Review and Summaries 2011

7. European Bioethanol Technology Meeting

On April 12 – 13 April the Association of Cereal Research organised its **7. European Bioethanol Technology Meeting** in Detmold. 195 participants of Austria, Belgium, Denmark, France, Great Britain, Hungary, Sweden, Switzerland, The Netherlands, USA and Germany visited this Meeting.

1. Raw Materials

1.1. **Jan Smits**, Delft (The Netherlands)
DSM's Integral Approach of Converting Agro-industrial Residues to Bioethanol


Jan Smits

During the past 200 years, our society and economy have become dependable on fossil hydrocarbons. The demand for fossil hydrocarbons however is higher than the production rate by mother earth. Therefore, hydrocarbons run out and become more and more expensive.

Alternatives for the fossil hydrocarbons oil, natural gas and coal as energy feedstock are available. Sun, wind and gravity are inexhaustible sources of energy that can replace fossil fuels to a large extend. For the production of materials, renewable agricultural products are alternative sources for fossil hydrocarbons.

The policy of DSM is to use in-house food and bio-technology know-how for the development of techniques that make it possible to produce materials from renewable agricultural sources. This policy fits in the company's mission to create brighter lives for people today and generations to come. To avoid interference with the food versus fuel discussion, the development of this technology focuses on the conversion of lingo-cellulosic agricultural residues and products. The cost effective production of bio-ethanol is the first step in this development.

Being a life sciences and material sciences company, DSM's competences fit very well in the value chain from feedstock to bio-ethanol and materials. To develop and enable the most optimal economical route, complementary technologies are required. Actively partnering with technology providers that excel in these complementary technologies along the whole value chain is therefore mandatory. This approach enables and requires integral technology development, but will ultimately deliver more value over the whole value chain than the sum of the values of each individual technological chain links.

Our thermo-stable cellulolytic enzymes are capable of long-lasting hydrolytic activities; almost without product inhibition. In this way, complete hydrolysis of the feedstock with moderate enzyme dosages is possible in less than 72 hours; allowing the fermentation to start with a maximum monomeric sugar load. This high sugar load will result in a rapid fermentation time of less than 2 days. In this way thermo-stable enzymes enable reduction of the overall process time to less than 5 days.

Thermo-stable cellulolytic enzymes act during the hydrolysis step but have a beneficial effect on pre-treatment as well. Due to the enzymes thermo stability, hydrolysis can start at higher temperatures, which require less cooling prior to hydrolysis and allows to start with hydrolysis earlier in time. This lowers the energy costs, reduces the process times, and will result in higher dry-matter contents in case the pre-treated feedstock is partly cooled by adding cold water.

After 72 hours of hydrolysis, enzyme activity of our thermo-stable cellulolytic enzymes remains present, allowing re-use of the enzyme activity after recycling. This has a significant effect on the enzyme dosage.

DSM's All-in-One yeast is capable of converting C6 and C5 sugars into ethanol. With this property, feedstock which is high in hemicellulose, like corn fiber and corn cobs, yield up to 50% more ethanol during fermentation. The All-in-One yeast ferments 5 most abundant sugars (glucose, galactose, manose, xylose and arabinose) from most commonly used feedstock in a single fermentation step. The use of this yeast does not only yield more ethanol, but makes the process less dependable on the sugar composition of the feedstock and thus suitable for a broader range of feedstocks.

Since the All-in-One yeast does not require oxygen or nutrient additions, operational performance of the whole process increases.

These examples show that the properties of the cellulolytic enzymes and the yeast are not only beneficial in hydrolysis and fermentation steps respectively, but also for other steps and the overall process performance. These examples support the approach of integral technology development as the best solution to enable the most optimal economical route in converting agro-industrial residues and products into bio-ethanol.

1.2. **André Koltermann**, Munich (Germany)

Biotechnological Production of Biofuels from Straw

André Koltermann

In a sustainable economy biofuels play an important part to reach sustainability in transport. World-wide bioethanol is the most common biofuel with a production volume of 85.8 billion liters in 2010. Cellulosic ethanol, a second generation biofuel made from agricultural residues, offers substantial reduction in CO_2 emissions and significantly increases the independence from fossil resources by domestic production.

Agricultural residues such as cereal straw or corn stover represent an ideal alternative renewable source for ethanol production. Their main component is lignocellulose which mainly consists of hemicellulose and cellulose, the world's most abundant biopolymer. Both are long chain sugar containing polymers that can be converted into sugar monomers by enzymatic treatment. The resulting cellulosic ethanol offers CO_2 savings of up to 95% with no additional land use or competition to food or feed production.

Agricultural residues hold a high potential – alone in the EU, every year more than 294 million tons of cereal straw are produced. Up to 60% of the straw can be taken from the field without endangering soil quality and humus balance. This amount would be sufficient to produce enough cellulosic ethanol to substitute more than 20% of EU gasoline demand by domestic production of cellulosic ethanol. Considering that engine and automobile technology will advance continuously underlines the huge potential of this new technology. And this can be achieved just by the utilization of an already existing renewable feedstock, without needing any additional land or production capacity.

Munich-based Süd-Chemie AG has developed the sunliquid® process for the production of cellulosic ethanol from agricultural residues at competitive costs. After pretreatment a highly optimized enzyme mixture breaks down cellulose and hemicellulose into sugar monomers. The biocatalysts are feedstock specific and optimized for the utilized process conditions, thus resulting in high sugar yields in short reaction times. Enzyme costs used to be one of the major cost factors for cellulosic ethanol production. The sunliquid® process has an integrated enzyme production, through which costs can be reduced by several times. Further, Süd-Chemie developed specialized yeast yielding 50% more ethanol due to its ability to simultaneously convert C5 and C6 sugars into ethanol in a one pot reaction.

The process energy is produced from the remaining lignin fraction. A new and proprietary downstream processing developed by Süd-Chemie saves up to 50% energy during ethanol separation compared to conventional distillation. The whole process is energy neutral as no additional energy source is needed and results in ethanol with CO_2 emission savings of almost 95%.

The next step on the way to commercialization is the confirmation of scale up of the technology in a demonstration plant. This represents the important intermediate step to large scale production plants with highest efficiency. In Straubing, Bavaria, Süd-Chemie is currently constructing a demonstration plant with the capacity to produce approximately 1000 tons of cellulosic ethanol from 4500 tons of wheat straw per year. The plant is expected to start operation by the end of 2011.

To exhaust the total straw potential in the EU, about 800 cellulosic ethanol plants would be needed. This would create around 200,000 new green jobs and save more than 88 million tons of CO_2 per year. To unlock the whole potential for this innovative technology, a political framework giving security to investors is indispensible. Especially for the construction of first production plants – so called first-of-its-kind plants – incentives are needed. These types of plants have high additional costs due to a certain uncertainty in the scale up process. Only with a stable political framework in the EU and Germany, Europe's leading position in the field of industrial biotechnology can be ensured.

1.3. **Timo Broeker**, **Marco Steffens** and **Jan Schneider**, Lemgo (Germany)
Energetic Conversion of Residues from Food Processing with a Focus on Bioethanol


Timo Broeker

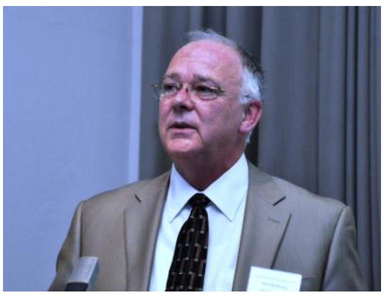
Sustainable energy production often faces the problem not to deliver positive balances after the production of raw material was counted in. Therefore the interest to convert residues from food processing into energy has become very popular lately. Besides biomethane production, a lot of residues are promising substrats for bioethanol production.

Different kinds of residues have been examined for the potential of bioethanol production, such as waste bread, bakery products, pudding, salty snacks and others. The problems to the standard process, which are caused by different ingredients, such as fat, were studied. The problem of retrograded starch and spoiled residues were examined. Ethanol yields of 43 ml / 100 g d.m. have been obtained using waste bread. Useful enzyme, yeast and supplements have been developed by our project partner, Erbslöh Geisenheim AG, so that a stable and reproducible process with Ethanol yields of 43 ml / 100 g d. m. was developed. Applying the α-Amylase on a highly viscos bread mash with 20 % d.m., the power demand of a stirrer was decreased by 91 % within 7 min. The Glucoamylase converted 90 – 96 % of starch into fermentable sugars. Overall Ethanol concentrations of 12 % vol. have been reached so far.

With the goal to make useful and sustainable balances, trails with bagasse for biomethane production have been made and show that energy balances could be improved by coupling processes.

2. Technology

2.3. **Bill McBride**, Brampton (Canada)
AutoHydrolysis: The Gateway to BioFuels, BioProducts, BioGas


Bill McBride

In bio-chemical pathways to conversion of biomass, fibre pretreatment is arguably the most important stage in the process. Its efficacy impacts capital and operating cost as well as the efficiency of all downstream processes.

Mascoma Canada is uniquely qualified in the field of fiber pretreatment for bio-refining applications given our 38 years of experience with numerous projects executed internationally. We have provided systems for use with variety of feedstock inputs. Although the desired process outcomes were also varied, most were related to

developing sugar streams from cellulose and hemi-cellulose.
AutoHydrolysis is an apt name for Mascoma Canada's steam explosion process. Unlike similar technologies it was developed specifically for bio-conversion outcomes. It is a single-stage, non-catalytic process that operates in vapour phase (high solids content) to maximize heat and mass transfer through the fibre. The resulting release of some of the acetyl groups provides an organic acid to begin hydrolyzing the hemi-cellulose.
The objective of effective pretreatment is to activate the cellulosic structure; in other words to expose the maximum number of sites for organisms (enzymes, bacteria, etc.) to break down the cellulose and hemi-cellulose to monomeric sugars.
In this presentation we will discuss the process benefits autohydrolysis contributes for biofuels production with reference to yield, rate, concentration, energy efficiency, hydraulic load and inhibition management.
Many bioproducts and advanced biofuels technologies are based on conversion of sugars. We will discuss briefly our process development for biomass-to-sugars based on autohydrolysis pretreatment and present information regarding capital and operating cost at small commercial scale.
This process development incorporated significant work on reducing visocosity with high solids content in downstream processes. The same approach is indicated for biogas production through anaerobic digestion whereby greater outputs can be achieved by introducing pretreated biomass into the reaction.

2.5. **Hartmut Brüschke**, Homburg (Germany), **Petra Lehnhoff**, Geisenheim (Germany**),** **Jan-Henryk Listewenik**, Köthen (Germany) and **Ingolf Voigt**, Hermsdorf (Germany)
Optimized Process to Produce Bioethanol from Starch Containing Raw Materials with Enzym Recyling and Membrane Separation Processes


Hartmut Brüschke

With the introduction of E10 gasoline the controversial discussion on the use of ethanol as fuel for combustion engines and the controversy of "table and tank" has started again. Although many of the arguments against biofuels lack objectivity and are full of prejudice, it is mandatory for a better acceptance of this fuel to prove that bioethanol is environmentally friendly and contains more energy than needed for its production.

Whereas in the production of ethanol from sugar cane at least the energy surplus and the positive $C0_2$ balance can easily be proven, this task is more difficult for the production of bioethanol from starch containing raw materials. With these raw materials there is no combined source for process energy as it is with bagasse in sugar cane processing plants, therefore external energy has to be supplied. In the past many of the older starch based ethanol plants often used more energy than they produced. With new developments the energy balance of ethanol plants can be improved even with external energy supply.

The main advantages in the recent technical development is found in the separation of all valuable ingredients from the raw material, in the purification of the substrate going into fermentation, and in the separation and purification of the product ethanol.

The combination of newly developed enzymes and the recovery of the enzyme by ultrafiltration membranes allows for a short saccharification time. The produced glucose is of high purity and can be fermented by a heat and ethanol tolerant yeast in a continuous mode. As a result short fermentation times are achieved and the formation of unwanted by-products is suppressed. By vacuum evaporation the produced ethanol is separated from the mash and preconcentrated. Most of the residual yeast is recycled in a concentrated form, a portion is freed from any residual alcohol, further concentrated, and dried and forms a high value by-product. The ethanol is concentrated in a single distillation column to concentrations fairly below the azeotrope and dehydrated by vapour permeation using high selective inorganic membranes. The thus possible reduction of the reflux ratio saves thermal energy for the distillation column to such an extent that the recovery of the heat of the produced dehydrated ethanol and that of the reflux by means of vapor compression allows for the operation of the distillation column without an additional heat input. The lutter water from the bottom of the distillation column is purified and used as process water.

3. By-Products

3.1. **Peter Williams**, Peterborough (Great Britain) and **Frank Taetz**, Oelde (Germany)
High Value Yeast Protein Recovery from Stillage

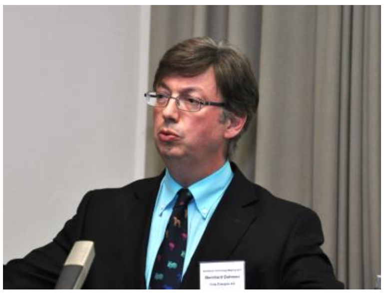
It is right in times of population growth and rising food cost to question whether it is appropriate to use crops which can be used for food as raw materials for renewable energy.


Peter Williams

This is exactly the case when wheat is used in the 1st generation bioethanol process and is fermented to produce ethanol. Wheat is an essential food crop; can we afford to use large quantities of wheat to produce ethanol? When wheat is fermented to produce ethanol it is often overlooked that once the starch of the grain has been fermented the remainder of the grain is used as feed for livestock. Approximately one third of the value of the grain goes into producing distillers dark grains and solubles which is an excellent livestock feed. Recent developments have demonstrated that it is also possible to recover the yeast generated in the fermentation process. Yeast is a valuable feedstock that can be used as a replacement for fishmeal. With further downstream processing it may be possible to further refine the recovery of wheat protein then to anaerobically digest the fibre to produce methane and to grow algae in the waste water stream. A combination of such processes into a bio refinery has the potential to dramatically increase the protein and energy recovered from a grain bio refinery to such an extent that the value of the co-products becomes greater than the value of the ethanol produced. Bio refining of the wheat produces a valuable source of feed protein as well as a valuable source of renewable energy. The uses of crops to provide both feed and renewable energy are not mutually exclusive. Furthermore in times of acute food scarcity the crop is immediately available to be diverted into use as food without any delay in cultivation. Adopting the bio refinery concept has great value for the future and it is only a short step to convert current bioethanol plants into bio refineries. In addition, adopting such a strategy will contribute greatly to the sustainability of 1st generation bioethanol production.

3.2. **Bernhard Dahmen**, Ochsenfurt (Germany)
Feed Quality of DDGS

Since the early days of industrial Ethanol production in Germany and Europe at the beginning of this century, it was clear to the feed industry, that they will get a very


Bernhard Dahmen

interesting protein source for feeding. It is one of the big benefits of Ethanol production that it not only lowers oil dependency; it also lowers GHG Emissions and the dependency on high quality protein sources. The main question was; what quality of DDGS will be produced?

Even before the beginning of significant ethanol production, the feed industry imported from time to time mainly corn distillers from the US, and in Great Britain distillers mainly from the Irish and Scottish whisk(e)y industry where highly appreciated by the feed industry. Today, there are about 16 factories in Europe with a production capacity for DDGS of about 3,5mmt. The main producers are France (1,1mmt), UK (800tmt), Spain (600tmt), Germany and the

Netherlands (each about 300tmt).The difference to the US, where most of the ethanol plants are producing on corn, is that in the EU, nearly every factory has its own technology and raw material concept. This makes it very difficult to compare value and use the qualities to their best application. It also reduces the possibility of substitution between different producers. On the other hand it may give customers a reliable value for money because some producers are using the chance to optimize the byproducts to meet the needs of the customers. From the nutritional point of view the important quality aspects are:

- Moisture 9-14%
- Protein 26-36%
- Fat 3,5-10%

Due to a nice taste (for Ruminants), DDGS are a favourable product especially in connection with high rapeseed meal shares. Beside this the amino acid composition and the value of undesired substances (for example Mycotoxins) have to be watched carefully.

In Europe, most of the DDGS are free of GMOs. The key aspects influencing the quality of the different types of DDGS are:

- Raw material
- Technology and strategy of milling (single/mixed)
- Drying technology
- Pelletizing technology

Beside this, the key questions for the quality of the products are:

- Philosophy of the producer
- Monitoring of raw materials and byproduct
- Quality systems in place
- Customer relationship management

As today there are already more than 3mmt sold to the European feed market, it is obvious, that DDGS form an important part of today's protein supply to the feed industry in Europe. Brand products like ProtiGrain® have set benchmarks for the quality and reliability of DDGS in Europe.

Today DDGS products are the number 3 of the European produced protein sources behind Rapeseed meal and Sunflowerseed meal. DDGSs are a benefit to the European feed market because they are substituting imported protein sources and they are a feed compound which is applicable for nearly all animal species. DDGS produced in Europe are a story of success as long as producers accept, understand and take on seriously their liability for feed quality.

4. Plant Management

4.1. **Oliver Teichert**, Norrköping (Sweden)
Odour Management in a Bioethanol Plant

While the use of renewable fuels in general is considered to be positive for the environment, there are elements in the production process that can release smelling components, an issue that can rapidly trigger the public opinion against bioethanol, at least in the direct surroundings of the plant.


Oliver Teichert

Already during commissioning and start up in 2001 Lantmännen Agroetanol AB (LAE) experienced how fast a public opinion can blow up to a media storm when exhaust from DDGS-driers were carried by the wind into a nearby residential area. When the County Administrative Board (CBA) prohibited the use the driers under certain weather conditions a Regenerative Thermal Oxidizer (RTO) was built after short consideration. But even though the RTO unit reduces VOC emissions with 98 % and a report by the

Örebro University Hospital identified components and declared detected concentrations in the drier-exhaust to be harmless it was hard to make peace with the neighbours and their demand for a zero odour plant.

In order to contain or combust any possible sources of odour the whole site was mapped and various measures were taken. In meetings with the neighbours and the CBA odour reduction strategies were discussed and a report system to collect all incoming odour complaints was established.

In 2003 a wider approach was taken and together with two other local companies an investigation was ordered to be performed by ÅF, a well-established technical consulting company. The purpose was to measure how often and to which degree odours from the participating companies were noticed by a trained panel of inhabitants in three different residential areas and relate the findings to wind conditions.

Strangely enough the number of odour complains declined after this rapport – only to rise again when LAE announced plans to expand production capacity.

At this moment the CBA had specific demands which had to be met to receive the environmental permit to start building Agro 2008. Even the neighbours came with suggestions how to reduce emissions of odour. In consideration with these demands and ideas the new production line was equipped with redundant RTO-units and a higher stack. Lately a system for continuous monitoring of VOC emission has been installed and currently calibrated in a 30 day testing period.

As long as the plant operates odour will remain to be a part of the daily life in the plant.
Odour management has to be maintained honestly and in an open way. It is useful to keep in mind – and accept the fact - that the human olfactory system is highly individual: one man's perception of odour can differ a lot from his neighbours.
Because of this it is important to be able to measure - not just to feel: numbers do not lie and enable us to prove our will to improvement and verify our actions. In any dialogue it is essential to establish a ground of mutual understanding– not just to talk.

In the end we have to be able not just to REspond or REact, but to see things coming and get time to avoid, reduce unwanted effects or to prepare other measures – last but not least our communication strategies.

4.2. **Michael Schüler**, Frankfurt am Main (Germany)
Operator Training System - Applications for Testing and Training

Michael Schüler

By using simulation tools parts of the control software can be tested already by the engineer during the configuration. Mistakes will not only be detected during putting the plant into operation. Furthermore these simulations can be used for the acceptance procedure (FAT) of the control system. Based on these simplified simulations Operator Training Systems (OTS) can be developed. Core part of an OTS is the process model that the behavior of the plant represents. Combined with the distributed control system (DCS) the plant operators can be instructed with a system that corresponds due to handling and behavior compared to the real plant. According to the requirements at any time different tasks and situations ca be trained. Both the proceeding during plant startup and shut down as well as during grade or load changes can be educated. Critical plant situations and process upsets like pump failures or the effect of heat exchanger fouling can be initiated by the instructor and be practiced over and over without any risk by the operators. Furthermore the handling of the automation equipment can be trained.

Based on an Operator Training System for the distillation part of a bio ethanol plant the possibilities of increasing the process understanding as well as the opportunity of cost reduction are highlighted. With the bio ethanol example the feasibility in production increase and quality improvement are demonstrated.

5. Enzymes

5.1. **Floor Kooy**, **Gerhard Konieczny-Janda** and **Pauline Teunissen**, Leiden (The Netherlands)
Benefits of Non-starch Polysaccharide Hydrolyzing Enzymes in the Fuel Ethanol Industry

Floor Kooy

Cereal grains, like wheat, barley and rye, contain high levels of non-starch polysaccharides (NSPs) like celluloses and hemicelluloses (pentosans, beta-glucans, arabinoxylans). Beta-glucans and arabinoxylans have high intrinsic viscosity due to their large water binding capacity. Each feedstock varies in NSPs composition, and differs consequently in their viscosity behavior. Table 1 shows the different amounts of NSPs in various feedstocks.

Table 1. *Non-starch Polysaccharides present in different feedstocks (g kg^{-1} dry matter)* [1,2]

	Corn	Wheat	Rye	Barley	
				Hulled	Hulless
Beta-Glucan	1	8	16	42	42
Cellulose	22	17-20	15-16	43	10
Soluble and Non-soluble NCP[3]	75	89-99	116-136	144	114
Total NSP	97	107-119	132-152	186	124

[1] Bach Knudsen, K. E. (1997) Animal Feed Sc. Techn. 67:319-338.
[2] Englyst et al (1983) J. Sci. Food Agric. 34:1434-1440
[3] Non Cellulosic Polysaccharides: pentosans, (arabino)xylans and other hemicelluloses

High viscosity has a negative effect on ethanol production, since it will limit the solid concentration that can be used in mashing and it will reduce the energy efficiency of the process.In addition, residual hemicelluloses may contribute to fouling in heat exchangers and distillation equipment.

Viscosity reducing enzymes can be added in different stages of the ethanol production process: mixing and/or saccharification/fermentation. Preferably the enzymes are added in mixing to breakdown initial viscosity. By using the enzymes in the fermentation step, breakdown of the oligosaccharides to their respective monomers is achieved. These monomers (glucose, fructose) can be fermented by the yeast to ethanol.

To breakdown the NSPs in wheat and rye, specifically formulated multi-component enzyme products have been developed. The major enzyme activities in these multi-enzyme products areendo-1,4-beta-glucanases (EC 3.2.1.4) and endo-1,4 beta-xylanases (EC 3.2.1.8), which catalyzes the endohydrolysis of 1,4-linkages in beta-D-glucans or the hydrolysis of 1,4 linkages in beta-D-arabinoxylans. In addition, a new product, OPTIMASH™ Barley has been developed for the beta-glucan hydrolysis in barley. Application tests using this latter product in barley/wheat mixtures will be discussed in the presentation.

5.2. **Rogerio Prata**, Franklinton (USA)
What's Under the DP4+ Peak?

Dry grind is most widely used method in the U.S. for generating fuel ethanol from grain. An estimated 13 Billion gallons of renewable fuel were produced in 2010 in 204 biorefineries located in 24 different states in the United States. Throughout the dry grind process from milling to fermentation and byproduct recovery, starch utilization and conversion to ethanol are monitored to evaluate process efficiency. Other important values such as % dry solids, organic acids and residual sugars including DP4+ are also monitored to enable informed decisions concerning plant operations. With the HPLC analysis of beer drop samples the DP4+ peak is often used as an indicator of fermentation performance in ethanol production plants. It is known from previous studies that the DP4+ peak contains ions, proteins, dextrins and other soluble compounds. The DP4+ peak is composed of all organic and inorganic molecules present in your sample that are capable of refracting light and have no affinity to the stationary phase in your HPLC column including dextrins with more than three glucose molecules. The contributions of the various compounds to the peak area are dependent on concentration, but they do not contribute equally to the peak area. In spite of the complex and ever changing composition of the DP4+ peak, routine interpretation of this peak is used to assess process efficiency. Interpretation of the DP4+ peak may lead to a cascade of decisions that may impact plant operations. Abnormal DP4+ concentrations may be presumed to be associated with potential yield loss. Plants that use DP4 + peak as an indicator of incomplete hydrolysis and fermentation need to understand that a sudden increase or decrease of DP4 + peak concentrations to an otherwise stable DP4 + peak baseline concentration may not only be indicative of incomplete dextrin hydrolysis and fermentations but also of process changes. Backset, chemical usage and sample handling, among other things, may also influence changes in DP4 + concentrations. Robust preparative chromatography step may be employed to understand the root causes of abnormal DP4 + peak concentrations without affecting the concentrations of dextrins and other important analytes.


Rogerio Prata

5.3 **Arjen van Tuijl**, **Kees-Jan Guijt**, **Vivek Sharma** and **Pauline Teunissen**, Leiden (The Netherlands)
Enzyme Systems Improving Process Robustness and DDGS Quality


Arjen van Tuijl

In the fuel ethanol industry, liquefaction is a crucial step to break open the starch granules and solubilize the sugars to be fermented to ethanol. Hydrolysis of starch into oligosaccharides is generally accomplished by a high-temperature jet-cooker step (90-126^{o}C), followed by a dextrinization step at a lower temperature (65-90^{o}C). Alpha amylase is added to randomly hydrolyze α-1,4-glucosidic bonds, reducing the viscosity of gelatinized starch and producing soluble dextrins and oligosaccharides. Factors influencing alpha amylase performance include temperature, pH, sodium- and calcium levels, but also the presence of inhibitors like phytic acid.

Phytic acid (phytate; *myo*-inositol 1,2,3,4,5,6, hexakisphosphate) is the primary source of inositol and storage phosphorus in plant seeds contributing ~70 % of total phosphorus. Phytic acid has a negative impact in liquefaction, as it acts as a chelator, binding metals like calcium, which is required by alpha amylase.

Another problem occurs with Dry Distiller's Grains with Solubles (DDGS), a co-product of the ethanol production process and a high nutrient feed valued by the livestock industry. High phytic acid levels in DDGS are a concern because the phosphorus in this form is unavailable to monogastric animals (swine, poultry, fish).

We recently introduced an improved enzyme system, combining a thermo stable alpha-amylase with a thermo stable phytase. Using corn this improved enzyme system shows superior performance compared to thermo stable alpha-amylase alone. However, small grains like wheat and barley do contain endogenous phytase, albeit not thermo stable.

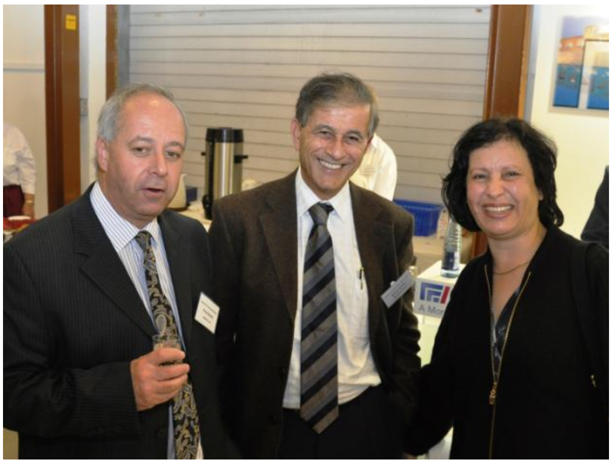
Therefore we investigated if benefits of the improved enzyme system also occurred with small grains.

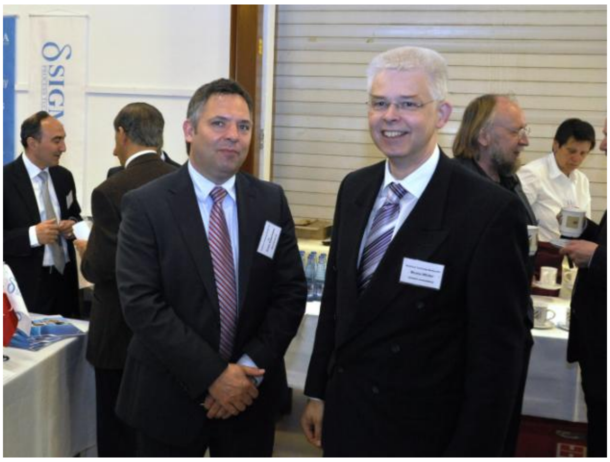
From our lab data it was obvious that the addition of thermo stable phytase does not only reduce viscosity and increase alpha amylase stability (activity), but also reduces phytic acid levels after liquefaction and generated free phosphate. We will present data showing reduced phytic acid levels on wheat as well as barley and at 85^{o}C as well as 95^{o}C. Especially at short mashing times (or relatively high temperature) the new enzyme system will outperform endogenous phytases present in small grains.

5.4 **Jan Wery**, Wageningen (The Netherlands)
Development of Efficient Second Generation Biofuel Enzymes Using the Fungal C1-Technology Platform

Jan Wery

Low-cost enzymes that efficiently hydrolyze residual (hemi-) cellulosic biomass into fermentable sugars are imperative for cost effective second-generation biofuel and chemicals production processes. These processes rely on the integration several cost-intensive technologies, such as thermo-chemical pretreatment of the biomass, enzymatic hydrolysis and microbial conversion of sugars into fuels or chemicals. The many different types of (hemi-) cellulosic biomass, pretreatment procedures and microbial conversion systems result in very diverse process conditions (e.g. feedstock of different composition, different pH's and temperatures). An enzyme system that is robust and active on a variety of (hemi-) cellulosic biomasses at different conditions is therefore highly desirable. Dyadic develops and owns the fungus *Chrysosporium lucknowense* C1 as a source and production platform of novel industrial enzymes. The C1-technology platform is characterized by a wide variety of different (hemi-) cellulose-active enzymes and a high potential to hydrolyse these complex materials. An extensive library of well-characterized single (hemi-) cellulose-active C1-enzymes has been constructed. The study of the synergistic interaction of these single enzymes enabled the design of very potent artificial enzyme mixtures. These results have led to the development of engineered C1-strains that hyper-produce enzyme mixtures which efficiently convert (hemi-) cellulosic biomass, such as corn stover, wheat straw, energy crop, DDGS and sugar cane bagasse into fermentable sugars. Importantly, the enzyme mixtures developed showed high activity at broad temperature and pH-ranges, which enables their use in very different biofuels and chemicals production processes. An overview will be given covering the different aspects of the development of C1 as a versatile enzyme production platform and, in particular, a producer of commercially competitive biofuel enzymes.



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