!

To produce fermentable sugars to feed the fermentation three types of raw material are discussed:

- Starch based (Corn or wheat)
- Sugar based (Cane sugar or beet sugar)
- Cellulosic (Bagasse, Straw, short plantation wood switch grass…)

Despite the arguments in favor for cellulosic feedstock, clear disadvantages have to be mentioned as well: Conversion cost will be higher compared to sugar or starch plants. On the other hand there are strong arguments in favor for starch containing feedstocks like corn or wheat:

- Relatively low conversion cost
- High yield per acre of land (land use aspects)
- Byproducts contributing to the food chain
- Profits generated by byproducts

So despite political opinion-making starch plants are still a very competitive and sustainable option!

Process design
One cognitive bias that can be observed quite frequently in the bio-industry is the underestimation of the downstream processing importance. To maximize the profitability it is decisive to squeeze the process design to a maximum yield with minimum utility consumption but without harming the process robustness. It requires knowledge and experience to do so.

Apparently it might be clever to involve an experienced engineering partner already in the planning phase of the project.

Dipl. Ing. ***Christian Abel*** *was born 1969 in Essen. He studied chemical engineering at the University of Essen, with special focus on Food-Technology and Biotechnology.*
Steps of his professional career are:
Product Manager for Food- Technology Fermentation and Gas-Liquid Processes at Heinrich-Frings GmbH in Bonn, Product-Manager Biofuels at the Lurgi GmbH.
Since January 2009 he works as Business Development Manager at GEA Wiegand, responsible for new developments and markets in renewable energy, food-tech and chemical engineering.

2. Technology

2.1. **Prashant Madhusudan Bapat** and **Nicholas Giffen**, Copenhagen (Denmark)
Novozymes Golden Batch: Finding gold by analyzing process data

Ethanol plants are constantly seeking ways to achieve higher profitability, especially on a more consistent basis. One area that can lead to increased consistency and performance is through the use of statistical process control. Ethanol plants produce vast amounts of data which contains valuable information. However, the full potential of this data is often not explored or realized. By utilizing the Golden Batch methodology of statistical process control, ethanol plants can turn vast amounts of data into valuable knowledge that can be used to make better business and operating decisions. Doing so will allow an ethanol plant to unlock a higher level of performance by more consistently achieving the perfect “Golden Batch”.

We will discuss what the Golden Batch methodology is, including some of the analytical techniques used in applying the method. We then show how these techniques are used to identify the recipe that creates the perfect Golden Batch for ethanol producers. Finally, we will conclude with a case study where the Golden Batch method was used in an ethanol plant to achieve a 1.25% ethanol yield increase and a higher level of consistency. The result is a significant increase in profitability without added cost.

Prashant Bapat PhD.
April 2014 onwards
Industry Technology Specialist : Bioethanol, Starch & Distilling
Novozymes Aps.
2009-2014
Scientist : Fermentation optimization, Novozymes Aps.
2006-2009
Post doctoral fellow : Systems biology department, Denmark technical university, Denmark
2000-2005
Ph.D. fellow: Department of Chemical engineering, IIT-Mumbai, India
1994-2000
Production officer: Fermentation, Lupin Limited, India

2.2. **Ioannis Papapetridis,** Delft (The Netherlands)
Carbon dioxide fixation by Calvin-Cycle enzymes improves ethanol yield in yeast

Background: Redox-cofactor balancing constrains product yields in anaerobic fermentation processes. This challenge is exemplified by the formation of glycerol as major by-product in yeast-based bioethanol production, which is a direct consequence of the need to reoxidize excess NADH and causes a loss of conversion efficiency. Enabling the use of CO2 as electron acceptor for NADH oxidation in heterotrophic microorganisms would increase product yields in industrial biotechnology.
Results: A hitherto unexplored strategy to address this redox challenge is the functional expression in yeast of enzymes from autotrophs, thereby enabling the use of CO2 as electron acceptor for NADH reoxidation. Functional expression of the Calvin cycle enzymes phosphoribulokinase (PRK) and ribulose-1,5-bisphosphate carboxylase (Rubisco) in Saccharomyces cerevisiae led to a 90% reduction of the by-product glycerol and a 10% increase in ethanol production in sugar-limited chemostat cultures on a mixture of glucose and galactose. Co-expression of the Escherichia coli chaperones GroEL and GroES was key to successful expression of CbbM, a form-II Rubisco from the chemolithoautotrophic bacterium Thiobacillus denitrificans in yeast.
Conclusions: Our results demonstrate functional expression of Rubisco in a heterotrophic eukaryote and demonstrate how incorporation of CO2 as a co-substrate in metabolic engineering of heterotrophic industrial microorganisms can be used to improve product yields. Rapid advances in molecular biology should allow for rapid insertion of this 4-gene expression cassette in industrial yeast strains to improve production, not only of 1st and 2nd generation ethanol production, but also of other renewable fuels or chemicals.

Ioannis Papapetridis
Did a BSc in Biology with a specialization in Molecular Biology and Genetics in Aristotle University of Thessaloniki in Greece. Did a MSc in Molecular Biotechnology in Wageningen University in the Netherlands. Currently a PhD candidate in the Industrial Microbiology section of Delft University of Technology, working on metabolic engineering of redox cofactor balancing in S. cerevisiae.

3. Analytics

3.1. **Patrick E. Williams** and **Ye Chen**, Franklinton (United States)
Methods of residual starch determination (NIR and advanced enzyme technology)

Due to the high cost of grain for bioethanol production, it is important to have efficient starch conversion to maximize ethanol production. During enzymatic starch hydrolysis in the dry grind corn process, not all starch is converted into fermentable sugars. The non-converted starch, which the industry terms as residual or resistant starch, is accumulated in distillers dried grain with solubles (DDGS). The amount of residual starch in DDGS is dependent on many factors in the process like temperature, pH, enzymes and duration of enzyme hydrolysis. Residual or resistant starch can be further categorized as starch inaccessible to enzyme due to entrapment in a non-digestible matrix, retrograded starch and ungelatinized starch due to poor liquefaction. Accordingly, a method to determine residual starch is important in order to determine changes in ethanol yield. One well-known method to determine residual starch involves using enzymatic hydrolysis. This method often uses a combination of alpha-amylase and glucoamylase which are commercially available in assay kits. Here we described using an improved enzymatic approach to determine residual starch utilizing a liquefaction enzyme (Avantec) and saccharification enzyme (Spirizyme Achieve). By

combining these two advanced enzyme technologies more residual starch can be accessed and converted to glucose. Subsequently, using these enzymes in the residual starch assay will result in higher residual starch when compared to the traditional commercial enzymatic method. The results obtained from the assay show that more starch is enzymatically accessible and can be tapped into in order to create more ethanol yield.

Near-infrared (NIR) spectroscopy is a fast and non-destructive analytical technique that provides chemical and physical information of various sample matrices. The combination of an NIR method and multivariate data analysis opens many promising perspectives for both qualitative and quantitative analysis. To increase laboratory throughput and provide better service to our customers, quantitative analysis of moisture, starch, crude protein, as well as fat and oil was explored on corn flour and DDGS samples using a DA 7250 NIR analyzer (Perten Instruments) and a multi-purpose FT-NIR analyzer (Bruker Corporation).

NIR spectra of corn flour and DDGS were calibrated against starch content, determined enzymatically, using spectral preprocessing techniques such as partial least squares regression (PLSR). Results showed the moisture and starch content in corn flour and DDGS can be predicted with a low root mean square error of prediction (RMSEP). The method was able to demonstrate RMSEP below 0.4% for moisture and 0.8% for starch, respectively. The robustness of the models were greatly enhanced by extending the range of the analytes. Prediction accuracy can also be improved by additional sample pre-processing steps. These models are a valuable tool for high-throughput monitoring of moisture and starch in biofuel industry during feedstock storage, handling, and processing.

Patrick Williams *is an Industry Technology Specialist serving the biofuels industry for Novozymes. A graduate of Appalachian State University, with a B.S. in Chemistry, Mr. Williams has spent time working in cellulosic biofuels R&D for Mascoma Corporation as well as vaccine manufacturing scale up for Wyeth/Pfizer.*

4. First Generation

4.1. **Maria Braune**, **Elias Grasemann, Arne Gröngröft, Marcel Klemm, Katja Oehmichen** and **Konstantin Zech**, Leipzig (Germany)
The production of biofuels in Germany – State-of-the-art and optimization approaches

In view of the debate on ensuring sustainable biofuel production systems, the existing biofuel plants will have to meet higher requirements. An additional incentive for the optimization of the GHG balance of existing biofuel production plants is given through the calculation of the German biofuel quota based on the GHG reduction potential as from 2015 (Biofuel Sustainability Directive, Biokraft-NachV). If these improvements are to be realized by process modifications, they are associated with additional investments and have impact on profitability of biofuel plants. Against this background, the aim of the project “Optimierungspotenziale von Biokraftstoffanlagen/Optimization potentials of biofuel plants” (funded by Fachagentur Nachwachsende Rohstoffe e.V.) was to investigate technical optimization potentials within the production plants. In the scope of the project bioethanol as well as biodiesel plants in Germany were examined. The presentation will focus on the studies made on ethanol production.

Within the project reference production concepts were defined based on an inventory of German bioethanol plants and intensive exchange with plant operators. The reference concepts were chosen to represent the plant portfolio in Germany as well as possible. Particularly large technical differences are in processing ethanol between starch- and sugar-based raw materials. Therefore, a reference concept was defined for each type of raw material. Wheat and sugar beets were chosen as model materials. The concepts were afterwards modeled as flowsheet simulations to calculate mass and energy balances. Based on a review of current literature different optimization approaches were identified with the goal to make the bioethanol production more efficiently in terms of minimizing GHG emissions. This research targets the exchange of plant components, the change in use of material and energy flows and the production of new marketable by-products. Several optimization approaches were qualitatively analyzed and described. Among them the following approaches were found as especially promising:

- Mechanical vapor recompression
- Biogas production from thin stillage/vinasse and beet pulp
- CO2 recovery and liquefaction

These optimization approaches were integrated into the simulation models and the resulting mass as well as energy balances were computed. The results formed the basis for the assessment of the resulting GHG reduction potential and production costs.
In all concepts the emissions from the raw material provision and the raw material processing (conversion pathway) are mainly responsible for the total GHG emissions. Thereby the heat demand of the product processing is the most GHG-intensive step. The high GHG emissions are due to the burning of fossil natural gas for heat supply. All in all the GHG emissions can be improved by the technical optimization approaches. The specific production costs of bioethanol are mainly determined by the variable costs, including raw, auxiliary and operating material costs. Noticeable is also that a significant part of the costs can be covered by the sale of by-products like DDGS and vinasse. The loss of these by-products through the conversion to biogas leads to deficiency in incomes and thus to higher production costs compared to the reference. The other optimization approaches show an improvement of the production costs compared to the reference concept.

A detailed presentation of the results will also be published as DBFZ-Report 22 “Die Biokraftstoffproduktion in Deutschland – Stand der Technik und Optimierungsansätze” in the course of the year 2015.

Maria Braune *obtained her M.Sc. in Biotechnology from the Anhalt University of Applied Sciences in Köthen, Germany, in 2012. In the same year she got an employment as a research associate at DBFZ - Deutsches Biomasseforschungszentrum in Leipzig, Germany. She is specialized in bioethanol processing technologies, optimization potentials of bioethanol plants as well as downstreaming processes.*

4.2. **Muriel Dewilde**, Gent (Belgium)
Bio Base Europe Pilot Plant – Turning grams into tons

In the emerging bio-based economy, companies and research institutes join forces and build innovation chains, to bring new bio-based products to the market and create value from scientific biotechnological results.

A critical step in the innovation chain is to translate scientific knowledge, generated at a laboratory scale, to an industrial process, to assess operating costs, specific strengths and weaknesses of the new biotechnological process and to produce the first ton quantities of the bio-product to test applications and sample the market.

Bio Base Europe Pilot Plant is an independent open innovation facility that provides bio-process development, scale-up and demonstration as a service to SMEs, large companies and research groups from around the world. With a wide range of modular equipment to flexibly perform biomass pretreatment, bio-catalysis, fermentation, purification and explosion proof green chemistry, Bio Base Europe Pilot Plant is a one-stop-shop where the entire production chain, from the biomass green resource up to the final refined bio-product, is performed under one roof.

Bio Base Europe Pilot Plant operates at a kilogram to multi-ton scale, with fermenters up to 15 m^3, glass lined chemical reactors up to 5 m^3, process vessels up to 50 m^3, continuous unit operations like centrifugation, membrane filtration, ion exchange, evaporation at the m^3/h scale.

Since the start-up in 2010, Bio Base Europe Pilot Plant has built and run, in close and confidential communication with the customer, process lines for the production of industrial enzymes, chemicals, food ingredients, bio-polymers, nutraceuticals, bio-plastics, biomaterials and bio-fuels from various biomass feed stocks.

Bio Base Europe Pilot Plant participates as a scale-up and demonstration partner in more than 10 publicly funded projects. Examples are BIOSURFING (biosurfactants), DEMOPROBIO (bio-butanol), NOVOSIDES (glycosylation enzymes) CHITOBIOENGINEERING (acetylated chitosan oligomers) and VISIONS (second generation bio-ethanol).

The presentation illustrates the open innovation piloting concept and infrastructure of Bio Base Europe Pilot Plant, as well as some of the private and public projects that were recently brought to ton scale.

Muriel Dewilde *holds a degree in chemical and biochemical engineering from the University of Leuven, Belgium.*
She has worked for ten years as a process engineer and product development scientist in the starch industry for companies as Tate & Lyle and Tereos Syral.
She is business development manager of Bio Base Europe Pilot Plant since the start-up of the open innovation pilot plant in 2010.

5. Second Generation

5.1. **Pieternel Claasen**, **Truus de Vrije, Miriam Budde, Patrick van Doeveren, Andrea Alberini** and **Koen Meesters,** Wageningen (The Netherlands)
Biochemical hydrogen production from 2nd generation biomass

For the transition to a sustainable economy, hydrogen and the hydrogen economy, are gaining increased interest. The advantages of the application of hydrogen as an energy carrier are most evident from the high conversion efficiency to electricity in fuel cells.
Currently the world is gearing towards implementation of hydrogen in stationary as well as transport sector. A prime example is the Japanese Ene-Farm scheme governing the installation of FC CHP units for domestic application. In 2012, FC CHP systems outsold the conventional engine-based CHP systems. Another example is the market

penetration of hydrogen filling stations, at 208 in April 2013. These are needed for the FC vehicles for which mass production is foreseen in Japan and Korea (The Fuel Cell Today Industry Review 2013).
However, to fully eradicate the unfortunate consequences of fossil resources, this hydrogen needs to be produced from renewable resources. To achieve this, energy from the sun, wind, geothermal- and hydropower and biomass can be used. Energy from the first 4 primary resources is in the form of electricity which is used for electrolysis to make hydrogen from water. Biomass can be used directly. The technology to produce hydrogen from biomass is either thermochemical or biochemical, more or less dependent on the moisture content of the biomass and infrastructural considerations.
In 2006, the EU FP 6 Integrated project HYVOLUTION received € 9 million of funding to establish biochemical production of hydrogen with high yield from various biomass feedstock (www.hyvolution.nl). The core of HYVOLUTION was the combination of extreme thermophilic and photo-fermentation. This was embedded in studies on biomass pretreatment, gas upgrading and system integration. The result was successful conversion of sugars from sugar, starch or lignocellulose feedstock with an overall efficiency of nearly 50% of the theoretical maximum of hydrogen per mol of hexose. With optimal heat and energy integration and improvements currently deemed feasible, a cost of hydrogen at circa € 60/ kg H2 was estimated. Because of the extensive impact of the photo-fermentation on the overall cost, a new project under the Fuel Cell and Hydrogen Joint Undertaking, HyTIME (www.hy-time.eu), has been started with a grant of € 1.6 million. This project is dedicated to increasing the productivity of the extreme thermophilic fermentation to 1-10 kg H2/ day. Instead of a subsequent photo-fermentation, the effluent of the dark fermenter is fed to an anaerobic digester to produce biogas to cover the energy demand. Biomass for HyTIME comes from verge grass, unsold food and straw. Gas upgrading and system integration studies are also addressed in this project.
The first results are stable, continuous fermentation using a designed co-culture of Caldicellulosiruptor sp. in a 5 L packed bed reactor. Hydrogen productivity was 9 g H2/ day at an hydraulic retention time of 3.4 h using glucose as feedstock and 6 g H2/ day at an HRT of 10 h using grass hydrolysate.

Pieternel Claassen is employed as senior scientist at Wageningen UR Food & Biobased Research. She has a full honours degree in Molecular Sciences and a Ph.D. in microbial physiology. After her Ph. D. she worked at Shell Research Centre in the UK on microbial desulfurization. In 1987 she returned to Wageningen to manage projects addressing the topic of energy and chemicals from renewable resources.

5.2. **Mirjam Kabel**, Wageningen (The Netherlands)
Basics in enzymatic (hemi-)cellulose biomass conversion and analysis.

In current perspectives, plant or aquatic biomass valorisation is an important aspect in the search for alternatives for the depletions of mineral oil-based fuels and chemicals; a key aspect for a Biobased Economy. Research in this field focusses at sustainable refining of available biomasses into their components for use in food, feed, biofuels or chemicals. The sources of such biomass are non-food agricultural side streams, like cereal straw, forest residues and vegetative grasses.
Refining of lignocellulosic biomass is currently hindered by the presence of recalcitrant structures, which are preventing microbial enzymes from degrading the carbohydrate polymers in lignocellulose. These recalcitrant structures even remain after hydrothermal pretreatments, although, less when pretreatments are assisted by added alkali or acid.

Nevertheless, milder conditions are preferred in view of a more sustainable and environmental friendly process. The recalcitrant structures in biomass can be divided into four types: A) highly substituted hemicellulose architectures shielding the access to cellulose and being resistant to breakdown to its constituent monosaccharides, B) lignin networks embedding cellulose and hemicellulose and thus restricting enzyme access, C) covalent hemicellulose-lignin junctions tying the two polymers together and hampering their removal, and D) highly crystalline cellulose microfibrils.
This presentation will cover general compositions of various industrial and agronomic by-products (e.g. corn stover, DDGS). Details are provided on recalcitrant carbohydrate structures present and enzyme activities required to hydrolyse these carbohydrates are reviewed. A focus will be put on the analysis-methods used, which includes HPAEC, MALDI-TOF MS and preparative and analytical LC-MSn.
As an example, it is shown how several oligosaccharides present in the supernatant of mild acid pretreated and enzymatically saccharified corn fiber that resist the current available enzymes were (semi)purified for structural analysis by NMR or ESI-MSn. The structural features of various recalcitrant oligosaccharides are presented. A common feature of almost all these oligosaccharides is that they contain (part of) an α-L-galactopyranosyl-(1→2)-β-D-xylopyranosyl-(1→2)-5-O-trans-feruloyl-arabinofuranose side chain attached to the O-3 position of the β-1-4 linked xylose backbone. Several of the identified oligosaccharides contained an ethyl group at the reducing end hypothesized to be formed during SSF (from ethanol formed). The ethyl glycosides found are far more complex than previously described structures. A new feature present in more than half of the oligosaccharides is an acetyl group attached to the O-2 position of the same xylose to which the oligomeric side chain was attached to the O-3 position. Finding enzymes attacking these large side chains and the dense substituted xylan backbone will boost the hydrolysis of corn fiber glucuronoxylan.

References

1. Appeldoorn, M.M., Waard, P. de, **Kabel, M.A.**, Gruppen, H. & Schols, H.A. (2013). Enzyme resistant feruloylated xylooligomer analogues from thermochemically treated corn fiber contain large side chains, ethyl glycosides and novel sites of acetylation. *Carbohydrate Research, 381*, 33-42. 10.1016/j.carres.2013.08.024
2. Murciano Martínez, P., Bakker, R., Harmsen, P., Gruppen, H., **Kabel, M.A**. (2015). Importance of acid or alkali concentration on the removal of xylan and lignin for enzymatic cellulose hydrolysis. *Industrial Crops and Products, 64*, 88-96. 10.1016/j.indcrop.2014.10.031

*Since 2011, **Dr.ir. Mirjam Kabel** is assistant professor at the Food Chemistry group of Wageningen University, The Netherlands. She obtained her PhD-degree in 2002. Her research field 'Biomass Carbohydrate Biochemistry' aims to understand the recalcitrance, mainly for enzymes, of lignocellulosic plant biomass at a molecular level, and, thereby, contributing to an efficient and sustainable biorefinery of biomasses; a key aspect for a Biobased Economy.*

5.3. **Prashant Madhusudan Bapat, Jan Bach Kristensen**, **Nicholas Giffen** and **Long Nguyen**, Copenhagen (Denmark)
Use of a Unique Cellulase to Tap New Starch Pools for Ethanol Production

Innovation in enzyme solutions and mechanical systems continue to enable ethanol producers to push the boundaries of starch utilization and deliver enhanced ethanol yield. Gaining access to previously untapped starch pools is paramount to moving beyond traditional enzyme solutions (i.e., α-amylase and glucoamylase) and ensuring conversion of this valuable substrate into ethanol, rather than distiller's grains.

Novozymes has recently developed a novel cellulase-containing glucoamylase that specifically taps into a new "fiber-bound" substrate pool where starch is either bound to

the corn fiber matrix or trapped within endosperm tissue thus rendering it inaccessible to traditional amylase enzymes. Additionally, this new glucoamylase has the added advantage of reducing residual sugars (DP2 and DP3) at the end of fermentation. The effectiveness of this new product has been demonstrated broadly at both the laboratory and industrial scale resulting in significant ethanol yield enhancement and subsequent reduction in post fermentation residual starch. We will present results from industry trials and electron microscopy work demonstrating novelty, mechanism and value of this advanced enzyme solution.

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April 2014 onwards
Industry Technology Specialist : Bioethanol, Starch & Distilling
Novozymes Aps.
2009-2014
Scientist : Fermentation optimization, Novozymes Aps.
2006-2009
Post doctoral fellow : Systems biology department, Denmark technical university, Denmark
2000-2005
Ph.D. fellow: Department of Chemical engineering, IIT-Mumbai, India
1994-2000
Production officer: Fermentation, Lupin Limited, India

5.4. **Michael Buck**, Stuttgart (Germany)
Effect of gluten on enzymatic hydrolysis of hemp and miscanthus

In bioethanol production, conversion of cellulose and hemicelluloses from biomass to monosaccharids burdens highest costs and efforts in whole processing. Especially binding of cellulases and hemicellulases to lignin surface causes low-level enzyme performance and necessitates high enzyme loads.
Lignin composes of different aromatic compounds. The three basic units are subdivided in p-coumaryl/hydroxyphenyl, coniferyl/guaiacyl, and sinapyl/syringyl alcohols. Hereby, the proportion of the three different basic units clearly influences enzyme binding. Hence, different lignin molecules can merge by self-assembling and probably constitute only one large polymer per plant.
Several approaches for decreasing enzyme binding to lignin consist of destruction, separation, modification or coating of lignin. Laccases degrades lignin and phenolic compounds. Protic ionic liquids dissolve lignin and leave a cellulose/hemicellulose residue.
Addition of non-ionic surfactants and BSA (bovine serum albumin) proves a decreased binding of enzymes. Supposedly, this effect causes by Hydrogen-Hydrogen and Sulfur-Sulfur bonds.
This study attempts a similar approach. Gluten is cheap and easy to access. Furthermore, gluten consists of different proteins and possesses similar binding sites like lignin. Hemp straw and miscanthus straw are chosen as model substrates by their characteristics. Hemp straw contains about 63% of cellulose, 14% of hemicelluloses, and only 15% of lignin. The basic polymers of miscanthus consist of 47% cellulose, 20% Hemicellulose, and a very high amount of 25% of lignin.
Chopped hemp straw and miscanthus straw were steam pretreated (155°C, 45 min.) and enzymatic hydrolyzed. Enzymes were provided by Erbslöh. Crude gluten was added to hydrolysates and mashes. Both, hemp straw and miscanthus straw presented an increased hydrolysis rate after addition of gluten early on hydrolysis (Fig 1, 2). However, addition of crude gluten did not effect fermentation. Nevertheless, further

experiments with addition of triticale grist increased hydrolysis rate (Fig 3, 4). Accordingly, gluten appears to enhance enzyme activity.

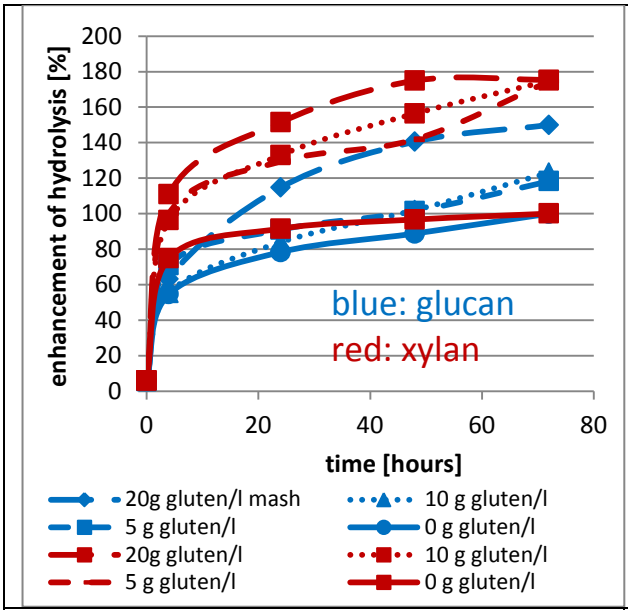


Fig 1: Hydrolysis of hemp with addition of gluten

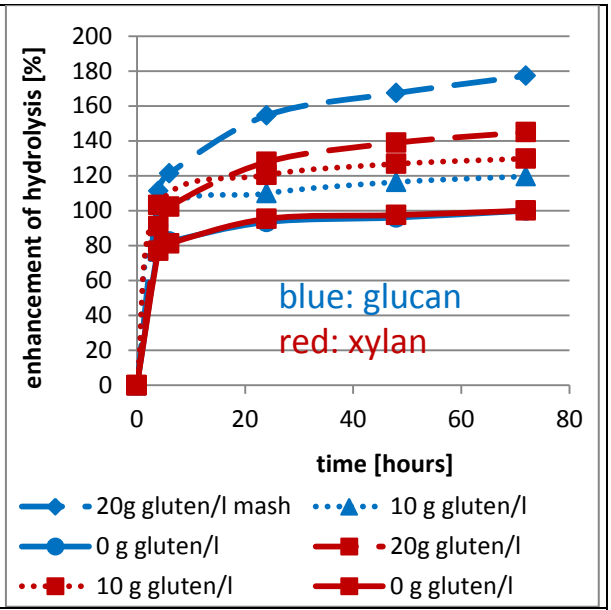


Fig 2: Hydrolysis of miscanthus with addition of gluten

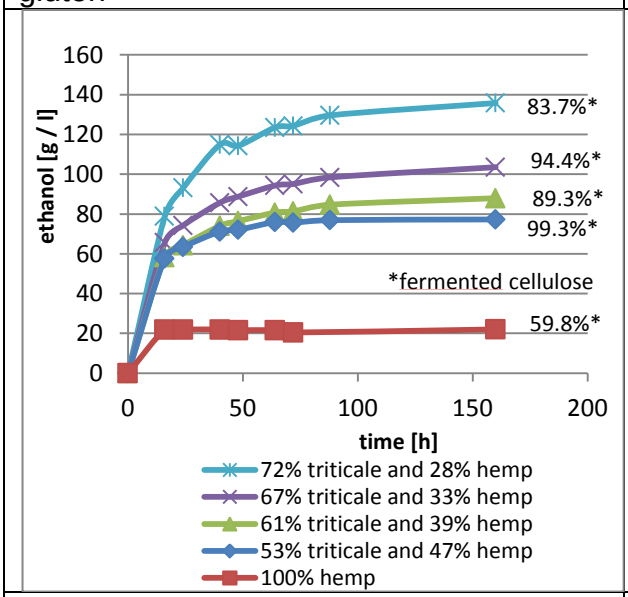


Fig 3: Fermentation of hemp combined with triticale grist

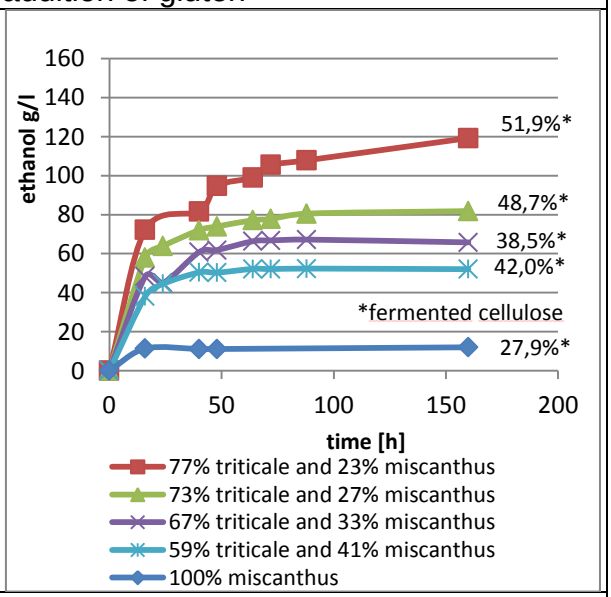


Fig 4: Fermentation of miscanthus combined with triticale grist

Future studies may answer if protease pretreated crude gluten or grain grist, respectively, improve those results. Furthermore, experiments should focus on enzymes with and without carbohydrate-binding module, other substrates, and pretreatment conditions.

Michael Buck
Diploma for agribiotechnology in Universität Hohenheim, Germany
Actually preparing doctor thesis in Institute for Food Sciences and Biotechnology, department of Yeast genetics and Fermentation Technology, Hohenheim
Topics: steam explosion, enzymatic hydrolysis, and fermentation of lignocellulosic substrates; eco-balances

5.5. **Padma Priya Ravi**, Stuttgart (Germany)
Influence of energy input and stirrer geometry on hydrolysis and subsequent C_5-fermentation in lab scale

Hydrolysis process of ethanol production from steam pretreated substrates faces various problems. Especially stirrers or propellers have to perform a difficult job early on hydrolysis due to low structural disruption of substrate. The main goal of this work was to attain the efficient hydrolysis process of 3 different substrates bamboo, wheat and hemp. Additionally, C_5 yeast fermentation of the three substrates was conducted to increase fermentation efficiency and economic utilization to produce bio-ethanol.
The three substrates were pretreated mechanically by milling (sieve 2x2 mm – 6x6 mm) and followed by steam explosion with water only at 155°C for 45 minutes. Furthermore the preheated feedstock was subjected to hydrolysis at 55°C for 48 hours using mixture of cellulases, β-glucosidases and xylanases supplied by Lyven. Hydrolysis was performed in an upgraded mash bath equipped with exchangeable propeller system and adjustable power supply

Manually operated parameters:

- Substrate
- Voltage
- Propellers
- Direction of propellers

Retrieved data:

- Power/Energy
- RPM
- Hydrolyzed glucose
- Hydrolyzed xylose

The hydrolysates were measured in HPLC (REZEX roa H+) for monosaccharids and organic acids. The three biomass hydrolysates with customized monosaccharids (glucose and xylose) were fermented with genetically modified C_5 yeast from Re2alko project implemented at Goethe Universität, Frankfurt for bio-ethanol production. Fermentation was carried out at 30°C on shaker with 110 rpm.
The experiments of hydrolysis of steam pretreated substrates depend on the physical parameters like rpm, sedimentation, direction of propeller, and flow pattern. Measurement setup has lower effect on hemp as there is only sedimentation of fine particles due to particle elasticity. In contrast to hemp, wheat and bamboo were tremendously effected by sedimentation. Energy consumption had no significant effect on hydrolysis grade. However, increased rotation speed of the stirrer resulted in increased sedimentation and spilling.
The yeast in C_5-fermentations simultaneously digested glucose and xylose in all three substrates. In these experiments, C_5-yeast fermentation ends at 80 g ethanol/L. Fermentation rate diminished after approx. 25 hours. Especially in hydrolysates enriched with high amounts of glucose and xylose, residual amounts of monosaccharids gets digested but not fermented to ethanol after approx. 50 hours.

Padma Priya Ravi
Studying Master in agricultural faculty in Universität Hohenheim, Germany
Actually preparing master thesis in Institute for Food Sciences and Biotechnology, department of Yeast genetics and Fermentation Technology, Hohenheim

5.6. **Reinhard Pätz**, Koethen (Germany)
Production of Ethanol with the High-Performance-Sequencing-Batch-Reactor-Technology

General systems of fermentation in first generation bio-ethanol plants, e.g. with starch containing raw materials, require fermentation times of 35 to 70 hours. To reduce this duration a new process called High-Performance-Sequencing-Batch-Fermentation (HSB) has been developed. The new concept is based on the integration of several strategies for increasing productivity, especially to achieve higher efficiency and shorter fermentation time, as well as lowering operation costs for energy supply. The integrative approach considers all stages of production: fermentation, bioreactor design, alternative distillation as well as further solution for processing.
An increasing ethanol productivity is achieved during the Sequencing-Batch-Fermentation process. This semi-continuous process consists of four steps that lead to a high biomass retainment. In particular harvesting the yeast after the fermentation process, as it is usually the case in batch or fed-batch processes, the yeast is retained in the fermenter as a very fast sedimentation step. This sedimentation is stimulated by a determined process integration self-immobilization of the yeast. After a few sequences only the fast settled yeast stays in the fermenter. Analyses of the fermentations have shown the yeast has a high activity and a high dry mass concentration. A stable process could be observed in long term fermentations with volumes of up to 30 liters.
The HSB-Reactor is a pneumatic system that operates with CO2-enriched air and only traces of oxygen. The CO2 is produced by the yeast and used in a closed loop in order to maximize the yield of the process. The traces of oxygen induce a slightly faster growing yeast compared to anaerobic fermentations and results in a high activity of the yeast. Due to the pneumatic system, parts of the product will be collected with the help of gas stripping at a concentration of ethanol up to 20 %.
A large benefit of the High-Performance-Sequencing-Batch-Reactor-Technology is the non-sterile fermentation process. Nevertheless, the fermentation is not contaminated and only adapted production yeast is able to grow and live in the stringent conditions in the HSB-Reactor. The main selection step is the settling process repeated two or three times a day. The majority of non-settled microorganism is rinsed up in the upper part of the fermenter and removed with the fermenter broth. Further parameters will inhibit the growth of contaminative microorganisms, in particular the low pH of 3-4, the high substrate concentration in the beginning of the fermentation process, and the high ethanol concentration at the end. In conclusion the HSB-process fermentation process is very recommendable to established production sites, e.g. starch producers, as a last step for the utilization of by-products.
The high productivity of the process is ensured by a combination of process parameters, such as the higher concentration of active production yeast, a higher feed concentration (e.g. glucose between 120 to 200 g/l), a shorter fermentation time (7 to 12 h), and a resulting higher ethanol concentration (e.g. between 70 to 100 g/l). The process is controlled by a computer-aided measurement of the CO2 concentration in the exhaust gas. Therefore, non-productive fermentation times and dysfunctions of the process are avoided. The productivity depends on the used substrate, for instance glucose led to an increased productivity of 8.3 g/(l*h) for ethanol.
The process has already been approved using industrial feed, e.g. hydrolysed starch, molasses, hydrolysed sugar beets, and by-products of starch production.
Further applications of the High-Performance-Sequencing-Batch-Reactor-Technology are possible. The fermentation process was successfully transferred to the production of carotenes with yeast, or the production of extracellular substances.

***Robert Reinhard Pätz**, born 24.04.1952*

09/70 - 08/74	*Full time study of chemical engineering* *Specialisation high polymere chemistry* *Technical University Leuna-Merseburg,*
03/79	*Promotion Dr. rer. nat., evaluation „god“*
Since 04/03	*Hochschule Anhalt (FH)/ Köthen* *Professor fot Bioprocess engineering*
06/02 – 03/03	*Institut für Energetik und Umwelt gGmbH* *Project manager anaerobic biotechnology* *Project leading Simulation of biogas accumulation* *Application of extracellular enzymes*
10/00 – 05/02	*EON ENERGY OF NATURE Projektgesellschaft für umwelttechnische Anlagensysteme mbH* *Head Biotechnikum* *Projektleitung/Projektakquisition für F/E-Projekte* *Biogasbildung/beta-Carotin/Phenylethanol/Enzymeinsatz*

6. Third Generation

6.1. **Wolfgang Schwarz, C. Held, B. Leis, B. Andreessen, V.V. Zverlov** and **S. Graubner** Freising (Germany)
Tackling with resilient cellulose

The growing demand for a sustainable alternative to crude oil which does not compete with food supply and also reduces the effects of climate change has resulted in the obvious but so far elusive need for a more complete utilization of the abundantly available plant biomass. The hydrolysis of plant biomass has the potential to deliver a variety of sugars, which then could serve as a basis for bio-based fuels and chemicals. However, plant biomass consists of a stable and naturally resilient matrix of cellulose, hemicellulose and lignin. To date, no economically competitive technology to hydrolyze cellulose with enzymes has been developed.
Filling this gap, our product FasCiPlex is an engineered enzyme complex which converts cellulose to sugar. Currently available cellulose degrading enzymes (cellulases) are mainly derived from the fungus Trichoderma, which has been intensively studied and developed for over 70 years. Major further improvements in fungus-derived cellulases are not expected.
Our product FasCiPlex is based on the most effective cellulase in nature, the cellulase complex (cellulosome) of the anaerobic bacterium Clostridium thermocellum. The cellulosome combines all enzymes required for cellulose degradation in a complex, thereby enhancing enzymatic synergy.
Tremendous research effort has already been spent on the characterization of the >70 native cellulosome components which are now available as a toolbox for the in vitro assembly based on our proprietary HTS screening system. The prototype FasCiPlex already outperforms marketed fungal cellulases. Our future research will concentrate on FasCiPlex’s adaption to cellulose sources commonly used in the industry, such as wheat straw. By Q2 2015 we will have developed FasCiPlex 3.0, optimized to wheat straw. An adaptation to different substrates such as wheat bran for the production of butanol may be a future goal.

Dr. Wolfgang H. Schwarz *has studied microbiology with the major of genetics at the Technical University of Muenchen and finished with a doctoral thesis on "DNA replication mutants of Salmonella typhimurium". He was a postdoctoral fellow for 4 years at the Kyoto-University, Japan, Virus-Research-Institute with molecular microbiological research. In Prof. W. Staudenbauers lab at the Technical University Muenchen he lead from 1982 on the research group for Microbial Biotechnology on genetics of thermophilic, anaerobic bacteria; cloning, expression and biochemical characterization of polysaccharide-hydrolyzing exo-enzymes; protein-engineering; determination of exo-proteins in clostridia (cellulosome of Clostridium thermocellum); new solvent producing bacteria from soil and acetone-butanol fermentation; and the role of hydrolytic bacteria in the biogas process. In the same institute, now lead by Prof. W. Liebl, he got the IdeAward of the Zeidler Research Foundation and a GO-Bio project from the German Federal Ministry of Education and Research for his research on the complexed cellulase system from Clostridium thermocellum and its application for the degradation of cellulosic plant biomass for biotechnology, which is to be transferred into a spinoff from the university. Major interest is on the enzymatic degradation of resilient polysaccharides in biological systems.*
He was member of the editorial board of the scientific journal "Microbiology" (SGM) and still is it at "Applied Environmental Microbiology" (ASM). He was speaker of the expert group "Bioenergy" of the Strategic Energy Technology Plan „Materials Roadmap enabling low-carbon technologies" of the European Commission, and is a frequently invited guest speaker at a number of international scientific meetings and task groups.

6.2. **Christian Schweitzer**, Leipzig (Germany)
Power to fuel: 3rd Gen Biofuel from CO2

Over the past years, the energy sector has been shifting more and more toward renewable energy sources. Today, the main portion of renewable energy is produced decentralised and intermittent.
Huge amounts of energy are required in the transport sector and at specific times and in areas of high population density. The resulting challenge for the energy sector is quite complex. It involves the coordination of energy availability to populations at the right time, which necessarily involves storage; the expansion of power grids and capacities; and political considerations as a further significant factor.
One approach to managing this complex challenge is to use energy for the production of chemical energy carriers using common renewable resources, particularly ones which are convenient for implementation in existing infrastructures and technologies.
On the other hand the existing Ethanol industry is faced to increase the GHG savings by carbon capture and utilization of in the fermentation regenerated green CO2.

The CO2mbined plant uses CO2 streams from biological Ethanol fermentation processes to produce E-Methanol. The plant directly faces the specific problem of fluctuated energies with the utilization of green CO2 for fuel production most notably in regard of Greenhouse gas emissions (GHG-E), fluctuated energy supply, power grid stability and sustainability of biofuels excluding without any additional land requirements.

In combination of the electrolyser with endo- and exothermal process steps the efficiency is increasing up to 88% of the inlet power. The endothermal process step is the biochemical fermentation and the exothermal process step is the Methanol synthesis.

The CO2mbined plant integrates the different streams to a closed loop of the utilization of Carbon and energy (therm, elect, mech) into a highly efficient plant.
The target for the prevention of an increasing land use for Energy is to increase the energy yield in the land use. With the CO2mbined plant the yield per existing used land will increase for about 50 %. CO2mbined plant is the most efficient and value-oriented processing of biomass and also not to danger the process of the existing bio fuel industry. It is the perfect completion of the existing Industries and prevents an extension of food/feed utilization for fuels. CO2mbined plant focus on existing mass flows by creating a new product.

CO2mbined plant offers an innovative technology to allow the introduction of alternative fuels and protect existing investments.

The CO2mbined plant proposes at least one unit for the fermentation of biofuels, wherein carbon dioxide is formed, and at least one second unit for the chemical conversion of carbon dioxide to hydrocarbon respectively methanol, wherein the second device for the chemical synthesis of a hydrocarbon is connected downstream of the first device for the biological production of ethanol. Using free O2 from the process of electrolysis increases the primary processes of the fermentation, thus allowing higher efficiency rates.

The long-reaching impact is the Planning, Construction and marketing of CO2mbined plant in large scale at existing and future installation. Hence the overall performance of market will be improved regarding to economical and normative chances. An already done market research leads to sensitivity in the relevant industries. A fundamental demand for the technology exists. But due to the lack of political evidence pointing leadership the market is not willing to invest in the technology at the moment. None the less the technology promises competitive advantages:

- Improvement of GHG-saving of the primary product of > 10%;
- Increase of the output capacities due to generation of an additional product generation from waste stream;
- adding value from waste stream results in RoI < 8 years; Return on sales > 20 % (EU wide sites) due to existing global Methanol market.

Conclusion
The CO2mbined plant is technical feasible and is able to provide in the integration of existing Bio-Ethanolplants additional value chains. This solution will increase the efficiency of the existing Industry and has the opportunity to reach the political target.
If the amendments of the RED and the FQD are going in above mentioned direction and E-Fuels get recognized as advanced Biofuel we will see a revival of Investments in the Biofuels sector in Europa based on proven technology.

Christian Schweitzer, Managing Director, **bse Engineering Leipzig GmbH**, was born in July 1964 in Aachen. There he completed his engineering studies at the Fachhochschule Aachen. He also received his Bachelor of Business Administration in 1999 from the St. Gallen Management Institute, Switzerland. Since 1995, Mr. Schweitzer is managing director of bse Engineering Leipzig GmbH.
The bse Engineering GmbH Leipzig works across whole Europe and is an independent, consultative and customer-oriented engineering company in the field of liquid and solid biomass.

With the successful establishment of the bioethanol plant in Zeitz in Germany in 2005 he joined the biofuel industry. Since that time Mr. Schweitzer has supported and implemented many bioenergy projects throughout Europe. Furthermore, Mr. Schweitzer released different articles about BioEthanol, sugar mills and (sustainable) utilization of biomass as well as held presentations on these topics at International Bio-Energy conferences.
At the moment Mr. Schweitzer is development the technical and economic integration of the chemical Energy storage of Methanol in the Ethanol Industry.

6.3. **Eckhard Boles**, Frankfurt (Germany)
From cellulosic ethanol to cellulosic butanol

With the first commercial plant in the world for the industrial production of cellulosic ethanol which was started up in Crescentino, North-Italy, by Beta-Renewables at the end of 2013, cellulosic ethanol has become reality. Since 2014, several companies in the world, especially in the US and Brasil additionally have started commercial production of cellulosic ethanol. The methods for pretreatment, enzymatic hydrolysis have been greatly improved, industrial yeast strains fermenting C5 sugars are available (e.g. CelluX from Lesaffre) and the whole process has been further optimized.

However, ethanol has some inherent disadvantages as a biofuel. Among third-generation biofuels, butanol isomers 1-butanol and isobutanol are regarded as more suitable gasoline substitutes due to their high octane rating and low hygroscopicity. Moreover, butanol is also a valuable compound for the chemical industry. However, in contrast to ethanol no microorganisms are known which produce butanol in sufficient amounts or are easy to cultivate in large scale production processes.

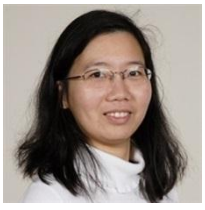
Baker's yeast S. cerevisiae is successfully employed in first and second generation ethanol production due to its high robustness. Whereas ethanol is its main product, yeast is also able to produce isobutanol and 1-butanol as byproducts although only in very low amounts (around 0.2 mg/g glucose). Nevertheless, these properties make S. cerevisiae an attractive organism for metabolic engineering to improve butanol production.

Companies such as Gevo, Butamax and Butalco, and several labs in research institutions worldwide have started to engineer yeast strains for more efficient 1-butanol or isobutanol production and to develop butanol production processes. Gevo is already announcing a commercial scale plant in Minnesota for yeast-based isobutanol production. However, the available processes are still based on food materials like corn and normal sugars. More efforts are needed to improve the process and to convert it to the fermentation of biomass waste streams.

Eckhard Boles *is a professor for microbial genetics and biotechnology at Goethe-University of Frankfurt, Germany. Since more than 20 years he is studying the central carbon metabolism of yeasts with a special emphasis on transport of metabolites across biological membranes. Via metabolic engineering he is developing yeasts for biotechnological applications, e.g. fermentation of lignocellulosic hydrolysates and production of biofuels and biochemical compounds. He is also developing new tools for metabolic engineering. Eckhard Boles has published about 100 articles in international journals, has edited a textbook about molecular mechanisms controlling transmembrane transport, has filed 13 patent applications and was a co-founder of the Swiss biotech company Butalco.*

6.4. **Melliana Jonathan**, **Mirjam Kabel**, Wageningen (The Netherlands), **Marco van Brussel** and **Martijn Scheffers,** Leiden (The Netherlands)
Isolation and characterisation of branched gluco-oligosaccharides from amylopectin and the mode-of-action of a glucoamylase from Hypocrea jecorina

For first generation production of bioethanol, starch is converted enzymatically into glucose, followed by fermentation of the glucose to ethanol. Starch is composed of two types of macromolecules: amylose and amylopectin. The linear amylose is composed of α-1,4-linked glucosyl units. The branched amylopectin has both α-1,4- and α-1,6-linked glucosyl units. Typically, α-amylase and glucoamylase are used to degrade starch into glucose. Hydrolysis of amylopectin by α-amylase and glucoamylase to glucose often has branched gluco-oligosaccharides as intermediates. The hydrolysis of these intermediates to glucose is slow and becomes a bottleneck because glucoamylase cleaves α-1,6-linkages more slowly than it cleaves α1,4-linkages. Hence, enzymes that are able to rapidly degrade these oligosaccharides are searched for. In order to test new enzymes, branched oligosaccharides are needed, but commercially available substrates are lacking. This research was aimed to produce, isolate and characterise branched gluco-oligosaccharides that are potentially used as substrates for glucoamylases.
Branched gluco-oligosaccharides were produced from potato amylopectin digested with α-amylase. Oligosaccharides with DP 5-7 were isolated and purified using preparative Size Exclusion Chromatography. MALDI-TOF MS was used to characterise the DP of the oligosaccharides. Furthermore, the branched oligosaccharides were labelled by 2-aminobenzamide (2-AB) or 2-aminobenzoic acid (2-AA) and characterised using UHPLC-MSn. The advantages of labelling the oligosaccharides include better ionisation in MS, detectability by UV absorbance, and the possibility to quantify the oligosaccharides based on molar amounts. The results also showed that the branched gluco-oligosaccharides gave different fragmentation patterns from the linear ones. Hence, the structure of the branched oligosaccharides could be characterised.
The characterised oligosaccharides were used as substrates to test the activity of a glucoamylase from Hypocrea jecorina as an example. The results clearly showed that α-1,6-linkages was very slowly cleaved, and the presence of such linkage hinders the cleavage of α-1,4-linkages in its vicinity. This finding highlights the advantage of using branched oligosaccharides as substrates for glucoamylases as a complement to smaller substrates such as panose, isomaltose and maltose.
As a conclusion, branched gluco-oligosaccharides with DP 5-7 were isolated and characterised. When used as substrates for glucoamylases, these oligosaccharides give information about the activity of the enzyme towards both α-1,4 and α-1,6 linkages, as a complement to smaller substrates such as panose and isomaltose.



Melliana Jonathan *is a postdoctoral researcher at the Laboratory of Chemistry, Wageningen University, The Netherlands. She obtained her PhD degree on the degradation of dietary fibres in 2013. Her current research is focused on the molecular characterisation of oligosaccharides using various analytical techniques including (U)HPLC and MSn with the aim to improve the efficiency of biorefinery processes.*

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08^{30} Beginning of the lectures

5. Second Generation

5.6. **Reinhard Pätz**, Köthen (Germany)
Production of Ethanol with the High-Performance-Sequencing-Batch-Reactor-Technology

6. Third Generation

6.1. **Wolfgang Schwarz, C. Held, B. Leis, B. Andreessen, V.V. Zverlov** and **S. Graubner** Freising (Germany)
Tackling with resilient cellulose

10^{00} Coffee Break

6.2. **Christian Schweitzer**, Leipzig (Germany)
Power to fuel: 3rd Gen Biofuel from CO2

6.3. **Eckhard Boles**, Frankfurt (Germany)
From cellulosic ethanol to cellulosic butanol

6.4. **Melliana Jonathan**, WG Wageningen (The Netherlands)
Isolation and characterisation of branched gluco-oligosaccharides from amylopectin and the mode-of-action of a glucoamylase from Hypocrea jecorina

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