Arbeitsgemeinschaft Getreideforschung e. V.

in cooperation with

Max Rubner-Institut
Institute of Safety and Quality of Cereal

and the

University of Hohenheim
Institute of Food Science and Biotechnology

12th European Bioethanol and Bioconversion Technology Meeting

April 12th – 13th 2016 in Detmold

Program

Evening Program

Exhibition

Participants

Summaries

Tuesday, April 12th 2016

08^{00} – 08^{30} Registration

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

1. Future

08^{45} 1.1. **Frederik De Bruyn,** Gent (Belgium)
Closing the gap in the Innovation Chain

09^{15} 1.2. **Rodrigo Ledesma-Amaro,** Versailles (France)
Engineering Yarrowia lipolyticato produce biodiesel from raw starch

09^{45} Coffee Break

2. Second Generation

10^{15} 2.1. **Pierre Basuyaux,** Marq en Baroeul Cedex (France)
Advancements in Cellulosic Ethanol Fermentation

3. Third Generation

10^{45} 3.1. **Maria Hingsamer,** Graz (Austria)
Biofuel from micro algae

11^{15} 3.2. **Christian Schweitzer,** Leipzig (Germany)
Synthesizing strengths of 1st and 3rd generation biorefineries. Electric Biomethanol as a biorefinery product

4. Fourth Generation

11^{45} 4.1. **Michael Klingeberg,** Obrigheim (Germany)
New Biorefinery Concepts – Utilization of CO_2

12^{15} Lunch Break

5. Technology

14^{00} 5.1. **Dirk-Michael Fleck,** Uzwil (Switzerland)
Innovative Grain Milling Technology for Biorefinery

14^{30} 5.2. **Prashant Madhusudan Bapat,** Copenhagen (Denmark)
Tracing starch throughout ethanol fermentation process

15^{00} Coffee Break

15^{30} 5.3. **Christian Pfitzner,** Braunschweig (Germany)
Studies for the assessment of ethanol yield of cereals by means of near infrared spectroscopy

16^{00} 5.4. **Rene Akkermans,** Duesseldorf (Germany)
Advanced Modelbased Control

6. Yeast Strains

16^{30} 6.1. **Hans De Bruijn,** Delft (The Netherlands)
Development of next generation yeast strains for ethanol production from lignocellulosic feedstocks

Continued on the penultimate page

Lunch

Lunch will be served in the exhibition hall:

The menu:

Tuesday, April 12th 2016

Leek soup with mincemeat cheese and mushrooms

Chorizo with tomato in glass

Mini Beefburger

Cucumber chunks with fresh cheese

Bruschetta appetizers

Gouda skewers with grapes

Party meatballs with skewers

Dessert: Bavarian cream with raspberries

Wednesday, April 13th 2016

Asparagus cream soup

Canapés with ham paté

Canapés with herb cream cheese

Canapes with trout filet

Canapés with Camembert

Dessert: Panna Cotta

Beverages:
Mineral water

Coca-Cola

Orange juice

Apple Spritzer

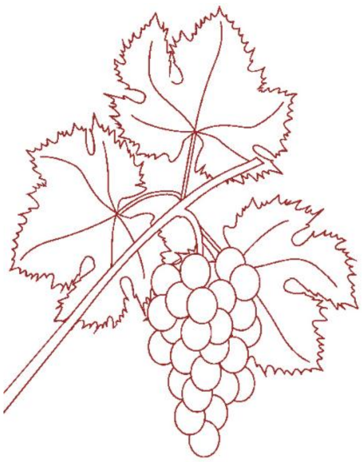
Bon appétit
and interesting conversations!

Bread & Wine

Tuesday, April 12th 2016

„Bread and Wine"-Get-together in the „Haus des Brotes" (Exhibition hall)

Wine



Baden
2011er Kirchberghof, Weingut Dr. Benz
Spaetburgunder Redwine, dry

Franconia
2013er Weingut Roth
Domina quality wine, dry

Palatinate
2014er Duerkheimer Riesling
Quality wine, dry

Rhinehessen
2014er Rivaner Kabinett
Prädikatswein, dry

Rhinehessen
2014er Weingut Knobloch
Dornfelder, quality wine

Wuerttemberg
2015er Schlossgut Hohenbeilstein
Lemberger, rosé, dry

Bread

Pretzels

Savoury Snacks

Mediterranean baguette and ciabatta

Small wheat rolls

19[30] **Social gathering** at "Strates Brauhaus", Lange Straße 35, Detmold

Please make your reservation until 4pm, if possible.

Thank you!

Evening Program

Monday, April 11th 2016

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

Tuesday, April 12th 2016

19^{30} **Social gathering** at the restaurant “Strates Brauhaus“, Lange Straße 35, Detmold

Wednesday, April 13th 2016

20^{00} **Social gathering** at the restaurant “Gastronomie am Hermannsdenkmal”, Grotenburg 50, Detmold (Teutoburg Forest)

Buffet

Starters:

- Rösti potatoes with pieces of cured ham,
- Sheep’s cheese wrapped in bacon,
- Tomato-mozzarella skewers
- Bruschetta fungi-toasted bread with mushrooms in an onion vinaigrette
- Salad buffet

Main dishes:

- Fillet of pork on a creamy mushroom sauce
- Chicken breast fillet with tomatoes, olives an sheep’s cheese

Accompaniments

- Basmati rice
- Potato croquettes
- Potato gratin

Dessert:

- Mousse of white & dark chocolate
- Tiramisu

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)

19^{30} h Meeting at parking place at Hermanns Denkmal, Grotenburg 50, Detmold and short walk to the Hermanns Denkmal

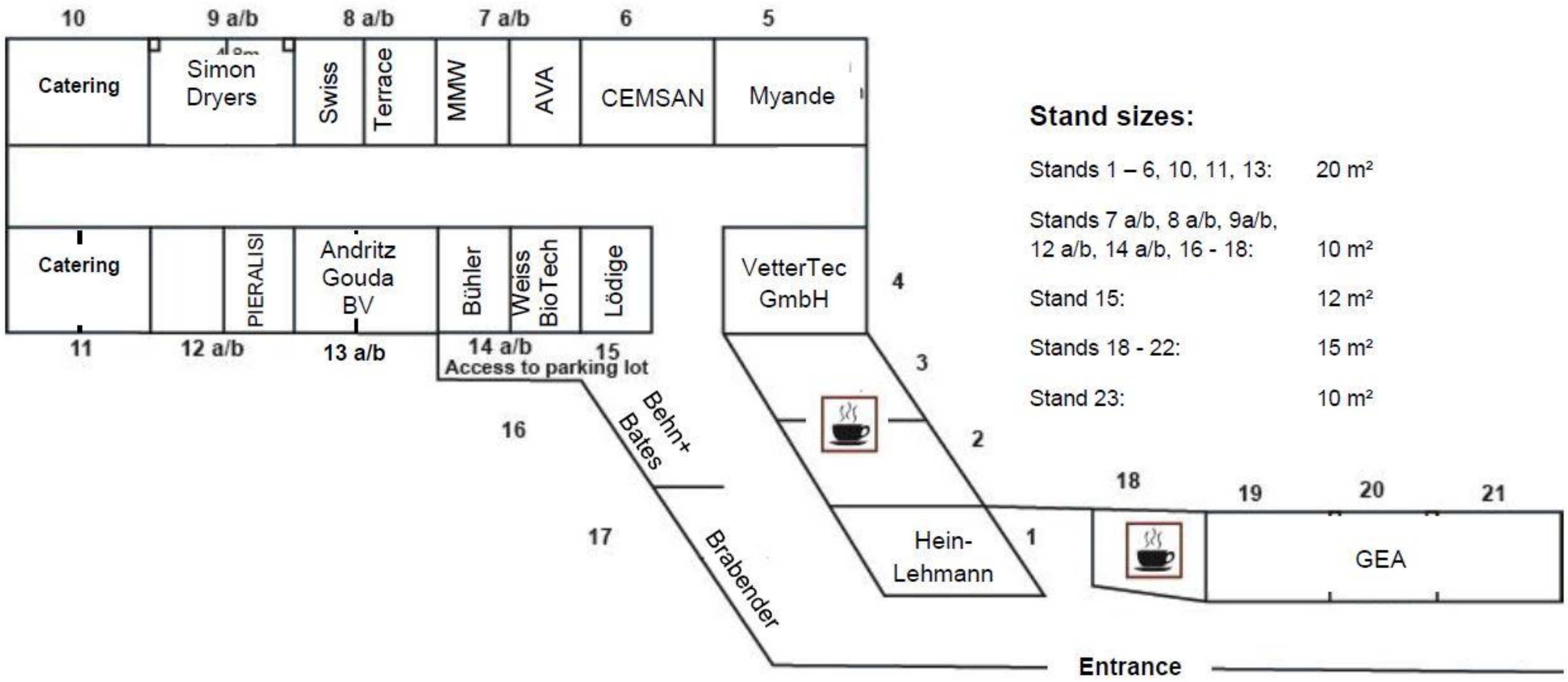
Departure: from 22^{00} h

Thank you!

Exhibition Hall Association of Cereal Research

Stand allocation

12th Bioethanol and Bioconversion Technology Meeting and 67th Starch Convention from April 12th – 14th 2016



Stand sizes:

Stands 1 – 6, 10, 11, 13:	20 m²
Stands 7 a/b, 8 a/b, 9a/b, 12 a/b, 14 a/b, 16 - 18:	10 m²
Stand 15:	12 m²
Stands 18 - 22:	15 m²
Stand 23:	10 m²

Exhibition

Andritz Gouda BV, PD Waddinxveen (Netherland)

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

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

Brabender GmbH & Co. KG, Duisburg (Germany)

Bühler GmbH, Braunschweig (Germany)

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

GEA Group AG, Oelde (Germany)

Hein. Lehmann GmbH, Krefeld (Germany)

Gebr. Lödige Maschinenbau GmbH, Paderborn (Germany)

MMW Technologie GmbH, Lutherstadt Wittenberg (Germany)

Myande Group Co. Ltd., Yangzhou, Jiangsu (China)

PIERALISI Northern Europe B.V., Eibelstadt (Germany)

Terrace International Inc., Bolingbrook (USA)

TUMMERS, Simon Dryers Technology, Nottingham (United Kingdom)

VetterTec GmbH, Kassel (Germany)

WeissBioTech GmbH, Ascheberg (Germany)

W. Kunz dryTec AG, SWISS COMBI, Dinitkon (Switzerland)

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Effective April 7th 2016

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Weber, Lydia, Dipl.oec.troph.
Wiege, Berthold, Dr.
Wolf, Klaus

Summaries

1. Future

1.1. **Frederik De Bruyn,** Gent (Belgium)
Closing the gap in the Innovation Chain

Bio Base Europe Pilot Plant is **multipurpose pilot facility** for the development, scale-up, demonstration and toll manufacturing of bio based products and processes at a kilogram to ten ton scale. The wide range of **state-of-the-art industrial equipment**, and the experienced and flexible team of 45 employees, serve customers from around the world in the field of biomass pretreatment, fermentation, downstream purification, bio-catalysis and explosion proof green chemistry. Bio Base Europe Pilot Plant is a European frontrunner in **industrial biotechnology**, and was therefore selected by the European Commission as a demonstrator multi-KET pilot line.

What is hampering industrial biotechnology and more in general the bio-based economy to take off? Well, although, SMEs and large companies can obtain financial support to scale-up their processes, for many of these companies and especially for SMEs, piloting is not the core of their activities. SMEs typically do not have the infrastructure to accommodate pilot lines, nor have the skilled personnel to run the tests. To obtain faster learning curves and shorter time to market, these activities are better outsourced. To allow this outsourcing, the necessary pilot infrastructure should be readily available, and companies should have easy access, without conflicts of interest with the organization or company that is running the pilot plant. Furthermore, the infrastructure available should be diverse and comprehensive, to allow the scale-up of a wide range of processes and finally, the pilot plant organization should have a critical mass of people to cover the many aspects of the bio-based economy.

With fermentation units up to 15 000 L scale, pilot scale biomass pretreatment equipment, chemical reactors up to 5000 L and an extensive set of pilot scale downstream processing equipment, BBEPP is often the missing link for companies to bridge the gap in the innovation chain.

This presentation will show how Bio Base Europe Pilot Plant addresses these concerns, how the pilot facility is set up, what bio-based processes are run and how it can help companies to bridge the gap in the innovation chain. Several **case studies** will be presented.

Frederik De Bruyn *received his Master's degree in Bioscience Engineering in 2010, at Ghent University, and his PhD in 2014 at the Centre for Industrial Biotechnology and Biocatalysis (InBio.be). He currently is Business Development Manager at the Bio Base Europe Pilot Plant (BBEPP) and focuses on the development, scale-up and custom manufacturing of bio-based products and processes to enable the transition to a sustainable bio-based economy.*

1.2. **Rodrigo Ledesma-Amaro,** Versailles (France)
Engineering Yarrowia lipolyticato produce biodiesel from raw starch

Microbial oils are promising alternatives to petroleum for the sustainable production of biofuels and chemicals. Among the possible producers, oleaginous yeasts are able to naturally accumulate high amounts of lipids (20-90% of the DCW). Currently, the most popular oleaginous yeast species for lipid production are *Lipomyces starkeyi*, *Rhodosporidium toruloides* and *Yarrowia lipolytica* .By far, the most studied and engineered oleaginous yeast is *Yarrowia lipolytica*, which has been previously modified to accumulate up to 90% of its DCW as lipids from glucose. Despite this high accumulation of biolipids, the process to produce them at large scale is not economically feasible, which has limited their use to high value lipids such as omega 3 fatty acids. Therefore, our aim is to reduce the production cost of biolipids in *Y. lipolytica* by 1) expanding the range of waste products and cheap materials that can be used as substrates and 2) the production of modified fatty acids with higher market value. Here we present the engineering of *Yarrowia* to produce lipids from raw starch.

Starch is one of the most abundant sugar on earth usually found in high amounts in many industrial waste effluents. It is a glucose polymer that requires at least two enzymatic activities to be degraded, alpha amylase and glucoamylase, none of them found in *Y. lipolytica*. After the heterologous expression of two synthetic genes coding for the mentioned activities, the modified strain was able to grow and produce a few lipids from raw starch. We therefore transferred this strategy to a metabolically engineered strain with more than 9 genes modified that allow it to accumulate high amount of lipids. As a result, the strain produces high amount of neutral lipids from raw starch and from a waste effluent, thus reducing the production cost of bio-oils. Additionally, we studied the properties of a biodiesel generated out of *Y. lipolytica* oils produced from starch.

Therefore, this multiple engineered yeast can serve as a starting point to further modification to produce high value lipids from cheap substrates.

***Rodrigo Ledesma-Amaro** studied Biotechnology and Chemical Engineered at the University of Salamanca, Spain. There, he did his PhD in metabolic engineering doing research stays at Ghent University (Belgium), Chalmers University of Technology (Sweden), INRA (France), EGE University (Turkey) and AIST (Japan). He is currently a postdoctoral fellow at MICALIS institute in Paris. His interests are in the interphase between synthetic, systems biology and metabolic engineering.*

2. Second Generation

2.1. **Pierre Basuyaux,** Marq en Baroeul Cedex (France)
Advancements in Cellulosic Ethanol Fermentation

Saccharomyces cerevisiae have been developed to meet the objective of simultaneous fermentation of these C5 and C6 sugars. In the early stages of the development of cellulosic ethanol, the primary challenge of Lesaffre in developing bioengineered yeasts was to overcome the challenges associated with cellulosic fermentation and to remove risk from within the process. Through classical and patented hybridization techniques Leaf (Lesaffre Advanced Fermentations) has now developed a new generation of (GMO) yeast for cellulosic conversion and is currently marketing their CelluX™ strain to the market. The main characteristics of CelluX™ are a higher resistance to inhibitors like acetic acid and phenolic compounds, faster fermentation kinetics and higher ethanol yield from xylose. Now that the industry is installing and the producers are deploying their technology, the focus of yeast producers such as Lesaffre is to improve the fermentation process to optimize producer’s productivity and profitability.

<u>Key points of the presentation</u>

- Yeast hybridization
- Principle of genome shuffling
- Areas of focus for fermentation improvement
- Product performance parameters and evaluation (examples / results provided)

3. Third Generation

3.1. **Maria Hingsamer,** Graz (Austria)
Biofuel from micro algae

Microalgae are currently considered to be highly attractive as a raw material for production of bioenergy and biomaterials in the future BioEconomy. However, a number of successful developments are still necessary before algae can reach commercial applications. These include the development of commercial production technologies, efficient energy, nutrients and water use, maintenance of stabile production conditions at commercial scale, and cost-competitiveness. Microalgae offer a number of possibilities for the provision of biogenic energy carriers. Specific characteristics of different algae species can be exploited for the production of various gaseous, liquid and solid bioenergy carriers, as well as heat and electricity. This can be combined with the co-production of non-energetic products such as Omega-3 fatty acids, fertilizer or animal feed.

Algae cultivation technologies can be divided into open and closed systems. Open systems, e.g. extensive ponds, raceway ponds, circular ponds, are those exposed to the atmosphere. Different types of closed systems are for example tubular, flat plate, or big bag photobioreactors. The combination of wastewater treatment and algal cultivation (integrated systems) offers significant synergies, and the demand for fresh water and external nutrients for algal cultivation can be reduced significantly. Conversion technologies (thermochemical, biochemical or mechanical and chemical processes) produce energy carriers from algal biomass. In the biochemical conversion, especially the methane fermentation to biogas and the ethanol fermentation to bioethanol are of interest. Possible technologies of thermochemical conversion are combustion for electricity and heat production, gasification to synthesis gas, pyrolysis to bio-oil, fluid catalytic cracking (FCC) and hydrocracking to fuels as well as hydrothermal processes (carbonization, liquefaction, gasification). FCC and hydrocracking convert the extracted algal oil to refined biofuels such as gasoline, diesel; the other technologies can convert the whole algal biomass. The mechanical and chemical conversion is the transesterification and esterification of algal oil to biodiesel.

The European project “FUEL4ME - Future European League 4 Microalgal Energy” - is driven by the urgent need of transforming the current energy system into a sustainable one, which pursues the European and global energy goals reducing GHG emissions, finding alternatives to fossil fuels and fostering the renewable energies. Microalgae are one of the most attractive sources of liquid transportation biofuels (e.g. biodiesel, hydrotreated vegetable oils (HVO)), since they can produce energy-rich molecules. FUEL4ME is developing and demonstrating an integrated and sustainable process for continuous biofuel production from microalgae, and making the algae-based biofuels (HVO) competitive alternatives to fossil fuels and to contribute to a future BioEconomy.

The big challenge for the future large scale and commercial production of algae for transportation biofuels within a BioEconomy is the up-scaling from demo size to commercial size. Therefore a modelling “Algae_Upscale 1.0” of the FUEL4ME process is done for specific parameters (technical, economic, environmental), which are most relevant for the up-scaling of the process; furthermore the modelling results in a framework for a sustainable future commercial HVO production from microalgae. This modelling approach includes the methodologies of Life Cycle Sustainability Assessment (LCSA).

Different cases with a production size of 100 kt/a HVO and coproducing PUFA are modelled, as the future commercial algae biofuel production needs huge volumes to receive significance on the energy market. The modelling uses three main targets, which have to be fulfilled simultaneously:

1. revenues ≥ costs,
2. reduction of GHG emissions ≥ 60 % compared to diesel,
3. cumulated fossil primary energy demand in relation to HVO energy content ≤ 30 %.

Based on this modelling a first set of specific parameters are identified to be most relevant for up-scaling from demo-scale (a few m²) to future large commercial scale (> 100 ha):

- algae biomass yield,
- algae oil yield,
- electricity demand,
- heat demand,
- investment costs,
- number of employees,
- nutrient recycling,
- CO_2 demand,
- revenues from algae oil and PUFA.

The results of the techno-economic and environmental assessment (LCA) within the modelling show that the PUFA production capacity has a strong influence on the economics and the target of 0.3 $MJ_{fossil}/MJ_{biofuel}$ is more difficult to be met than > 60% GHG reduction according the RED methodology. The modelling is used to identify obstacles for an efficient process and helps to guide the development of the FUEL4ME process in the desired direction.

The production of bioenergy from algal biomass is still in development. Research and development needs are high especially in the cultivation, harvesting, and processing. Pilot and demonstration projects are being actually implemented. For a commercial large-scale implementation it is essential to further optimize the cultivation and processing of algal biomass and to reduce the auxiliary energy demand and the associated costs needed for cultivation.

Acknowledgement: The work is part of the project "FUEL4ME – FUture European League 4 Microalgal Energy". The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 308983; and the finalized project "Algae - A Future Renewable Energy Source? – Current Status and Future Perspectives for the Austrian Energy System", funded by the Austrian Climate and Energy Fund.

***Maria Hingsamer** holds a diploma in Environmental System Sciences with the special field Geography at the University of Graz. Since 2010 she has been working as scientist and project leader at JOANNEUM RESEARCH. Her main research areas are life cycle sustainability assessment (LCSA), environmental assessment with a focus on biofuels, biorefineries and algal biomass for energy production.*

3.2. **Christian Schweitzer,** Leipzig (Germany)
Synthesizing strengths of 1st and 3rd generation biorefineries. Electric Biomethanol as a biorefinery product

Introduction

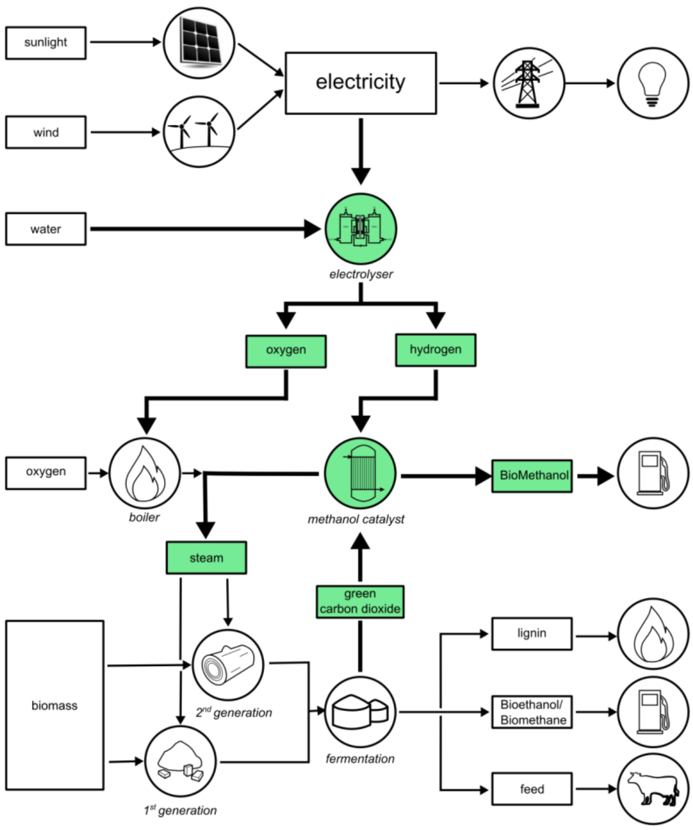
Over the past years, the energy sector has been shifting more and more toward renewable energy sources. Today, the main portion of renewable energy is produced decentralised and intermittent.

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 handthe existing Ethanol industry is faced to increase the GHG savings by carbon capture and utilization in the fermentation regenerating green CO_2.
Bse has done a pre-engineering to evaluate the opportunities for a realistic solution on the technical level under the view of the political aspects. The result is the combined plant.

The CO_2mbined plant uses CO_2 streams from biological Ethanol fermentation processes to produce Renewable Methanol. The plant directly faces the specific problem of fluctuated energies with the utilisation of green CO_2.

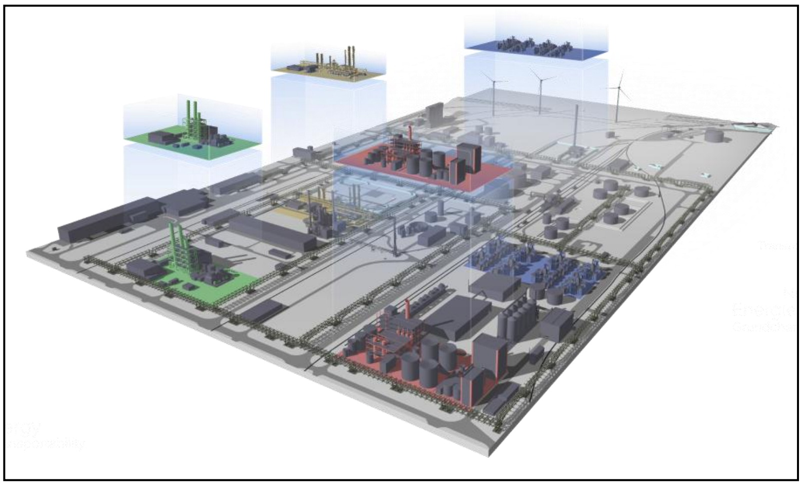
CO_2mbined plant is solving the problems which increase in 5 – 10 years, when renewable energy part is linking with EU renewable energy goals of 27 % at the final energy consumption by 2030. The CO_2mbined plant will be installed in order to process continuous green CO_2 of biochemical origin (biomass) under fluctuated electrochemical conditions in a thermo-chemical process to produce Methanol.
The interconnection of the different process units creates a high efficient energy and resource cascade.



The challenge of the Future Energy sector is the management of using the fluctuated power supply from wind, solar source to replace fossil fuels. The option is to create a chemical Energy storage, which should be also used in the transport sector, and should be transportable with high energy density and marketed in existing value chain. This is reachable by using waste green Carbon dioxide from biological ethanol fermentation. The figure shows the way to store excessive power, when production exceeds grid demand: 1. Divert power to electrolysis. 2. Convert H_2 to methanol. 3. Divert O_2 to boiler furnace. **CO_2mbined plant** uses this principle to process green CO_2 into a high value fuel (BioMethanol, Renewable Methanol or E-Methanol).

Technical Aspects

The CO_2mbined 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 O_2 from the process of electrolysis increases the primary processes of the fermentation, thus allowing higher efficiency rates.



E-Methanol produced from green CO_2 and power from renewable sources is a promising potential source of clean energy for transport. CO_2mbined plant enables the industrial usage of

green CO_2 as a new processing on Ethanol plants, thus enlarging for the first time the biomass utilisation by processing the complete biomass streams including CO_2. The result is increasing the yield of utilisable biomass by almost 50 % per acre of cultivated land.
Additionally, E-Methanol from the Carbon capture and Replacement (CCR) improves the GHG savings compared to fossil fuels up to 79 % for Ethanol from sugar beet and up to 74 % for Ethanol from wheat.

$$E = e_{ec} + e_l + e_p + e_{td} + e_u - e_{sca} - e_{ccs} - e_{cc} - e_{ee´}$$

Political Aspect
The described E-Methanol production is a new type for fuel production that promise GHG reduction, re-industrialisation and integration of renewable energy via energy switch power to transport fuel. These "renewable liquid and gaseous transport fuels of non-biological origin" (PtX) and "carbon capture and utilisation for transport purposes" (CCU) will get greenhouse gas emission Default Values by the European Commission according to Article 7a No. 6 of Directive 98/70/EC of the European Parliament and of the Council of 13 October 1998 relating to the quality of petrol and diesel fuels and amending Council Directive 93/12/EEC (FQD) last amended by Directive (EU) 2015/1513 of the European Parliament and of the Council of 9 September 2015.

I. Default Values
To enable their use in the fuel markets these Default Values should be defined as an essential element to the normative implementation. To make the definition practical it cannot be coupled for each technical method with an application (see ANNEX I Part 2 of Council Directive (EU) 2015/652 of 20 April 2015 laying down calculation methods and reporting requirements pursuant to Directive 98/70/EC of the European Parliament and of the Council relating to the quality of petrol and diesel fuels). Up to 120 million different options would emerge taking into account the different criteria (power sources, power connection, electrolysis technologies, by-product utilisation, carbon source, CO_2 capture technologies, conversion technologies, fuel use, 1st stage synergistic technologies).
To determine the greenhouse gas emission Default Values of fuels whose energy content comes from electricity, it is suitably to determine a calculation method for the Default Values. This calculation method could be based on the existing methodology for biofuels (bright text is not relevant):

Methodology for Greenhouse gas emissions of biofuel according ANNEX IV C. FQD

$$E = e_{ec} + e_l + e_p + e_{td} + e_u - e_{sca} - e_{ccs} - e_{ccr} - e_{ee´}$$

E = total emissions from the use of the fuel
e_{ec} = emissions from the extraction or cultivation of raw materials
e_l = annualised emissions from carbon stock changes caused by land use change
e_p = emissions from processing
e_{td} = emissions from transport and distribution
e_u = emissions from the fuel in use
e_{sca} = emission savings from soil carbon accumulation via improved agricultural management
e_{ccs} = emission savings from carbon capture and geological storage
e_{ccr} = emission savings from carbon capture and replacement
$e_{ee´}$ = emission savings from excess electricity from cogeneration

The base principle of the calculating method for the greenhouse gas emission Default Values of PtX and CCU process is the use of power from the grid. Since the energy carrier hydrogen is generated from power, power has to be defined as a raw material. If the national CO_2 footprint of the power mix of the domestic consumption is basis (for Germany 609 gCO2/kWh, i.e. 169 gCO2/MJ), the target of 60 % savings is not technically feasible.

Definition (Suggestion)

e_{ec}	=	emissions from the extraction or cultivation of raw materials *respectively of the energy carrier = hydrogen generated with power with CO_2 emission of the Member States power mix*
e_p	=	emissions from processing includes process energy with *gCO2/kWh (electrical power as auxiliary power for the process)*

The required power for production of hydrogen is, however, not auxiliary power that is needed for the process. Under auxiliary power is understood the power, which is necessary for the process control, pumps, motors.
Since the demand for PtX and CCU process is the provision of system services, the ability of the system integration is a potential element for determining the Default Value. We therefore see emission savings through flexibility in the power grid as an additional criterion.

Definition (Suggestion)

e_{fl}	=	*emission savings from provision of flexibility in the power grid*

How is the flexibility measured?

- **Ability** of the technology to the flexibility of the current consumption in 1 – 100 %

The more flexible the PtX and CCU process can respond to the current market situation, the greater is the ability to integrate renewable energies in the electricity market. This capability should be considered in the CO_2 emissions intensity of the member states power mix, e.g. as divisor to the national CO_2 emission intensity.

Formula for the calculation of Default Value PtX/CCU (Suggestion)

$$E = e_{ec} \,/\, e_{fl} + e_p + e_{td} + e_u - e_{ccr} - e_{fa}$$

The threshold of greenhouse gas emissions of the fuel can be achieved, if renewable energies are there that have to be integrated in the power grid. Consequently, the higher the share of renewable power in the system and the national CO_2 emission intensity of power reduces, the lower the flexibility of the system must be and a change of energy in all sectors including the transport sector is thus possible.

II. Methodology

In addition to the calculation of the Default Value above, the methodology for transport fuels could be adjustment to reflect the single evidence of fuels by stabilisation of the power grid. Other subgroups of flexibility should be considered and integrated into the calculation method, weighted and be deducted.

e_{fa}	=	*emission savings from performed flexibility and integration of renewable power in the power grid. The value consists of e_{ec} divided by e_{fl} multiplied with all sub groups. Sub groups:* *1) By grid operator prequalified flexibility, factor 0.00 – 1.00;* *2) at the control energy market offered balancing power, factor 0.00 – 1.00;* *3) at the control energy market accepted tender of flexibility, factor 0.00 – 1.00;* *4) at the control energy market actual called flexibility, 0.00 – 1.00.*

The above formula for calculating the Default Values of PtX/CCU could be extended to minus flexibility work.

Methodology: Greenhouse gas emissions from the production and use of (bio-)fuels (Suggestion)

$$E = e_{ec} \;/\; e_{fl} + e_{p} + e_{td} + e_{u} - e_{ccr} - e_{fa}$$

Conclusion

The CO_2mbined plant is technical feasible and is able$_2$to provide existing Bioethanol plants additional value chains. This solution will increase the efficiency of the existing industry and has the opportunity to reach the political targets for GHG savings of fuels and renewable energy integration in the sectors power and transport fuel.

E-Methanol from green CO_2 is recognised in the EU legal framework. If the implementation of the amended Fuel Quality Directive is going in above mentioned direction, we will see a revival of Investments in the Biofuels sector in Europe based on proven technology.

We invite you to support the described idea and are looking for mandates from the Industry to discuss the options with the relevant legal bodies across EU.

***Christian Schweitzer** 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 develops the technical and economic integration of the chemical Energy storage of Methanol in the Ethanol Industry.*

4. Fourth Generation

4.1. **Michael Klingeberg,** Obrigheim (Germany)
New Biorefinery Concepts – Utilization of CO_2

Objective:

The SÜDZUCKER subsidiary CropEnergies operates three 1st generation bioethanol factories in Belgium, Germany and in the UK which produce sustainable biofuel. During fermentation of glucose towards ethanol carbon dioxide (CO_2) is liberated. Dependent on the production process (separation of gluten, biomass boiler fed with bran) the ethanol factories produce the ethanol more or less sustainable and the Biofuel Sustainability Directive (Biokraft-NachV) requires a further reduction of the CO_2 emission. In the bioethanol plant of Zeitz already 100,000 t of CO_2 are dried, compressed and sold into the beverage industry. However, in order to further improve the sustainability of the factory trials were carried out to capture CO_2 and to convert it into higher value products.

Strategy:

Südzucker together with CropEnergies has joined a project which was supported by the Federal Ministry of Food and Agriculture (BMEL). The aim of the project was to build up a biorefinery concept which uses pure CO_2 emitted by the bioethanol plant and to feed the gas into flat-panel-airlift reactors for the cultivation of starch producing algae. After growth the algae are harvested, disrupted and fractioned. The starch is converted in the bioethanol factory towards glucose and fermented to ethanol while the proteins are recovered. Residues are converted in a biogas fermenter into methane, the sludge is separated into mineral containing water which can be recycled into the bioethanol plant while the concentrated sludge is used as fertilizer. Further trials are currently performed in a project supported by the Federal Ministry of Education and Research (BMBF) with bacteria of the genus *Acetobacterium* which is able to grow on solely CO_2 and H_2. During growth acetic acid is produced which later should be converted by genetically modified organisms into biobased chemicals. In the presentation preliminary results will be presented.

Dr. Michael Klingeberg, *born 1960 in Hannover, Studies of Biology at the University of Cologne and Goettingen with special focus on Microbiology, Biochemistry and organic chemistry, PhD thesis on the purification and characterization of a protease from the archaebacterium Thermococcus stetteri, Scientist at Novo Nordisk, Denmark – Head of the working group Microbiology in the R&D department of SÜDZUCKER AG in Offstein/Pfalz. Among others responsible for the support of the ethanol factories of the Südzucker subsidiary CropEnergies concerning fermentation and enzyme application.*

5. Technology

5.1. **Dirk-Michael Fleck,** Uzwil (Switzerland)
Innovative Grain Milling Technology for Biorefinery

Grain is more than just an important source for food and feed. It is also increasingly being used as a source for Biorefinery products. Innovative Grain Milling Technology is required to gain the best utilization for all grain ingredients.

Biorefinery plants produce a wide range of different products: ethanol, native and modified starch, different glucoses and proteins such as vital wheat gluten, lysine and feed products, e.g. DDGS, bran or wheat feed. The complexity rises with the number of finished products. The requirements for processing grain are extensive: high yield, control of raw material, product traceability, high quality finished product and fulfilment of food safety requirements.

Raw material costs are 70 – 80 % of the OPEX in a Biorefinery plant. The milling process is the very first processing step of a long bio refining process chain. Therefore the milling process has a direct and significant impact on the performance of the subsequent process and the finish product quality. However, grain is a natural raw material where different types and levels of quality exist. And even the same type of grain, e.g. wheat, has a wide spread of quality, depending on the origin, the harvesting year or the condition in which it was stored.

One of the main tasks of a milling system is, to manage this fluctuation in raw material characteristics in order to produce a constant finish or semi-final product on a high yield. While a fully automated milling system ensures that it can be operated with minimum workforce, ~~but~~ it does not necessarily ensure a perfect milling result, i.e. a constant product quality. Frequent product sampling, analysis and a well-educated headmiller are necessary to ensure an optimal performance of a mill.

But still a failure in the milling system, such as the breakage of a sieve in a sifter for example, can spoil a whole daily production of high value vital wheat gluten by bran particles. Or, the individual influence of manually taking samples, doing the analysis or making the adjustments of the mill, can lead to a suboptimal milling result.

The following introduced smart process control systems can take-over the main control of ~~this~~ this regulation. Intelligent systems doing immediate corrective intervention in the on-going production process.

As part of this presentation, we explain how the following innovative technology manage the complexity and the requirements of Grain Milling for Biorefinery:

- Intelligent Process Optimization Sensors ensure the online measurement of the product during processing.

- The new Antares Plus roller mill with integrated measurement and control device keeps the grinding effect at the optimum.

- Latest SORTEX optical sorters are a cornerstone in separating contaminated grain and ensuring food safety.

- WinCos automation system guarantee product traceability.

About Bühler:
Every day, billions of people come into contact with Bühler technologies to cover their basic needs for foods, mobility, or communication. With our industrial-scale process technologies and solutions, we contribute significantly to feeding the world's population, setting the focus on food security and food safety. Bühler flour mills process around 65% of the wheat harvested worldwide into flour. Its contribution to processing rice and producing pasta, chocolate, or breakfast cereals is similarly important. Moreover, Bühler is a leading solution provider of die casting, wet grinding, and surface coating technologies, with an emphasis on automotive, optics, electronics, printing & packaging inks, and glass applications. The solutions provided for these industries are distinguished by high energy efficiency and sustainable mobility. As a leading technology group, Bühler invests up to 5% of its sales revenue in research and development. Bühler is proud of its Swiss roots, with 10,600 employees in some 140 countries generating sales of CHF 2.3 billion for the 2014. The family-owned company Bühler is particularly committed to sustainability.

Dirk-Michael Fleck, *Account Manager Biorefinery, Buhler AG, Dirk has graduated in Mechanical Engineering in 1997 and gained an MBA degree in 2006. He has been with Buhler for almost 20 years, started in the Feed Technology Industry, where he spent four years in Japan. Since 2005, Dirk is responsible for the grain based Ethanol and Biorefinery market at Buhler. He has managed to gain a vast experience in numerous successfully realised projects.*

5.2. **Prashant Madhusudan Bapat,** Copenhagen (Denmark)
Tracing starch throughout ethanol fermentation process

Ethanol yield is one of the most important metrics for the bioethanol industry. Often, ethanol yield estimation is based on ton of dissolve solids (t DS). Although this methodology is fast, it does not give accurate estimation of ethanol yield as DS measurement is influenced by liquefaction DE as well as backset solids. Moreover, as the typical ethanol yield improvement ranges from 0.1% to 3%, any small deviation in DS measurement has big influence on ethanol yield estimation.

Ethanol yield estimation based on the total starch at the beginning of the fermentation process can reduce some of the uncertainties mentioned above. In this work, we tried to estimate ethanol yield based on total starch. Two starch hydrolysis methods, enzymatic and acid, were used to measure total starch at the beginning of the fermentation (after liquefaction), at 10 hours and residual starch at the end of fermentation (drop) as well as in DDGS.

Although the acid hydrolysis method is fast and simple, we found that it is not specific to starch, it is not specific to substrate condition, and the acid to substrate ratio had profound influence on the final starch measurement. Here in this work, we tried to understand the plausible reasons behind non specificity of acid hydrolysis. Finally, ethanol yield was measured based on total starch values obtained from these methods and then was compared to the theoretical maxima.

Prashant Madhusudan Bapat, *is from Mumbai, India. He did his graduation in microbiology. During his doctoral work (IIT-Mumbai), he worked on mathematical model based fermentation process optimization. He joined Novozymes in 2009 as an optimization scientist. Currently, he is working as an Industry technology specialist at Novozymes and serving their starch as well as bioethanol customers.*

5.3. **Christian Pfitzner,** Braunschweig (Germany)
Studies for the assessment of ethanol yield of cereals by means of near infrared spectroscopy

Aside of other relevant agronomic characters ethanol yield is an important trait for breeding and processing of cereal varieties. However, the quantitative analysis of this trait is very laborious and therefore, investigations in utilizing near infrared spectroscopy (NIRS) as method for the fast estimation of ethanol yield were initiated. Calibration experiments using an extensive set of samples including samples from field trials for the assessment of the value for cultivation and use by the Federal Plant Variety Office (Bundessortenamt) as well as breeding stocks of commercial German breeders received by the GFPi (Gemeinschaft zur Förderung von Pflanzeninnovation e. V.) were carried out.

The ethanol yield of winter wheat, winter rye, and winter triticale could not be estimated by NIRS with satisfactory accuracy. The reason for this is an insufficient precision of the reference analysis in combination with an obviously too small variability of the trait. To overcome these restrictions an alternative approach could be the estimation of the ethanol yield potential based on the stoichiometrically deduced sum of starch and sugar content by NIRS. This approach resulted in preliminary calibration experiments in much better prediction models even if applied on whole seed samples.

Christian Pfitzner *(Dipl. Ing. agr.), Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Braunschweig, he is a specialist for application of near infrared spectroscopy (NIRS) in agriculture.*

5.4. **Rene Akkermans,** Duesseldorf (Germany)
Advanced Modelbased Control

Rockwell Automation model-based software drives profitability
Our Pavilion8 Model Predictive Control (MPC) is widely used throughout the process industry to maximize the performance of plants, delivering higher throughput at improved efficiencies, with lower energy consumption, and reduced emissions.

Our MPC solutions create value by continuously optimizing the key process parameters online, closed loop, keeping the plant in the most economical operating point every minute, 24/7. To be able to do that, the underlying MPC technology needs to be able to predict the plant behavior, account for plant disturbances, be able to incorporate plant constraints and operator knowledge.

Pavilion8 MPC provides hybrid modeling functionality that allows the user to do this including empirical data and first principles equations when building a model. Hybrid modeling results in a more robust, more accurate model across the entire range of operating parameters. This capability allows users to build robust models faster with higher accuracy than alternatives.

Our experienced team of engineers work with leaders in the BioEthanol and BioDiesel Industry to implement industry-specific solutions that deliver results. Pavilion8 solutions consistently deliver a faster time to value and greater sustained value than alternatives, year-over-year.

Rockwell Automation Biofuel Solutions
The Biofuel industry faces a wide spectrum of challenges, including varying demand and tight operating margins, increased competition, and increasingly stringent quality specifications. Manufacturers must constantly strive to find the best approaches to optimize operations and lower costs in order to remain competitive in the marketplace. The key is to find solutions that provide better and faster results across market swings, deliver quicker project execution, and longer sustained value.

Pavilion8 provides a suite of advanced process control and optimization applications across all key areas process, knowing;

Ethanol Solution applications include:
- Milling/Cook and Fermentation Control
- Evaporator and Dryer Control
- Distillation and Sieve Control

Biodiesel Solution applications include:
- Transesterification Reactor Advanced Control
- Methanol Rectification Advanced Control
- Glycerin Drying Column Advanced Control
- Fatty Acid Stripper Advanced Control among others

Benefits
We have helped major producers increase production and/or yield, reduce costs, enhance product quality and maintain environmental compliance. By leveraging the most modern technological capabilities and domain expertise available.

Rene Akkermans, *Pavilion Account Executive EMEA, Rockwell Automation, Mr. Rene Akkermans is the Account Executive for their Pavilion8 Model Predictive Control (MPC) business in the Biofuels Industry(s) in EMEA. He is based out of Brussels headquarters in the Belgium. Rene has more than 17 years of consulting and process automation experience in a spectrum of industries ranging from Bioethanol, Biodiesel, Starch up to Polymer Industry and is graduated in Information and Economics. Rockwell Automation, the world's largest company dedicated to industrial automation and information, makes its customers more productive and the world more sustainable. Biofuels Industry Experience: Efficient production of renewable fuels is essential to a holistic energy policy and this remains a challenge in this among many strongly cyclical industries. Saving energy reduces costs of production and increases your net positive CO2 footprint. Even more important is any shift in plant yields, which is the most costly input to making products. Finally managing production capacity in cyclical markets means that you can run faster when margins are excellent and turn-down rates and run efficiently when margins shrink. All of these objectives we have improved with a higher level of MPC Control.*

6. Yeast Strains

6.1. **Hans De Bruijn,** Delft (The Netherlands)
Development of next generation yeast strains for ethanol production from lignocellulosic feedstocks

Ethanol is considered to be a sustainable alternative to fossil transport fuels due to its reduced carbon-footprint and the benefit that it can be produced from renewable resources such as agricultural residues. Key to economic viability of ethanol produced from lignocellulosic biomass is low-cost production and application of enzymes to hydrolyze lignocellulosic carbohydrates into monomeric sugars, which subsequent are converted efficiently into ethanol with high productivity and high yield using advanced engineered yeast strains.

DSM is a frontrunner in the development of these bioconversion technologies. Its on-site manufactured enzyme cocktail is uniquely active at high temperature and low pH, offering process and cost benefits as well as product differentiation. DSM was also the first to prove and commercialize C5 sugar-fermentation using *Saccharomyces cerevisiae* on demo & commercial scale, and its strains are implemented in cellulosic ethanol production processes globally.

In taking cellulosic EtOH fermentation to a level beyond the now widespread basic (xylose isomerase-based) xylose fermentation technology, DSM and its collaborators have developed several key technologies for which a broad IP position was obtained.

A selection of these technologies has already been introduced in novel commercial yeast strains, offering significantly accelerated C5-fermentation rates, improved robustness and an expanded substrate range to the market. This presentation offers a glimpse of new innovative technologies finding their way towards commercial implementation.

Pentose conversion in engineered *S. cerevisiae* typically occurs only when glucose is nearly exhausted , as the native yeast sugar transporters involved in pentose uptake (Hxt1,-2, -4,-5, -7 and Gal2) have 10-100x higher affinity for glucose than for xylose. Subsequently, after glucose exhaustion pentose conversion continues at a relatively low rate. This results in extended fermentation time caused by xylose tailing, and this effect is amplified by increased sensitivity to hydrolysate-derived fermentation inhibitors during low-flux C5 fermentation. To address this, DSM in collaboration with the Molecular Microbiology Group of the Rijksuniversiteit Groningen, has engineered native sugar transporters with improved affinity and/or specificity for xylose, enabling glucose/xylose co-fermentation.

As ethanol yield on lignocellulosic biomass is a powerful means of reducing production costs, DSM has developed and commercialized industrial strains capable of fermenting all major monomeric sugars present in typical biomass hydrolysates (glucose, xylose, arabinose, galactose, mannose). To progress beyond this point, metabolic pathways have been integrated that enable the conversion of previously untouched carbon sources into additional ethanol. As a constituent of hemicellulose released upon its hydrolysis, acetic acid is inevitably present in lignocellulosic hydrolysates and severely inhibits ethanolic fermentation. However, by anaerobically converting acetic acid from the hydrolysate to ethanol , an alternative redox-sink is created, abolishing the requirement of the yeast to produce the undesired byproduct, glycerol, to compensate for redox-equivalents generated during anaerobic growth.

Besides the ethanol generated from this acetic acid, carbon from glycerol byproduct formation is channeled to additional ethanol. This proprietary DSM technology, developed by the Industrial Microbiology Group of the Technical University of Delft, was successfully integrated into C5-fermenting strains and demonstrated on lignocellulosic hydrolysates. The concept was subsequently augmented with a pathway to anaerobically convert externally added glycerol to EtOH, producing additional redox equivalents and thereby enabling increased conversion of acetic acid. The glycerol supplement is envisioned to be provided through biorefinery waste-streams. As this combined technology both detoxifies the fermentation medium while increasing the EtOH yield, an industry-wide problem is in effect turned into a yield-improvement opportunity.

DSM has built a strong technology base for improving both efficiency and yield of conversion of lignocellulosic carbon sources to EtOH. It will continue to actively develop innovative technologies, and implement these into next generations of commercial yeast strains.

***Hans de Bruijn**, Fermentation Scientist at the DSM Biotechnology Center in Delft, working on the development and application of yeast strains for cellulosic ethanol. Started working on this topic in 2007 at Royal Nedalco, along with work on optimization of 1st generation (potable) grain ethanol. Joined DSM through the acquisition of this R&D group (a.k.a. C5 Yeast Company).*

7. Raw Material

7.1. **Reinhard Pätz,** Köthen (Germany)
Potential for utilization of starch by-products

In our R&D work we used so-called c-starch of Jäckering Mühlen-und Nährmittelwerke Hamm as substrates for different fermentations in labscale.

First application was production of colourants for food and feed industry. So we could reach good results for beta-carotene production with the fungal mixed culture of *Blakeslea trispora* up to more than 8 % of dry matter of fungal biomass. The process is well developed and shall be realized in near future. With yeast *Rhodosporidium toruloides* we could increase protein content, also with red colour in a high cell density fermentation process up to 140 g l^{-1} DYM. This colour consists of torulene, beta-carotene and torularhodene, new and till now not used colourants. A new fermentation technology for fungal growth was used for technological investigations.

With the same substrate we tested different bacterial and yeast strains for production of different kinds of biofuels. Highest productivity we could reach for ethanol production. But also butanol and 2,3-butanediol, an intermediate for biofuels and chemicals, could be fermented in labscale with good productivities without technological optimization.

Ultimately polyhydroxyalkanoates could be fermented with the by-product of starch production. Thus we could show that the c-starch is a good substrate for biotechnological processes.

***Dr. Reinhard Pätz,** born 1952 in Zschepplin, full time study of chemical engineering and specialisation high polymere chemistry at the Technical University Leuna-Merseburg, PhD-Student polymeranalogous reactions at Technical University Leuna-Merseburg, Researcher Bioflocculants at Institute of technical Chemistry, Head of Department Bioprocess engineering at Institute of Biotechnologie (IBT) Leipzig Academy of Sciences, Engineering Consultant for biotechnological processes environmental processes, Head of Biotechnikum Projectleader Biogas beta-Carotene-Production other biotechnological processes at EON Energy of Nature Projektgesellschaft für umwelttechnische Anlagensysteme mbH, Professor for Bioprocess Engineering at Hochschule Anhalt (FH)/Köthen*

7.2. **Wim van der Wilden,** Wageningen (The Netherlands)
From Waste to Wealth

Detailed information will be presented at the convention.

8. Enzymes

8.1. **Arjen van Tuijl,** Leiden (The Netherlands)
Next Generation Glucoamylase products for SSF Ethanol Production

Throughout the years, DuPont has introduced different generations of glucoamylase products into the marketplace with increasingly better performance. First generation glucoamylase products contained glucoamylase alone, whereas second generation contained glucoamylase in combination with fungal alpha amylase.

With DISTILLASE® PLUS DuPont now introduces a third generation glucoamylase product, developed to generate high yields in Dry Grind Ethanol Production. DISTILLASE® PLUS is a carefully designed blend of four activities: Glucoamylase, Alpha Amylase, Trehalase and Protease. These activities all work together to hydrolyse starch liquefacts into glucose and facilitate yeast in optimal production of ethanol from this glucose.

For an optimal fermentation, as much starch as possible should be converted. This can be achieved by optimizing alpha amylase dose in liquefaction and glucoamylase dose in fermentation. Very high conversion of starch (low residual starch) is often reached with higher alpha amylase and glucoamylase dosages. Possible downsides of higher enzyme dosages, high peak glucose and high glycerol, can be overcome by 'staged' addition of DISTILLASE® PLUS (at 0 hours and 8 hours of fermentation). In this way peak glucose can be controlled, thus decreasing glycerol and increasing ethanol by up to 2.3%.

Combining DISTILLASE® PLUS with an optimized dosing strategy results in highest ethanol yields and thus extracts the most value from grains.

DuPont is happy to assist producers to apply and optimize DISTILLASE® PLUS dosing to 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.*

8.2. **Patrick E. Williams,** Franklinton (USA)
Improving plant profitability through the application of new enzyme technologies

Novozymes' new advanced liquefaction product, Avantec® 2.0 E, sets a new standard for your plant's performance while simplifying operations and diversifying operational possibilities. Avantec® 2.0 E's unique formulation can significantly increase ethanol yield and corn oil yield beyond the performance of any other competitive enzyme solution. The product provides unparalleled viscosity reduction in cook at low pH conditions, and the potential for increased throughput, giving your plant the flexibility to run at higher solids or higher run rates. Simplification of the ethanol production process through reductions in urea or ammonia usage and the elimination of protease additions to fermentation are also benefits of the product. Plants using the product have seen immediate production gains, new operational simplicity and flexibility, and the potential for increased profits.

***Patrick Williams** is a Technical Service Manager for Novozymes. His group supports the biofuels industry in Europe, the Middle East, Africa, and Asia Pacific. A graduate of Appalachian State University, with a B.S. in Chemistry, Mr. Williams has spent time working in conventional biofuels, cellulosic biofuels, as well as the pharmaceutical industry.*

8.3. **Wolfgang Aehle,** Zwingenberg (Germany)
Metagenome Approach for New Enzymes

Industry is using enzymes for a wide variety of processes under conditions that cover almost the complete pH-scale from alkaline to acidic and the temperature range of water in its liquid phase. Enzymes for industrial use are not chosen due to the catalysed reaction, but based on the effect that they cause during the preparation of the food. The starch-hydrolysis of α-amylases e.g. is used for the standardization of flour or as anti-staling enzyme in the baking industry, as liquefying agent during the production of high fructose corn syrup, or in the fruit juice industry for yield enhancement or anti-haze agent, to name a few. The conditions of use are consequently as diverse for a given enzyme functionality as is the effect that the user expects from the application of the enzyme. Access to wide enzyme diversity is key to the successful discovery and development of any new enzyme due to the variability in the application conditions and the diversity of effects that are expected by the users.

The natural diversity of cultivable microorganisms has proven to be a good resource for new proteins. The accessibility of this resource, however, depends on the ability to cultivate organisms under laboratory conditions. It has been estimated that less than 1% of all microbial species can be cultivated by men (Torsvik et al. (2002)). Consequently the vast majority of the existing microbial biodiversity cannot be exploited with traditional approaches. Modern molecular biology has therefore developed methods to assess the vast amount of non-cultivable biodiversity. These technologies allow to screen or sequence the so-called metagenome (coined by Handelsman et al. (1998)) of various habitats. A metagenome is the collective genomic information of all microorganisms (Bacteria, Fungi, Algae, Protists and Archaea) indigenous in a given habitat at a given (sampling) time point. There are habitats with a wide genomic diversity like uncultivated forest soils or pasture land, which contain the genomic equivalent of 6000 to 8000 genomes of E. coli per cm^3 of soil. The diversity in ecological niches with a high selective pressure in contrast, is generally much lower. E.g. in a salt-crystallizing pond only 7 *E. coli* genomic equivalents were found per cm^3 of soil (Torsvik et al. (2002)). The genetic information of a given metagenome can be recovered by directly isolating DNA from environmental samples without the need of cultivation. The DNA can then be deposited in gene libraries that are subsequently screened for desired enzymatic activities. A compilation of enzymatic activities recovered by this approach can be found in Lorenz and Eck (2005).

A metagenome based approach to enzyme discovery is certainly not limited by the immense enzyme biodiversity. It is therefore important to use the right discovery strategy for a given application. A few approaches (e.g. Gabor et al. (2012)) will be presented and their advantages will be highlighted in view of possible applicability for enzyme discovery for the food industry.

Born in 1957, Dr. Wolfgang Aehle obtained his Ph.D. in organic chemistry from Braunschweig Technical University in 1986. From 1987, at a PostDoc position at the German Research Centre for Biotechnology (GBF) in Braunschweig, Dr Aehle specialised in the field of protein structure, computer assisted protein design and protein engineering within an industrial collaboration with Solvay enzymes (Brussels, BE). In 1991 Dr Aehle accepted a position as protein engineer in the R&D Department of Gist-Brocades (now part of DSM) in Delft, The Netherlands. When the industrial enzyme division of Gist-Brocades was *sold to the American biotech pioneer Genencor International in 1995, Dr Aehle became employee of the company's R&D department in Leiden (NL). In 2008, Dr Aehle joined BRAIN AG, where he is responsible for the corporate development in the field of performance proteins and enzymes. During his industrial career, Dr. Aehle served as senior scientist, department head and project leader in the development of industrial enzymes. At BRAIN Dr Aehle became responsible for the acquisition of new collaborations and execution of projects in the enzyme field and performed the technical due diligence during one of BRAIN's acquisitions.*

9. Chemicals

9.1. **Peter Punt,** Zeist (The Netherlands)
Metabolic pathway engineering for organic acid production in Aspergillus

Among the compounds listed as top building blocks chemicals in particular organic acids have gained industrial interest for biobased production. Many of these organic acids, traditionally also being food ingredients, are directly derived from the central metabolic pathway in every living cell, the so-called tricarboxylic acid (TCA) cycle. The volume-wise largest almost exclusively biotechnologically produced compound, besides ethanol, is citric acid, which is a precursor for most of the other organic acids. As the yields for citric acid in *Aspergillus niger* are very close to the theoretical yield, the citric acid pathway is a promising backbone for organic acid production using genetic engineering in this established and FDA-approved fungal host. Based on these considerations we embarked on a research program for producing itaconic acid using *A. niger*. For *A. niger* this acid, which is produced in very few human- or plant-pathogenic fungi, is a non-native product.

At the start of our research, we elucidated the genetic basis of the biochemical pathway for itaconic acid, which is derived from the TCA cycle, using cis-aconitate as its precursor. Using a transcriptomics approach, we did not only identify the biochemical pathway gene but also mitochondrial and plasma membrane transporters, confirming that the pathway in the native host *A. terreus* is compartmentalized. The three identified genes, forming a small gene cluster, were quite dissimilar to any *A. niger* gene. Overexpression showed that all three were relevant for itaconic acid overproduction in *A. niger* (Li et al., 2011 & 2013). Molecular genetic and process technological research performed on the newly developed *A. niger* itaconic acid-producing strains resulted in the identification of several leads for further improvement of the itaconic acid titres and the reduction of by-product formation. Array analysis in *A. terreus* and RNAseq transcription analysis in *A. niger* strains expressing the itaconic acid biochemical pathway, revealed the potential relevance of the pentose phosphate pathway and a hitherto unidentified gene cluster related to organic acid production. Overexpression of a gene from this latter gene cluster allowed further increase in itaconic acid titers and reduction of citric acid coproduction.

***Peter J. Punt,** PhD is professor in Industrial Biotechnology at Leiden University and has a core expertise in Molecular Microbiology and Fungal Biotechnology. Peter's passion is to look for what fungi can accomplish in their unique lifestyle. His curiosity focuses on the width and breath of what these organisms provide as industrially applicable opportunities. Within Dutch DNA Biotech Peter is Chief Technology Officer.*

Memos

Memos

Wednesday, April 13th 2016

7. Raw Material

08^{30} 7.1. **Reinhard Pätz,** Köthen (Germany)
Potential for utilization of starch by-products

09^{00} 7.2. **Peter Punt,** Zeist (The Netherlands)
Fungal Cell Factories for Protein Production

09^{30} Coffee Break

8. Enzymes

10^{00} 8.1. **Arjen van Tuijl,** Leiden (The Netherlands)
Next Generation Glucoamylase products for SSF Ethanol Production

10^{30} 8.2. **Patrick E. Williams,** Franklinton (USA)
Improving plant profitability through the application of new enzyme technologies

11^{00} 8.3. **Wolfgang Aehle,** Zwingenberg (Germany)
Metagenome Approach for New Enzymes

9. Chemicals

11^{30} 9.1. **Peter Punt,** Zeist (The Netherlands)
Metabolic pathway engineering for organic acid production in Aspergillus

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