

Review and Summaries 2010

6. European Bioethanol Technology Meeting

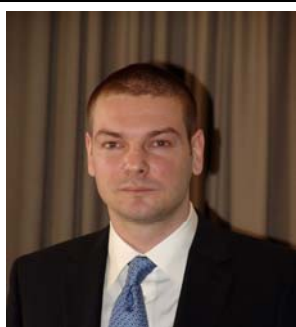
On April 20 - 21 the Association of Cereal Research organised its **6. European Bioethanol Technology Meeting** in Detmold. 163 participants of Austria, Belgium, Denmark, France, Great Britain, Hungary, Sweden, Switzerland, The Netherlands, USA and Germany visited this Meeting. The visit of the Bioethanol Meeting was highly affected due to the Island volcano because air traffic in Europe was impossible.

1. Raw Materials

1.1. **Ferdinand Pohl, Frank Hartmann, Carsten Reinbrecht and Thomas Senn**, Stuttgart (Germany)

Potential of Various Wheat, Rye and Triticale Cultivars for Fuel Ethanol Production

The objective of this study was to explore the performance of wheat (*Triticum aestivum*), rye (*Secale cereale*) and triticale (*x Triticosecale*) as a feedstock for fuel ethanol production and to compare the prediction accuracy between two near infrared (NIRS) spectrometer systems for determining fermentable sugar profile and also ethanol yield. The total sample set contained 724 samples of 10 types of wheat, 24 types of triticale and 6 types of rye, which were grown on 10 locations in Germany from 2006 to 2008. Correlations were established between ethanol yield and eleven grain components and properties: moisture, ash, protein, oil, fibre, starch, free sugars, fermentable sugar profile, falling number, bulk density and thousand-kernel weight. These correlations form a valuable basis for selection of appropriate cultivars on different habitats with respect to ethanol production. Based on the results obtained the costing of winter cereal production was made up.



Ferdinand Pohl
(Universität Hohenheim)

For NIRS analysis samples were scanned as intact or ground seeds and their reference values regressed against different spectral transformations by partial least squares regression (PLS1). Ground seeds were scanned on a dispersive NIR spectrometer (1100 to 2498 nm at 2 nm resolution). Intact seeds were scanned on a dioda array NIR spectrometer (960 to 1690 nm at 2 nm resolution). Principal Component Analysis revealed that samples could be classified by year, location and species. Partial Least Squares regression indicated that there was a little advantage in accuracy when using ground seeds. It is shown that near infrared spectroscopy is an appropriate and useful tool for analysis of biomass both in cereal breeding programs and for the evaluation of feedstock in ethanol plants.

1.2. **Eduard Taufratzhofer**, Tulln (Austria)

Selecting the Right Raw Material for Bioethanol Production

The appropriate selection of raw material is crucial for the economical operation of a bioethanol plant. Apart from the price of the raw material, the alcohol yield and the required fermentation time for a full conversion must be taken into consideration. In a joint research project, Zuckerforschung Tulln GmbH and Saatzucht Donau GmbH should find suitable types of wheat for the production of bioethanol. The previous methods used to determine the alcohol yield did not take into consideration fermentation speed and a delay time of the fermentation. In order to be able to test a large number of samples, a fermentation test was developed which is able to serve as a preliminary screening for the assessment of the different types. Afterwards, the selected types are compared with one another in laboratory fermentation with a measuring unit of 20 litres.



Eduard Taufratzhofer
(Agrana Stärke GmbH)

This test system is also extremely suitable for testing various qualities of raw materials, blends or other additives to the substrate as to their influence on the alcohol yield and fermentation performance. The fermentability of various yeast strains can also be tested. It is still in discussion whether sprouted grain is suitable for bioethanol production from an economical point of view. The crucial factor is the germination phase. It can be shown that determination of the starch content only is not enough to estimate correctly the actual alcohol yield in all cases.

2. Eco Balance

2.1. **Albrecht Schaper**, Klein Wanzleben (Germany)

Production of a Sustainable Biofuel-Certificate of Sustainability from the View of a European Bioethanol Producer

In spring 2008 the European leaders agreed on a roadmap for the promotion of renewable energies and the reduction for greenhouse-gas emissions (GHG). The target was to reach a share of 20% renewable energy on the total energy consumption until 2020 as well as a 20% GHG reduction and a share of 10% renewable energy in the transport sector. As a result in June 2009 the European Commission published several new directives, which translates the roadmap into legislation. Two Directives includes the guidelines for the implementation of the 10% renewable energy target in the transport sector, one is the renewable energy directive (RED), the other the fuel quality directive (FQD). To guarantee that the needed biofuels have a better ecological balance than the actually used fossil fuels, it was also agreed, to promote only such biofuels for the transport sector, which are produced in a sustainable way. Both directives, RED and FQD, include now a set of equal sustainability criteria, which have to be transformed into national law in all EU Member States until the end of 2010. The set of criteria includes protection of areas with high biological diversity, high carbon stocks, peatland, etc. The sustainability must be proven on the complete agriculture production chain and should deliver sustainable crops for the biofuel production. Also the greenhouse gas emissions from farming, the conversion into biofuels and all transports until the biofuel reached the end user are monitored and must be below a fixed threshold. A standard fossil fuel mix was set as a reference for the threshold.



Dr. Albrecht Schaper
(fuel 21 GmbH & Co. KG)

From the very beginning the German biofuel legislations were demanding the implementation of sustainability criteria, but due to the European regulation, the implementation was delayed. In autumn last year, the German government translated as first national EU Member State the Sustainability criteria from the RED and FQD into German law and from July 1st it must be applied for biofuels sold on the German market. As Germany is the first mover in Europe in the transformation of the regulations of RED and FQD into national law, not everything is fixed yet and the system will start with some black boxes. As the timing between the publishing of the German ordinance and the start of the system is very short market participants are not sure, if everything can be put into force until July 1st. To fit with the criteria certification schemes must be installed and certification bodies must be trained with the systems, before industry can be audited and certified. As the complete value chain from the farm, via traders and biofuel producers must be audited, it is a challenge for biofuel producers to find sufficient certified material from summer onwards. As the crop of 2009 is excluded from the system, the market participants can gain some time by using “old” crop as long as possible. But from mid of July the first new crop will enter the warehouses and the certification system must be ready to work.

2.2. **Thomas Senn**, Stuttgart (Germany)

The Ecobalance of Regional Coproduction of Fuel Ethanol and Biogas Considering the EU-RED

As shown before, ethanol production can be carried out very effectively in regional plants regarding energy balances and sustainability. The high energy efficiency is obtained by the combination of ethanol production with the production of biogas. Sustainability is achieved by crop rotation growing triticale, wheat or rye and maize. The seeds then are used in the ethanol plant, straw and maize silage are used in the biogas fermentation. Based on the data from our experiments at the Hohenheim distillery, a first scenario was calculated, where the crops are grown on 1000 ha level. Two thirds hereby are used for Triticale production, one third is used for the production of energy maize. LCA-calculation is carried out with the software tool GaBi 4. In this scenario, ethanol is produced only from triticale seed using a dry-milling process. Straw and maize silage is used in biogas fermentation.



Dr. Thomas Senn
(Universität Hohenheim)

This kind of ethanol production from grain then leads to an energy balance, showing an output:input-relation of about 5:1. Regarding the CO₂ emissions and the Greenhouse Warming Potential the regional ethanol production results in emissions less than 2,0 t CO₂-equ. / ha*a. So it can be shown, that the production of ethanol in combination with the biogas production on regional scale is one of the most effective bio-energy pathways, resulting in less than 20 kg of CO₂ emissions per GJ. Compared to the use of gasoline or diesel, this is a reduction of CO₂ emissions of roundabout 75% or about 10 t / ha*a. In summary we could demonstrate clearly the great potential of regional ethanol and energy production from arable land. And compared with the large scale ethanol production there are clear benefits in energy gain per ha as well as regarding the CO₂ reduction potential, what is very important regarding the EU-RED sustainability initiative.

2.3. **Christof Wetter** and **Elmar Brüggig**, Steinfurt (Germany) Greenhouse Gas Emission-free Production of Bioethanol

Conventional plants for the bioethanol production use fossil energy sources like fuel oil or brown coal. The plant examined in this work for the steam production at the agricultural farm Glitz-Ehringhausen produces the steam required for the farms's own distillery from the combustion heat flow of the combined heat and power units pursued with biogas. By using this innovative technology one can renounce on fossil energy sources as much as possible and the greenhouse gas decrease potential of the so produced bioethanol improves considerably. The excess waste heat of the combined heat and power units is used less or only insufficiently in many biogas plants. Often the thermal energy of the combustion heat flux is released via the chimney to the environment.

The production of thermal energy from biogas plants will be increased by using this energy in a steam generator and new potentials are created for the use. Thereby the economic efficiency of the plant improves and fossil energy sources can be substituted. Other advantages of the concept are the small-scale closing of material and energy circulations and a high regional added value. This will be achieved due to the close linking of the farm with the distillery and the biogas plant. The study supported by the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) will analyse the energy use in form of steam, the economy of the concept und the aspect of the sustainable production in view of the avoidance of climate-damaging greenhouse gas emissions. In addition, optimisation potentials are determined and tested for their technical and economic possibilities. The aim of the study is to optimize the new concept of process-engineering and to evaluate it economically and ecologically, so that it can be applied to similarly structured farms.



Prof. Dr. **Christof Wetter**
(Fachhochschule Münster)

2.4. **Ralf Thomann**, **Christa Luft** and **Rosemarie Schneeweiß**, Nuthetal, **Stephan Icking** and **Henrik Hiob**, Reichenbach (Germany) Economical and Ecological Improvements in the Production of Ethanol from Grain using Roller Mills

Ethanol made from grain has been and will continue to be an important raw material for chemical engineering, pharmaceuticals, cosmetics, food and fuel. The carbon footprint of the ethanol is

important. It is drastically influenced by the consumption of energy needed for the distillation process. The use of up to 20-30 L of fuel oil is the accepted standard for the distillation of 100 L of ethanol (86%). The use of hammer mills is the standard procedure for the production of bioethanol in large distilleries in Germany. The advantages of these mills are: low investment costs, low requirements for the cleanliness of the grain and high performance. Hammer mills have a much higher energy input than roller mills because they destroy the stable fiber structure of the bran to a larger extent. However, this is not necessary any more because modern fermentation processes and enzymes are now able to convert unreleased starch particles in the roller mill flours into glucose and ethanol. After hammer milling the remaining DDGS has less fibrous structure and can be separated from the thin stillage only insufficiently even with high performance decanters. The DDGS of the coarse roller milled flours, however, can be excellently separated by simple screw pressing. The analysis of the press cakes (DDGS) made from the roller-milled grain show a dry matter content of 29,3 % for rye, 31,2% for wheat and 30,7% for triticale. After drying the DDGS can be processed into pellets.



Dr. Ralf Thomann
(Institut für Getreide-
verarbeitung GmbH)

The investigations took place within the scope of a research project, sponsored by the FNR, Gülzow. A pilot scale equipment was used under practical conditions at the distillery in Seyda near Wittenberg. The analytical data of the pellets from rye, wheat and triticale are presented. First trials on thermal-physical properties of the pellets have been concluded. The final goal of the project is to use the pellets for in-company thermal processes needed for the generation of processing steam for example. The pellets can also be used for heating purposes at other users. In 2010, the project will focus on a further adjustment of the milling and fermentation processes and the optimal thermal utilization of the pellets.

3. Technology

3.1. **Herbert Pertl und Dirk-Michael Fleck**, Uzwil (Switzerland) Efficient Grinding Systems for the Ethanol Industry

Starch based agricultural products, mainly grains are still the main sources for the worldwide Ethanol industry. But grain is also a valuable raw material used for our food and feed consumption. It is therefore an obligation of the Ethanol industry to process these valuable raw materials in a most sustainable way. This goal can be reached, e.g. by producing value-added co-products from the same grain and/or processing the grain in a most efficient way. The presentation “Efficient Grinding Systems for the Ethanol Industry” is showing solutions for today’s and tomorrow’s grain based ethanol market. Milling of the grain is the first major step in producing grain based ethanol. And as wisdom of the miller says: “If doing something wrong at the front of a process, it is nearly impossible to correct this further downstream”, or in other words: an optimal milling system, which is adoptable to the various grain types and qualities, is essential for a highly efficient ethanol production.



Herbert Pertl
(Bühler GmbH)

With the evolutionary step of implementing the first roller mill base grinding section into large scale ethanol plants, the Buhler group in Switzerland could gain experience and results on that “new” technology. Compared with the traditional single hammer mill grinding, the roller mills can produce a more precise flour granulation. This even at a lower energy consumption of more than 30%! The results showing also that availability of the grinding section could be increased significantly: whereas hammer mills need several shut-downs per year for changing worn out screens and beaters, a roller mill equipped with especially hardened rolls can operate constantly throughout an entire year without any maintenance stop. The presentation will provide a view insight into the latest results from the industrial field.

How can a state-of-the art milling system increase the overall efficiency of an Ethanol plant? – This question will be answered in the second section: The Selective Milling System, which incorporates

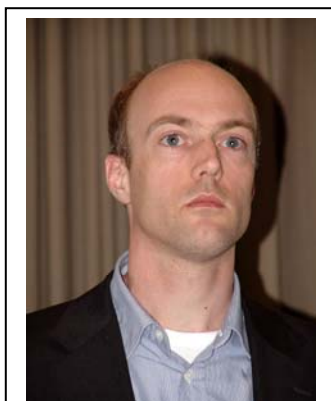
a plansifter, enables to extract fibrous bran fractions. Separating these non-fermentable products before the liquefaction process saves enormous energy in the alcohol but mainly in the DDGS drying process. The separated bran can then be either sold directly to feed producers or it can be fed back to the stillage stream between the decanter and the dryer, which reduces the drying energy up to 50%. Another attractive solution is using the bran as a CO₂ reductive fuel on a biomass boiler and producing the steam needed for operating the plant. One step into the future is to integrate the so called "second generation" process into a traditional, first generation, plant. Such a "Hybrid Plant" can convert both into Ethanol: the starchy fraction of the grain and the fibrous, cellulosic material like bran. The Selective Milling System is one brick which enables this step into future even applicable for existing, first generation, plants.

The third part of the presentation will show further interesting alternatives based on an innovative milling system: The corn fractionation allows to gain 6-9% of germs as a co-product. The germs are enriched in lipids and can be processed further into vegetable oil or Biodiesel. Also the separation of gluten from protein rich grains like wheat is a milling based solution which can increase the overall profitability of an Ethanol plant and minimizing its dependency on fluctuating raw material prices.

3.2. **Wouter Huijgen**, Petten (The Netherlands)

The Organosolv Process for Pretreatment and Fractionation of Lignocellulosic Biomass for Fermentation and Lignin Valorization

The development of cost-effective lignocellulose biorefineries requires optimum valorisation of all major structural biomass components: cellulose, hemicelluloses, and lignin. Most pretreatment technologies aim primarily to increase the enzymatic digestibility of the cellulose fraction for the fermentative production of second generation biofuels like bioethanol.



Wouter Huijgen
(ECN Biomass)

In these processes a lignin-rich residue is typically produced containing also minerals, non-converted sugars, and process chemicals. This type of residue is mostly only suited for heat and power generation. By separating lignin prior to conversion of the cellulose fraction, a high quality lignin may be produced with suitable characteristics for higher-value applications such as the production of phenolics for resins. Organosolv pretreatment is capable of effective separation of lignin from lignocellulose while largely preserving the chemical structure of lignin. ECN is developing an organosolv fractionation technology with the aim to separate all lignocellulose constituents in one process step with sufficient quality for further conversion into fuels and chemicals.

The first part of the presentation will give a general introduction in the organosolv process as a pretreatment method for the production of second generation bioethanol. The second part of the presentation will discuss highlights of the ECN organosolv work to date, including recent developments and results. An extensive parametric study has been performed including different types of lignocellulosic biomass, organic solvents, and catalysts. Organosolv was found to lead to good fractionation and pretreatment of lignocellulosic biomass, particularly of straws. A strongly improved accessibility of the cellulose fraction for enzymes was achieved (e.g., up to 92% enzymatic glucose yield after organosolv pretreatment of barley straw). In addition, organosolv effectively delignified lignocellulose and lignin was successfully isolated from the organosolv liquor. Quality assessment of the produced lignins showed interesting characteristics for follow-up chemistry such as high purity and relatively low mean molecular mass and low polydispersity.

3.3. **Johan van Groenestijn**, Zeist (The Netherlands)

A New Method for Dilute Acid Pretreatment and Fermentation of Wheat Straw Under Extremely High Dry Matter Concentrations

The success of yeast-based second generation bioethanol production depends on our ability to convert lignocellulosic biomass into fermentable sugars cost-effectively. The policy of governments and companies is to shift from starch and sugar as feedstocks to residue biomass such as wood, straw and bagasse, due to considerations on the environment, costs, availability and food market interference. Worldwide at least 10 different processes are under development for the pretreatment of lignocellulose (breaking the bonds between lignin and polysaccharides) and subsequent

hydrolysis. TNO is working on thermal/mild acid, dilute acid, concentrated acid and alkaline pretreatment. Two challenges are: how to prevent accumulation of pretreatment by-products that inhibit fermentation processes (e.g. furfural) and how to carry out the processes at high dry matter concentrations (which is important for the economy of fermentation processes). The approach is to heat acidified lignocellulosic biomass in a relatively dry state and at the same time evaporate more water and the most important inhibitors. The novel method is based on passing super heated steam through heaps. This makes the biomass highly accessible to hydrolytic enzymes. The pretreated material can be added to fermenters, in which enzymes and yeasts liquefy the biomass and convert the polysaccharides into ethanol. It was proven that these types of fermentation can be loaded with extremely high concentrations of biomass.



Johan van Groenestijn
(TNO Quality of Life)

Dilute acid pretreatment of wheat straw could be carried at 30-60 % w/w dry matter by passing superheated steam through heaps of acid impregnated straw (2% w/w sulfuric acid). This method allowed an efficient heat transfer and a few minutes heating at only 165°C was sufficient to break the polysaccharide-lignin bonds. After enzymatic hydrolysis more than 95% of glucose and xylose could be recovered as monomers. 90% of the xylose already was converted to monomeric form during pretreatment. Alternatively, if only 0.4% sulfuric acid was used, the same results can be reached by heating 15 min at 160°C. The pretreated wheat straw did hardly contain any furfural, HMF, acetic acid and levulinic acid, the four most important fermentation inhibitors, as a result of evaporation of these compounds. This phenomenon can be used to develop a combined pretreatment and detoxification process. Energy balances have been made for a full scale process. The energy required to evaporate water from the straw and produce excess steam can be reused in ethanol distillation. Fed batch simultaneous saccharification and fermentation (enzymes and micro-organism in one reactor) was successfully carried out to convert pretreated wheat straw of high dry matter content into ethanol using *Saccharomyces cerevisiae*. During the fermentation 270 g wheat straw dry weight was added per L broth. It can be concluded that passing superheated steam through heaps of biomass yields a fermentable substrate and forms the basis for high dry matter concentrations in fermenters. The ability to feed fermenters with streams of pretreated lignocellulose with a high dry matter concentration is important to reach high product concentrations (e.g. 14% ethanol v/v) and an economical down stream processing.

3.4. **Christian Abel**, Ettlingen (Germany)

Best Generation Biofuels: Energy Integrated Bio-refinery Concepts & Streamlined Project Execution to Reduce Project & Market Risks

Building a fuel ethanol plant requires proper planning and an experienced engineering partner. Optimizing the feasibility of your plant could be not good enough in today's business. Depending on the sustainability criteria or regional market conditions, an optimal balance between co-product revenues and GHG-savings has to be obtained. CHP, biogas or stillage incineration could become valuable process alternatives. However, the most important risk mitigation is to optimize your start up period and your plant operation. We will show the different process options available today to optimize energy and co-product handling. Furthermore for the first time a total process plant simulation has been realized for an ethanol plant. This simulator center allows realistic off-line testing and training of operation personal.



Christian Abel (GEA Wiegand GmbH)

3.5. **Rene Strandgaard**, Gentofte (Denmark)

Production of 2. Generation Bioethanol and Other Products – Technology and Development at a Demonstration Facility in Denmark

In 2004, Inbicon was formed as a 100% owned subsidiary of DONG Energy and today Inbicon is part of DONG Energy's strategy to convert from fossil fuels into renewable energy. Historically, it began in the mid 90s when Danish energy companies were committed by law to co-fire biomass (mainly wheat straw) at coal-fired power plants. This resulted in challenging tasks with respect to straw logistic and handling and high temperature corrosion in boilers. R&D work was established to

solve these challenges and one of the tests was a process for pretreatment in which salt and components are washed out of the biomass. Further process development with respect to developing and producing second generation bioethanol suitable for co-firing was tested by adding enzymes to the pretreated biomass.



Rene Strandgaard
(Dong Energy)

In 2003, Inbicon built its first pilot plant with a capacity of 100kg per hour. The purpose was mainly to further develop and improve the pretreatment process (washing and cooking). In 2005, the pilot plant was expanded to ten times its original capacity, capable of processing 1 t of straw per hour. The purpose was not only to further improve the pretreatment process but also to make sure that the process was working in a continuous operation mode with respect to both pretreatment process and downstream processes like liquefaction, fermentation and distillation. In 2008, Inbicon started the construction of a demonstration plant in Kalundborg (DK) with a capacity of 4 t/h of wheat straw. In December 2009, it was officially inaugurated in connection with the COP15 conference in Copenhagen, and during the second half of 2010 it is expected to reach full capacity. As a result

hereof, Inbicon now has:

- 'Cheap' and quick possibility to test feedstock, 100 kg/h.
- Up-scaling of a full process test under continuous operation, 1 t/ h.
- Full scale test/production at demonstration plant, 4 t/h.

The Inbicon process has since been optimized for wheat straw, proven on corn stover, miscanthus, residues from palm oil production and other soft cellulosic biomass. In addition to producing ethanol the Inbicon Biomass Refinery also produces high quality lignin pellets suitably for co-firing at power plants and C5 molasses suitable for cattle feed or conversion into other products. In 2010, Inbicon signed its first license agreement granting Mitsui Engineering and Shipbuilding from Japan the right to use our technology. A spin-off from this technology is the use of thermal pretreatment and enzymatic hydrolysis on unsorted household waste and thus obtain higher energy efficiency and improved recycling of metal, glass and crop nutrition.

4. Yeasts

4.1. **Michael Spandern** und **Chris Richards**, Milwaukee (USA) The Hidden Costs of Propagating Yeasts

Due to the convenience and the lack of availability of fresh cream, most ethanol plants were (are) designed with a yeast propagation unit connected to the mash stream. This process itself creates:



Michael Spandern
Agribusiness Consulting

- Process requirements (dilution, treatments etc.),
- Additional cleaning,
- Additional workload,
- Additional contamination risk,
- Complexity of the system.

Historically there has been no other alternative to propagation. One of the advantages of "preparing" or "conditioning" the yeast was to eliminate the lag phase of fermentation. This is beneficial in that yeast made are better suited and prepared in the exponential phase thus outweighing the issues above. Cream yeast could be an alternative,

however previous bakers cream yeasts as an option is not very viable due to the very short shelf life. However, with the emergence of a new stabilized liquid yeast this issue has been eliminated. During a cost benefit analysis of a direct pitch trial, it became clear that there are numerous direct costs due to yeast propagation that are not normally taken into consideration. Generally these factors are usually hidden in or are accounted for in other budgets besides "fermentation" and aren't really thought of having to deal directly with fermentation and therefore aren't really accounted.

In addition to the direct costs, there are a number of indirect costs associated with the actions or operating procedures of the propagation equipment that affect the profitability of the plant by reducing either yield or throughput. If the propagation process is considered as a cost center and all the actions and processes accounted for, then it becomes clear how much these direct costs are involved. It is possible to calculate an accurate cost benefit analysis of the propagation process and compare this to the additional yeast cost associated with directly pitching the yeast into the fermentor. This presentation takes some practical examples and calculates the theoretical costs based on

4.2. Yves Gosselin, Marcq-en Baroeul (France)
 Influence of Dry Yeast Shaping in Fuel Ethanol Production

Fermentation time reduction is a key point in the ethanol production process today. The faster the fermentation starts the less contamination risks are faced with during the process, which ensures maximum efficiency in the fuel ethanol plant. The presentation will demonstrate how the yeast drying process influences fermentation. *Saccharomyces cerevisiae* yeast is a unicellular microorganism the size of which is 20 to 50 μm . The yeast cell wall is made of glucanes, mananes, chitine and is not quite extensible. Its role is close to the one of an external skeleton. Beneath the cell wall, is the cytoplasmic membrane. The latter is made of a phospholipid bilayer sheet and protein complexes. The cytoplasmic membrane contains cytosol, cellular organites (Golgi apparatus, les mitochondria, vacuole), nucleus. The latter exchange their reactives and products with the cytosol thanks to their membranes which are also made of a phospholipid bilayer sheet and protein complexes. The functionality of the membranes and their associated proteins is often obtained thanks to their ternary conformation. Water plays an essential role in the ternary conformation of macromolecules. Since it is the latter which will be eliminated during the drying process, the destruction risk is very important. Therefore yeast production and drying objectives are:

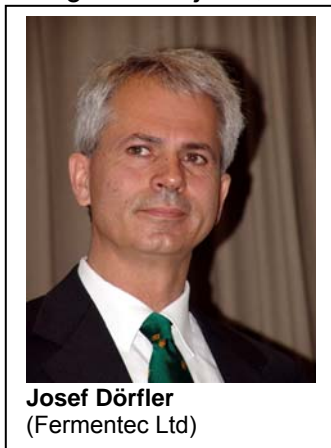


- preparing cell contents to be dried and functionality after rehydration,
- conditioning the yeast organism for the application in which it will need to perform. In the case of biofuel rapid bioconversion of sugars to ethanol.

Particular attention needs to be taken in the feed batch nutrition management of the yeast biomass produced in the fermenter...".Our presentation will show you the recent results in the Lesaffre Biotech Center obtained during yeast shaping processes of fuel ethanol yeasts.

4.3. Josef Dörfler and Henrique Amorim, Piracicaba (Brazil)
 Yeast and Bacteria: Strategies of Survival and Competition in Alcoholic Fermentation

The Brazilian processes of fuel ethanol production are characterized by fermentations with musts of sugar cane juice, molasses or a mix of both, high contents of yeast cells (10-15% w/v), short



fermentations (6-12 hours), alcohol concentration between 8-12% (v/v), centrifugation of the beer, recycling of the yeast cells and an acid treatment of the concentrated yeast to kill bacteria before to start a new fermentative cycle. Concerning the ethanol production, 420 Brazilian distilleries produced 27 billion L in 2009. 80% of these distilleries are fed-batch processes and just 20% are continuous fermentation processes. Furthermore, Brazilian distilleries have used selected yeast strains as PE2 and CAT1 in their fermentation processes. These strains have shown the highest rate of dominance and persistence in industrial fermentations when they where were introduced in comparison with Baker's yeast and laboratory strains. The Backer's yeast and laboratory strains don't tolerate the stressful conditions of industrial fermentations being quickly replaced by selected strains or wild yeasts. In 2009, 154 distilleries started the

fermentation process with these two selected yeast strains, PE2 and CAT1.

Despite of the fast fermentations, bacteria and yeast cells compete by the same substrate: easily fermentable sugars such as glucose and fructose. Sucrose is hydrolyzed by the yeast invertase yielding glucose and fructose readily available to yeast and bacteria. Moreover, selected yeast strains compete with wild yeast (*Saccharomyces* and non-*Saccharomyces* species). These microorganisms have developed different strategies of survival and competition in alcoholic fermentation processes. Some yeast strains are foaming while other are flocculating and leave unfermented sugar in the beer. Besides PE2 and CAT1, new yeast strains have been selected to ethanol tolerance as well as new proprieties and attributes have been discovered for selected yeast strains such as the inhibition of bacterial metabolism (mannitol), reduction of yeast-bacteria flocculation, aluminium tolerance, production of vitamins and others.

Bacteria and wild yeasts cause serious problems to industrial fermentations with cell recycle and its control is essential to obtain high fermentative yields. When the strategies favour bacteria, fermentative yield drops. For each 1×10^7 bacteria/mL, the fermentation yield falls by 0.6%. Bacterial contaminations between 10^7 and 10^8 /mL are common in the beer but some distilleries have worked with contaminations below 10^6 bacteria/mL. Yeast and bacteria developed different strategies to survive and to compete in the industrial fermentation processes. To understand these strategies may open new doors to improve the production of fuel ethanol.

5. Enzymes

5.1. **Lau Andersen**, Bagsvaerd (Denmark)

Different Raw Materials in Fuel Ethanol Production: Viscosity Reduction, Starch Hydrolysis and Yields

Beside sugar cane (Brazil) different grains are used as raw materials for fuel-ethanol production.



Lau Andersen
(Novozymes AS)

Globally this is closely connected to climatic conditions as well as availability and costs. Corn is by far the most extensively used feedstock in the US, but wheat, barley, rye and triticale are also relevant sources in different geographic areas such like Europe, Canada, Australia and to certain extend China. Especially wheat, barley and rye require an additional viscosity reducing step, as far as they contain higher amounts of water soluble and insoluble beta-glucans and pentosans. These substances are present in the differing raw materials in differing ratios and have to be reduced by enzymatic degradation to guarantee low mash viscosities which are basic for economic fuel-ethanol production. The ethanol yields of different starch containing raw materials can be seen in direct correlation to the ingredients of the raw materials, especially the starch contents but are also strongly dependent on the process engineering and the

effectiveness of the enzymatic degradation. The presentation will include the process steps of fuel-ethanol production using different raw materials with special focus on enzymatic degradation, presenting data from laboratory trials and industrial scale applications with different enzymes, due to the viscosity reduction, the starch hydrolysis and their influence on the yield.

5.2. **Pauline Teunissen, Kees-Jan Guijt, Vivek Sharma and Gerhard Konieczny-Janda**, Leiden (The Netherlands),

An Improved Enzyme Product for Low pH Liquefaction of Dry Milled Grain

The manufacturing of ethanol from cereal crop by enzymatic conversion of starch to fermentable glucose and fermentation by yeast is now a well established process. The first phase of the process is the addition of alpha-amylases to the liquefaction of the milled grains. Alpha-amylases hydrolyses the α -1,4-glucosidic bonds to reduce the viscosity of gelatinized starch producing soluble starch and oligosaccharides. Conventional alpha-amylases perform best at specific process conditions; the recommended pH is normally between 5.6-5.8, the recommended temperature 83-85°C. Deviations from the recommended conditions can result in inactivation of the enzyme. Other factors that have an impact on alpha-amylase performance are the presence of enough free calcium and the absence of inhibitors like phytic acid. Phytic acid (inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate) present in wheat and corn complexes minerals like calcium and

zinc and inactivates enzymes used to convert starch to fermentable glucose. Phytic acid can be hydrolysed by phytase, releasing free phosphate and inositol which cannot longer complex with calcium. Endogenous phytase is present in small grains (like wheat, barley), but not in corn and sorghum.



Pauline Teunissen
(Genencor, a Danisco
division)

An improved enzyme system has been developed with enzymes produced from selected strains of *B. licheniformis* and *T. reesei*. The enzyme system is a combination of an alpha-amylase and a phytase. The new developed enzyme system combines phytic acid hydrolysis with an excellent performance at low pH and increased performance at high temperature. The improved enzyme system clearly shows improved starch hydrolysis for corn, as measured by DE development, at all pH levels tested. Also for wheat, although endogenous phytase is present, increased starch viscosity reduction is observed at low pH. In addition, significant alpha amylase survival is observed in low pH

conditions when using the improved enzyme system compared to the conventional alpha-amylase. Using this improved enzyme system pH adjustments for liquefaction can be eliminated or reduced prior to the saccharification/fermentation step. This will result in a lower cost for sulfuric acid and/or ammonia. An environmental benefit will be that less sulfur will be present in produced DDGS.

5.3. **Martine P. van Gool, Henk A. Schols and Harry Gruppen**, Wageningen (The Netherlands), **Ildiko Vansco and George Szakacs**, Budapest (Hungary) Screening for Novel Enzyme Activities to Degrade Complex Xylan

Xylan rich lignocellulosic biomass offers an attractive option for bioethanol production. In order to convert this xylan rich biomass to sugars, enzymatic hydrolysis is considered the most environmentally sustainable technology. Until now most commercial enzyme preparations are lacking one or more of the enzymes needed for the complete hydrolysis of the biomass. Objective of this study was to use fast screening methods to find novel and powerful fungal enzymes in soil samples collected world-wide by Budapest University. Crude fermentation supernatants were screened on their enzyme activity. Wheat arabinoxylan (WAX) and eucalyptus wood xylan hydrolysate were used as model substrates during this screening. WAX is composed of a linear backbone of β -linked D-xylopyranosyl units, which were either nonsubstituted, monosubstituted at O-3 or disubstituted at O-3 and O-2 with α -L-arabinofuranosyl units. Eucalyptus wood xylan hydrolysate contains both 4-O-methylglucuronic acid and/or acetyl substituents, which makes it difficult to be degraded.



Martine P. van Gool
(Wageningen University)

All digests were analyzed by High Performance Anion Exchange Chromatography (HPAEC) and the eucalyptus xylan hydrolysate samples also by Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (Maldi-TOF MS). The degradation patterns on HPAEC of WAX could be divided in 4 different groups and distinguish the presence of different arabinose hydrolyzing enzymes and xylanases working in synergy. All fermentation supernatants were also able to degrade eucalyptus xylan hydrolysate to a certain extend, indicating the presence of glucuronosidases, acetyl xylan esterases, endo-xylanases or β -xylosidases. Despite the high level of substitution the fermentation supernatants were quite successful to degrade the complex xylans. This study shows that a combination of HPAEC and Maldi-TOF MS is powerful in screening for novel/interesting enzyme activities.

Some pictures from the meeting

