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Advanced Biofuels in a Biorefinery Approach

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Henning Jørgensen (Ed.)

FACULTY OF SCIENCE
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Welcome

The conference organizers welcome you to Copenhagen and the conference "Advanced Biofuels in a Biorefinery Approach". Essential for the conference is to have a balanced mix of academic and industrial representation thereby providing updates on developments and status at all levels from lab to deployment and commercialization. We therefore appreciate your attendance.

The conference is organized by the University of Copenhagen and Bio4Bio, a centre for development and implementation of biotechnology for bioenergy consisting of 8 Danish universities and companies. The conference is also coordinated with the International Energy Agency's two networks: Bioenergy Task 39 (commercializing liquid biofuels from biomass) and Task 42 (biorefineries). We are therefore pleased to welcome alle the international participants from the two networks. Two special sessions at the conference will be dedicated to these networks to provide updates on their activities.

The program consists of more than 50 presentations divided into 2 plenary sessions, 10 parallel sessions - of which two are IEA Bioenergy sessions - and a poster session with around 60 representatives. The sessions will focus on liquid biofuels, biochemicals and materials mainly derived from biochemical processes and cover the latest developments in advanced biofuels and biorefinery technologies from field to final products including sustainability assessments.

We are also pleased to offer a complimentary tour (limited seats) to two well-known companies within the biorefinery area: DONG Energy's Inbicon demonstration plant and Novozymes' production factory. Both companies are examples of the latest developments in biorefining of biomass into advanced fuels and chemicals.

We are very grateful to our sponsors who have enabled us to have a low registration fee, thereby maximizing the attendance and provide the complimentary tour.

We welcome your participation at the conference and hope it will be an informative and stimulating event. Thanks for attending.

Henning Jørgensen
Conference Chair
Associate Professor, University of Copenhagen

IEA Bioenergy

IEA Bioenergy is an international collaboration set-up in 1978 by the International Energy Agency (IEA) to improve international co-operation and information exchange between national bioenergy RD&D programmes. IEA Bioenergy's vision is to achieve a substantial bioenergy contribution to future global energy demands by accelerating the production and use of environmentally sound, socially accepted, and cost-competitive bioenergy on a sustainable basis, thus providing the increased security of supply whilst reducing greenhouse gas emissions from energy use. Currently, IEA Bioenergy has 24 Members and is operating on the basis of 12 Tasks covering all aspects of the bioenergy chain, from resource to the supply of energy services to the consumer.

Task 39 IEA Bioenergy

IEA Bioenergy Task 39 is a group of international experts working to commercialize sustainable transportation biofuels. Bioenergy and biofuels are important components within a country's green energy portfolio.

The goal of Task 39 is to provide participants with comprehensive information to assist with the development and deployment of transportation biofuels. The Task coordinates both technical and the infrastructure issues related to biofuels. To meet this goal, the Task objectives are to:

- Provide information and analyses on policy, markets and implementation issues that help encourage the adoption of sustainable conventional biofuels and help commercialize advanced liquid biofuels as a replacement for fossil-based fuels
- Catalyze cooperative research and development projects that will help participants develop improved, cost-effective processes for the production of advanced liquid biofuels
- Provide information dissemination, outreach to stakeholders, and coordination with other related groups

Currently the task member countries are Austria, Australia, Brazil, Canada, Denmark, Finland, Germany, Italy, Japan, Netherlands, New Zealand, Norway, South Africa, South Korea, Sweden, United Kingdom, USA.

Task Leader: Jack Saddler, Canada, and Jim McMillan, USA

For more information visit www.Task39.org

IEA Bioenergy | Task 42 Biorefinery

IEA Bioenergy Task 42 Biorefinery deals with knowledge building and exchange within the area of biorefining, i.e. the sustainable processing of biomass into a spectrum of marketable Bio-based Products and Bioenergy. The Task was started in 2007, and is now very successfully in operation involving Australia, Austria, Canada, Denmark, France, Germany, Ireland, Italy, Netherlands, Turkey, United Kingdom, United States of America. One activity has been to set a common international framework on biorefining (i.e. definition, classification system, state-of-the-art in participating countries). Lately the focus of the activities has been on the integral technical, economic and ecological assessments of full biofuel-driven biorefineries; the analysis of the types of Bio-based Chemicals that potentially could be co-produced with secondary energy carriers; to organise a Biorefining Summer School to get both industrial stakeholders, policy makers and students acquainted with the principles, current state-of-the-art, and future possibilities of applying the biorefining approach as base for a Bio-based Economy.

Task42 will continue in the next triennium (2013-2015) with main focus on tackling market deployment aspects for integrated biorefineries, supporting stakeholders in the energy sector finding their position within a future Bio(-based) Economy, optimal sustainable use of biomass for Food and Non-food applications, and dissemination and training activities.

Task Leader: Rene van Ree, Netherlands

For more information visit www.iea-bioenergy.task42-biorefineries.com

General information

Registration
On-site registration and distribution of meeting packets to pre-registrants will take place in the lobby Tuesday from 8:00 am. Programs will be distributed at the meeting to all attendees.

Internet
Wireless internet will be available free of charge. See the reception desk in the lobby to get your free password.

Speaker ready
Speakers should contact the speakers' desk well in advance to ensure that your presentation is uploaded to the computers in the conference room. Speakers' desk is located in the lobby area in front of the conference rooms.

Poster presenters
Poster presenters should put up their poster before Tuesday noon. You are welcome to leave your poster up until Wednesday afternoon. Poster boards will be marked with numbers to identify your poster. Authors should be available during the poster session to answer questions, expand on the material and take part in discussions.

Poster session
The posters session and reception will take place on the second floor on Tuesday at 6-9:00 pm.

Meals:
Breakfast: Breakfast will be served in the lobby in front of the conference rooms at 8-9:00 am.
Lunch: Lunch will be served in the restaurant "Østerbro" on the third floor
– sponsored by Novozymes.
Conference dinner: Dinner will be served in the restaurant "Østerbro" on the third floor
– sponsored by Genencor and DONG energy

Fitness center
The use of the fitness center and swimming facilities in the adjacent DGI Byen will be free of charge for all conference attendees during the conference.

Tour to biorefinery facilities
There is a complimentary tour to visit the Inbicon advanced bioethanol demonstration plant and the production plant of Novozymes in Kalundborg. Only limited seats are available and preregistration is required. People that have got space on the tour will be contacted directly. There might be last minute cancellations and you are welcome to contact the people at the registration desk to get on a waiting list. The final list of participants in the tour will be posted at the registration desk prior to lunch Wednesday. Busses for the tour will depart from CPH Conference at 8:30 am and we expect to be back in Copenhagen at 4:00 pm. Please contact the registration desk so we can ensure that you are on the right bus. Lunch will be provided on the tour – sponsered by Statoil Norway.

Hotels

DGI-byen hotel
Tietgensgade 65
1704 København V

Zleep Hotel Centrum
Helgolandsgade 14
1653 København

Zleep Hotel Astoria
Banegaardspladsen 4
1570 København V

Transportation

Train from Kastrup airport to CPH Conference. Trains departs every 10 minutes from Copenhagen Airport Kastrup station on platform 2. Arrival at København H (central station) app. 14 minutes later. See the map for directions from the central station to CPH Conference.

Train tickets are available from the DSB ticket office above the railway station in Terminal 3. Tickets and travel cards are also available from ticket machines in the station area. The ticket (3 zoner) is 36 DKK from the airport to downtown Copenhagen. Ticket and travel cards allows you to travel on train, S-train, bus and metro throughout the greater Copenhagen area. For more information and to plan your trip visit www.dsb.dk or www.rejseplanen.dk/bin/query.exe/en.

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Conference tour to biorefinery facilities

The tour and lunch is sponsored by Statoil Norway.

Thursday March 1st is organized a tour to two biorefinery facilities located in the city of Kalundborg, around 1½ hours drive from Copenhagen. The tour is complimentary for people registered for the conference, however there are limited seats. On the tour will be served a complimentary lunch. Busses will depart from CPH Conference at 8:30AM and we expect to be back in Copenhagen at 4 PM.

On the tour you will visit the largest production site of Novozymes in Denmark. Here will be a tour through the production facility showing the fermentation, separation, purification and formulation of enzymes. The next visit will be the Inbicon Biomass Refinery where straw is converted into ethanol, solid biofuel and feed. We hope also to have a visit to the DONG Energy low temperature biomass gasifier, the Pyroneer demonstration plant (not confirmed).

The city of Kalundborg is known for the "Kalundborg Symbiosis", which is the world's first working industrial symbiosis. In Kalundborg Symbiosis, public and private enterprises buy and sell waste products from industrial production in a closed cycle. The residual products traded can include steam, dust, gases, heat, slurry or any other waste product that can be physically transported from one enterprise to another. A residual product originating from one enterprise becomes the raw material of another enterprise, benefiting both the economy and the environment. For more information visit www.symbiosis.dk/en.

Novozymes

Novozymes is the world leader in bioinnovation and the largest producer of industial enzymes. The business is industrial enzymes, microorganisms, and biopharmaceutical ingredients. Novozymes has more than 5800 employees globally and the headquarter is located in

Photo: Novozymes

Bagsværd, Copenhagen. The production site in Kalundborg is the largest in Denmark and employs more than 600 people. It is co-located with the production site of Novo Nordisk and together the site occupies 1,000,000 m2.

For more information visit www.novozymes.com

Inbicon Biomass Refinery

Inbicon is a fully owned subsidiary of DONG Energy, Denmark's largest energy group with more than 5100 employees. The development of the core technologies started in late 1990's and the first pilot plant was put in operation in 2003. The Inbicon demonstration plant was opened at Kalundborg and integrated with the Asnæs Power Station, Denmark's largest, in late 2009. Inbicon has their research and pilot scale facilities in Skærbæk, Jutland. Inbicon's core technology is a three-stage process: mechanical conditioning of the biomass, hydro-thermal pretreatment, and enzymatic hydrolysis. A key component of the technology is the ability to operate all steps at high solids concentrations.

For more information visit www.inbicon.com

Facts about the Inbicon Biomass Refinery

Raw materials:

4 MT/h equivalent to about 30,000 metric tons of straw a year

Enzymes supplied by by Danisco Genencor and Novozymes

Annual production:

5.4 million liter (1.4 million gallons) of cellulosic ethanol

11,400 metric tons of lignin pellets

13,900 metric tons of C5 molasses

Employees:

30 employees

Costs:

Total construction costs: About 400 million DKK (54 mio. EUR; $76.7 million USD).

Photo: Inbicon

Program

Tuesday 28th

8:00-17:00 Registration
8:00-9:00 Breakfast
9:00-9:15 Opening
9:15-12:10 Plenary session 1: Biorefineries seen from an ecological, economical and industrial point of view.
Chairman: *Claus Felby, University of Copenhagen, Denmark*
9:15-9:35 *Anders Eldrup*, TBA, DONG Energy
9:35-10:00 *Paulo Cesar de Campos Barbosa*, Overview of the Petrobras biofuels program, Petrobras

10:00-10:20 Break

10:20-10:55 *Jacob Sterling*, The role of biofuels in a diversified fuel supply for the shipping industry, Mærsk Line
10:55-11:20 *Antonio Bonomi*, The Brazilian Sugarcane Industry: a favourable environment to introduce Lignocellulosic ethanol production technologies, Laboratório Nacional de Ciência e Tecnologia do Bioetanol – CTBE
11:20-11:45 *Wout Boerjan*, Engineering poplar trees for the biorefinery, Gent University
11:45-12:10 *Claus Fuglsang*, Commercialization of cellulosic ethanol - a reality, Novozymes

12:10-13:10 Lunch

13:10-14:50 Parallel sessions 1 and 2

Parallel 1: Plants and biomass – input for the biorefinery.
Chairman: *Søren K Rasmussen, University of Copenhagen, Denmark*
13:10-13:35 *Christian Bukh*, Characterization of Brachypodium cinnamyl alcohol dehydrogenase, University of Copenhagen
13:35-14:00 *Jakob Magid*, Effects of genetic and environmental factors on recalcitrance of winter wheat straw, University of Copenhagen
14:00-14:25 *Kasiviswanathan Muthukumarappan*, Novel Integration of Preprocessing and Densification of Different Biomass Feedstocks,
14:25-14:50 *Thomas Didion*, Perennial grasses, flexible feed and biomass supply, DLF Trifolium

Parallel 2: Biomass processing and pretreatment.
Chairman: *Guido Zacchi, Lund University, Sweden*

13:10-13:35 *Guido Zacchi*, Pretreatment of biomass for production of fuels and chemicals, Lund University

13:35-14:00 *Benjamin Levie*, Producing Concentrated Cellulosic Sugars at Scale, Catchlight Energy

14:00-14:25 *Xiongjun Shao*, Kinetic modeling of xylan hydrolysis in countercurrent and cocurrent liquid hot water flowthrough pretreatments, Oak Ridge National Laboratory

14:25-14:50 *Leif J. Jönsson*, Lignocellulose-derived inhibitors of enzymes and microorganisms, Umeå University

14:50-15:20 Break

15:20-17:00 Parallel sessions 3 and 4

Parallel 3: Enzymatic hydrolysis in biorefineries.
Chairman: *Liisa Viikari, Helsinki University, Finland*

15:20-15:45 *Liisa Viikari*, Recyclable enzymes for the hydrolysis of lignocelluloses, University of Helsinki

15:45-16:10 *Mian Li*, Driving new advances in cellulosic enzymes for biorefinery development, Genencor

16:10-16:35 *Johan Börjesson*, Improving enzyme performance in biomass hydrolysis, Novozymes

16:35-17:00 *Peter K Busk*, Peptide Pattern Recognition, a new alignment-independent method predicts the function of glycoside hydrolases with high precision, Aalborg University

Parallel 4: Sustainability.
Chairman: *Warren Mabee, Queens University, Canada*

15:20-15:45 *John Neeft*, Sustainability and GHG criteria for lignocellulosic biofuels – the role of BioGrace, NL Agency

15:45-16:10 *Don O'Connor*, Indirect Land Use Change – How Good are the Models?, (S&T)2 Consultants Inc.

16:10-16:35 *Niclas Scott Bentsen*, Thermodynamic optimization of the use of biomass for energy services, University of Copenhagen

16:35-17:00 *Gerfried Jungmeier*, The Integration of a Sustainable Advanced Bioethanol Production in the Value Chain of a Pulp and Paper Biorefinery – European Perspectives based on an Austrian Case Study, Joanneum Research

18:00-21:00 Evening poster session and reception

Wednesday 29th

8:00-8:30 Registration and breakfast

8:30-10:10 Parallel sessions 5 and 6

Parallel 5: Fermentation technologies and systems biology.
Chairman: *Lisbeth Olsson, Chalmers University of Technology, Sweden*

8:30-8:55 *Lisbeth Olsson*, Robust microorganisms – the key to successful lignocellulose based ethanol production, Chalmers

8:55-9:20 *Inge Minneboo*, Development of engineered S. cerevisiae strains enabling C5-sugar fermentation for cellulosic ethanol production in the framework of the KACELLE project, DSM

9:20-9:45 *Birgitte Rønnow*, Rapid xylose and glucose fermentation by engineered S. cerevisiae for commercial producton of cellulosic ethanol, Terranol

9:45-10:10 *Willie Nicol*, Continuous Biofilm Reactors for Organic Acid Production: Influence of Shear Homogeneity on Biofilm Productivity, University of Pretoria

Parallel 6: Biomass recalcitrance and conversion.
Chairman: *Lisbeth G. Thygesen, University of Copenhagen, Denmark*

8:30-8:55 *Mads T. Hansen*, Biophysical factors involved in biomass recalcitrance, University of Copenhagen

8:55-9:20 *Svein Jarle Horn*, Cellulose cleavage by a novel type of copper oxidases Norwegian University of Life Sciences

9:20-9:45 *Junyong Zhu*, Fundamentals and practices for efficient production of biofuel from lignocelluloses, USDA Forest Service

9:45-10:10 *Mette Lange*, Discovery of novel GH61 and attempt to correlate subfamily grouping with taxonomy and physiology of producing organism, Aalborg University

10:10-10:30 Break

10:30-12:10 Parallel sessions 7 and 8

Parallel 7: Biorefinery technologies and integration.
Chairman: *Ioannis Skiadas, Aalborg University, Denmark*

10:30-10:55 *Stefano Macrelli*, Techno-economic evaluation of integrated ethanol production from sugarcane, Lund University

10:55-11:20 *Birgitte K. Ahring*, Development of a Biorefinery concept for integrated production of Biomedicals, Biochemicals, Food, Feed and Fuels from selected plant materials (BIOREF). Washington State University

11:20-11:45 *Arturo Sanchez*, Co-production of ethanol, hydrogen and gas in a 2G biorefinery based on agro-wastes. Conceptual design and NPV analysis in mid-size economies, Unidad de Ingenieria Avanzada

11:45-12:10 *Martin Jeppesen*, Introduction to HGBiofuels, Inbicon

Parallel 8: IEA Bioenergy Task 42.
Chairman: *Rene van Ree, Wageningen UR, Netherlands*

10:30-10:35 *Rene van Ree* (Task leader Task 42) – Updates on Task 42 activities

10:35-11:00 *Gerfried Jungmeier*, Innovative Biofuel-driven Biorefinery Concepts and their Assessment – An outlook until 2025 in IEA Bioenergy Task 42 "Biorefinery", Joanneum Research

11:00-11:25 *Patrick Walsh*, Value added Products from Biorefineries – Bio-based Chemicals, Galway Mayo Institute of Technology, The National University of Ireland
11:25-11:45 *Gil Garnier*, Biorefinery developments in Australia, Monash University
11:45-12:10 *Isabella de Bari*, Current status of biorefineries in Italy - R&D activities, ENEA Research Centre

12:10-13:10 Lunch

13:10-14:50 Parallel sessions 9 and 10

Parallel 9: IEA Bioenergy Task 39.
Chairman: *Jack Saddler, University of British Columbia, Canada*
13:10-13:25 *Jack Saddler* (Task Leader Task 39) – Update on Task 39 activities
13:25-13:40 *Warren Mabee*, Updates on the implementation of biofuels, Queens University, Canada
13:40-14:05 *Ian Suckling*, Liquid Biofuel Production in New: Zealand: Opportunities and Challenges, Scion, New Zealand
14:05-14:30 *Dina Bacovsky*, Biofuels Demoplants, BIOENERGY 2020+ GmbH
14:30-14:50 *Jim McMillan*, Status of Production of Advanced Liquid Biofuels in the United States, NREL

Parallel 10: New materials and chemicals from biomass.
Chairman: *Ed de Jong, Avantium, Netherlands*
13:10-13:35 *Ed de Jong*, Biomass conversion into YXY (furan) building blocks for polyester applications, Advantium
13:35-14:00 *Keld Ejdrup Andersen*, Biorefining for high value food and feed ingredients, University of Copenhagen
14:00-14:25 *Mickel Jansen*, Low pH Fermentation to Succinic Acid, the Basis for Efficient Recovery, DSM
14:25-14:50 *Joachim Venus*, Pilot plant facility for the scale-up of continuous mode lactic acid fermentation, Leibniz-Institute for Agricultural Engineering Potsdam-Bornim

14:50-15:20 Break

15:20-17:15 Plenary session 2 – Deployment of biorefinery technologies.
Chairman: *Jim McMillan, National Renewable Energy Laboratory, USA*
15:20-15:35 *Anne Grete Holmsgaard*, Biorefining Alliance - local solutions to global needs, Biorefining Alliance
15:35-16:00 *Michael Persson*, Experience from the Operation of the Inbicon 2nd Generation Bioethanol Demonstration Plant, Inbicon
16:00-16:25 *Gisle Johansen*, A Biorefinery approach to production of lignocellulosic ethanol and chemicals from low value biomass, Boregaard
16:25-16:50 *Sune Wännström*, SEKAB experiences from scaling up and commercialization of CE-technology, Sekab
16:50-17:15 *Tony Sidwell*, From Sugar Mills to Biorefineries - the sustainable future for the sugar industry, AB Sugar

17:15-17:30 Closing remarks

18:30- Conference dinner

Overwiew of the Petrobras biofuels program

Paulo Cesar de Campos Barbosa

Petrobra Biofuel
Av. Chile, 500-29o andar, 24240-260, Rio de Janiero, Brazil.

paulo.barbosa@petrobras.com.br

Created in 2008, Petrobras Biofuels, a Petrobras subsidiary company, is already an important player in Brazilian biofuel market and is working on development of new projects and new technologies to improve its contribution to sustainable biofuels.

In this presentation Petrobras strategies for biofuel will be discussed in the context of sustainable bioenergy technology, market and regulations.

The role of biofuels in a diversified fuel supply for the shipping industry

***Jacob A. Sterling**, Head of Climate and Environment*

Mærsk Line
Esplanaden 50, DK-1098 Copenhagen K, Denmark

Jacob.Sterling@maersk.com

The current annual market for shipping fuels is approx 170 billion USD, corresponding to a fuel volume of 250-350 Million USD. Currently, this is almost 100% fossil fuels. With upcoming strict regulation on sulphur emission and an increasing focus on CO_2 emissions, there is potentially a significant role for biofuels in shipping as biofuels are low in both sulhur content and CO_2 footprint.

Maersk Line is the world's largest container shipping company with an annual fuel consumption of 10 million tones. Maersk Line is committed to reducing its CO_2 footprint and so far performance improvements have been achieved through fuel efficiency improvements. In the longer term, Maersk Line would very much like to see sustainable biofuels become a commercially available low-carbon fuel for shipping, and we would therefore invite for a discussion on how to accelerate the development of sustainable biofuels for the shipping market.

The Brazilian Sugarcane Industry: a favorable environment to introduce Lignocellulosic ethanol production technologies

Antonio Bonomi

Laboratório Nacional de Ciencia e Tecnologia do bioethanol – CTBE
Camoinas, Brazil

antonio.bonomi@bioethanol.org.br

One of the greatest concerns of society and governments nowadays regards the large scale production of alternative forms of energy, such as biofuels, which are able to reduce greenhouse gases emissions and improve energy security when compared to their fossil counterparts. However, issues about biofuels sustainability, including environmental, economical and social aspects, have been raised around the world.

A comprehensive strategy to evaluate the sustainability of different biofuels production routes using sugarcane as raw material is under development at the Brazilian Bioethanol Science and Technology Laboratory (CTBE) integrating different computer platforms such as Aspen Plus, SimaPro and electronic spreadsheets. This tool, so called the Virtual Sugarcane Biorefinery (VSB), allows the comparison of technical, economic, social and environmental impacts of different production technologies regarding the production of bioethanol, sugar, bioelectricity and other products, such as the ones derived from termochemical conversion, sugarchemistry, alcoholchemistry and lignochemistry. Since the agricultural phase is also being modeled and integrated with the industrial phase, the impacts of the agricultural technologies on the industrial phase (and vice-versa) are also evaluated in the VSB.

The first results obtained with the VSB are shown in this presentation. The evaluation of first generation ethanol production coupled, or not, with electricity and sugar production is presented, along with process optimization and an analysis of the flexibility of annexed plants and harvest extension using sweet sorghum. Second generation ethanol production from sugarcane bagasse and trash through the biochemical route is evaluated as well, compared with electricity production. The integrated first and second generation ethanol production from sugarcane is compared with a stand-alone second generation plant using sugarcane bagasse and trash as feedstock.

So far, the results obtained with the VSB allowed comparison of different technologies for first and second generation ethanol production from sugarcane, through evaluation of technical, economic and environmental impacts. Some already structured applications of this tool are sensitivity analysis, life cycle assessment and calculation of economic parameters, such as internal rate of return. The present results indicate that the Brazilian Sugarcane Industry is a favorable environment to introduce Lignocellulosic ethanol production technologies.

Engineering poplar trees for the biorefinery

[1]Van Acker, R., [1]Storme, V., [1]Goeminne, G., [1]Ivens, B., [2]Custers, R., [3]Aerts, D., [3]Soetaert, W., [4]Ralph, J., [5]Santoro, N., [6]Leple, J.-C., [6]Pilate, G. and [1]**Boerjan, W.**

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Global warming, environmental disasters, and increasing oil prices have catalyzed a world-wide trend to use plant biomass as a renewable source for liquid biofuels and bio-based materials. Plant biomass can be processed into bio-ethanol by enzymatic depolymerization of the cell wall polysaccharides into simple sugars, followed by fermentation. However, the presence of lignin in the cell wall is an important recalcitrance factor. Lignin is an aromatic polymer made by the combinatorial coupling of monolignol radicals. It provides strength to the cell wall, but also limits access of the cell wall carbohydrates by cellulases. One approach to overcome this hurdle is to engineer lower lignin amounts or alter its composition to make lignin more susceptible to the costly chemical pretreatments. Down-regulation of cinnamoyl-CoA reductase (CCR) in genetically modified poplar results in reduced lignin content and improved saccharification of the woody biomass, when grown in greenhouse conditions. Field trials are necessary to evaluate whether the results obtained in the greenhouse can be extrapolated to field conditions. After a long regulatory calvary, field trials have been initiated under short rotation coppice culture to evaluate their potential as raw material for bioethanol production. The latest scientific results from these trials will be presented.

Commercialization of cellulosic ethanol – a reality

Claus Crone Fuglsang, *Managing Director*

Novozymes, Inc.
1445 Drew Ave, Davis CA 95618 United States

ccf@novozymes.com

After a successfully demonstrating biomass conversion to ethanol at pilot scale in 2011, the biomass to ethanol industry is entering the commercial deployment phase. Several producers have broken ground to construct the first wave of commercial cellulosic ethanol facilities with 25 MGPY capacity. In US alone, it is projected that the tenth to quarter billion gallons of cellulosic ethanol fuel would be produced and would be available for blending in the automotive fuel/gasoline by end of 2013. Also, in China and EU companies are moving ahead with establishing commercial scale facilities using biomass as a feedstock.

The progress of these companies will be discussed as will the continued improvements in process and especially enzyme technologies which will help drive down cost of producing cellulosic ethanol by increasing the conversion yield from the cellulosic and hemicellulosic feedstock and increase the capacity utilization of the industrial scale B2E facilities by allowing for high solids load. Examples of specific improvements will be presented together with their impact on ethanol production cost.

Characterization of Brachypodium cinnamyl alcohol dehydrogenase

***Christian Bukh**, Pia H. Nord-Larsen and Søren K. Rasmussen*

Department of Agriculture and Ecology, Faculty of Science,
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Lignin is a major recalcitrant factor in the production of bioethanol from lignocellulosic biomass. The object of this project is to isolate and clone genes involved in the biosynthesis of lignin in grasses to facilitate reduction of recalcitrance of temperate grasses. We have cloned the five cinnamyl alcohol dehydrogenase (CAD) genes from the Brachypodium distachyon genotype Bd21-3 and studied their expression during plant development.

Of the five isolated CAD genes two (BdCAD1 and BdCAD3) was successfully expressed in E. coli as His-tagged proteins. One of these proteins BdCAD1 exhibits 86% identity to CAD2 proteins from maize and sorghum. Phylogenetic analyses of known CAD proteins further group BdCAD1 together with CAD4 and CAD5 from dicot Arabidopsis. This branch of CAD proteins has been implicated in the lignification of vascular tissues. Mutations in maize and sorghum CAD2 proteins have been linked to the brown midrib phenotype which is associated with reduced lignin content and increased digestibility. BdCAD3 exhibits 53% identity on protein level to BdCAD1 but does not group with the same branch of CAD proteins. The his-tagged expressed proteins was purified to >95% and characterized by different physical-chemical techniques. Furthermore, their ability to convert coniferyl aldehyde to coniferyl alcohol and vice versa was investigated under different pH-values and at different temperatures.

Keywords: biofuel; lignin; cellulosic; cinnamyl alcohol dehydrogenase; CAD; Brachypodium distachyon; protein expression; protein purification.

Effects of genetic and environmental factors on recalcitrance of winter wheat straw

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Recalcitrance of lignocellulosic material is important for ease with which it can be used for numerous different purposes including fermentation to produce bioethanol and as feed-stuff. In addition, recalcitrance is important for the way it is decomposed in soil. We have found a large variability between wheat straw samples depending on factors as variety, climate and nutrient supply.

We have built an archive (1400 samples) of winter wheat straw material, derived from modern as well as historical varieties, grown at different locations under varying climatic conditions and fertilizer regimes. This material (or selected parts of it) is being examined using a suite of methods, in order to determine the variability in recalcitrance, and its relation to genetic traits and environmental conditions. The methods employed include but are not limited to: a.) presoaking in acetic acid solution, liquid water pretreatment at 195ºC for 10 min, and subsequent enzyme loading, in order to assess the potential sugar yield, b.) digestion in pepsin-HCl solution and subsequent treatment with Novozym 51454 in order to assess ruminant degradability, c.) decomposition in soil under controlled moisture and temperature conditions, giving a microbial ecosystem measure of variability in recalcitrance.

Results demonstrate a variability of 30-40% in digestibility and up to 26% in sugar yield with significant and substantial effects of both environmental conditions and genetic traits. We assess that genetic selection for an improved biofuel feedstock within wheat straw is possible, with total sugar yield heritability of 57% and no disadvantageous correlations to other quality traits. Furthermore there are preliminary indications of diminishing variability due to fine grinding and milling of straw, indicating that variability in tissue ultrastucture may be of some importance. Considering the variability in lignin, cellulose and ash content we are surprised by the observed variability in total sugar yield. We are currently exploring cell wall composition on subsets of the archive using CoMPP. The archive can potentially be used for numerous different investigations and is available for the international research community for similar and other purposes in the future.

Novel Integration of Preprocessing and Densification of Different Biomass Feedstocks

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Our goal is to develop and validate the performance of an integrated pretreatment and compaction process to reduce the logistical hurdles facing second generation biofuels. This process will link AFEX pretreatment with a novel pelletization process to produce dense, solid biomass PAKS that retain their original composition, are more susceptible to hydrolysis and fermentation, and can use existing transportation/handling infrastructure.

In preliminary trials we have subjected corn stover and switchgrass to AFEX pretreatment. After pretreatment, samples were PAKed using the ComPAKco device. The preliminary evaluation of AFEX-treated and ComPAKco densified PAKs revealed some interesting findings. We found that all four samples showed higher angle of repose than the normal value of 45°. For the billeted samples this was related to their lower roundness (i.e., more sharp edges on surface), compared to their AFEX only counterparts. The AFEX treated and PAKed samples of corn stover and switchgrass possessed lower porosity, water adsorption index, water activity, and moisture content compared to the samples only subject to pretreatment, meaning the former will be more storable. The lower porosity and higher bulk density and true density of the billets will also reduce shipping costs.

Perennial grasses, flexible feed and biomass supply

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With high productivity, efficient resource utilization and environmental friendliness perennial grasses are very well suited to become a strong pillar in future biomass supply.

DLF Trifolium as one of the largest grass seed producer is engaged in several activities optimizing grass varieties for bioenergy conversion technologies like biogasification or second generation bioethanol production. In breeding for bioenergy traits germplasm collections will be screened for biomass productivity under low input management. At the same time DLF is implementing Genome Wide Selection to tie up phenotypes to genotypes as the preferred tool to achieve breeding success with complex traits like yield, biomass convertibility or high productivity under low input.

The presentation will describe some of the initiatives, research projects and collaborations DLF Trifolium has facilitated and is involved in. The common denominator in these activities is to develop sustainable grass varieties optimized to support the increased demand for biomass for feed, food and energy.

Pretreatment of biomass for production of fuels and chemicals

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The pretreatment of biomass is a crucial step for production of fuels and chemicals based on the sugar platform pathway as it has a large impact on all the other steps in the process. The primary aim is to make the cellulose accessible to enzymatic attack and several pretreatment methods have been developed, comprising methods working at low pH, i.e., acid based, at medium pH (without addition of catalysts), or at high pH, i.e., with a base as catalyst. Many methods result in high sugar yields, above 90% of theoretical for agricultural residues while more recalcitrant materials like hardwood, and especially softwood, require dilute-acid pretreatment to reach high sugar yields. The pretreatment method also affects the composition and structure of the hemicellulose and lignin, which can be of great importance depending on the intended use for these fractions of the raw material.

So far most studies on pretreatment have assessed the efficiency of the pretreatment by enzymatic hydrolysis of the solid fraction at low solids content and high enzyme dosages and the main purpose has been to produce sugar solutions for fermentation to ethanol. The various pretreatment methods need in the future to be reassessed at more industrial-like conditions considering the whole integrated process taking into consideration the influence on all process steps and also which other co-products, like chemicals and materials, that have to be produced. In this presentation, various pretreatment methods are discussed and how assessment should be performed to reach optimal conditions applied primarily to ethanol production but also considering the effect of the pretreatment on the possibility to produce other co-products.

Producing Concentrated Cellulosic Sugars at Scale

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Catchlight Energy is the Chevron / Weyerhaeuser joint venture whose mission is to commercialize the production of liquid transportation fuels from sustainable forest-based resources. Catchlight Energy has developed a unique sulfite-based pretreatment process to achieve excellent yields of concentrated sugars and lignosulfonates from a variety of potential forest-based feedstocks, including softwood, hardwood, and switchgrass. The combination of mature technology components and feedstock flexibility offers a practical alternative for achieving biofuel facilities at significant scale and increases the potential for pulp mill retrofits.

Sugar conversion yields on Southern Pine forest residual, a highly recalcitrant feedstock, exceed 80% with levels of inhibitors like HMF and furans that are below the levels that require conditioning. The results to date have achieved sugar titers over 10% and hydrolysis times less than 96 hours at reasonable enzyme loading levels. Keys to these results are less intensive pretreatment conditions and more effective chemical reactions occurring. Novel process development both upstream and downstream of the hydrolysis results in low water use, high enzyme efficiency, and concentrated product streams, enabling advanced biofuel conversion technology.

Kinetic modeling of xylan hydrolysis in countercurrent and cocurrent liquid hot water flowthrough pretreatments

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Flowthrough pretreatment, in which the solids residence time is longer than that of the liquid, has been shown by many investigators to effectively remove hemicellulose and lignin and generate highly reactive substrate. Countercurrent flowthrough pretreatment, in which liquid and lignocellulosic biomass flow in opposite directions, could be employed to increase the carbohydrate concentration in the flowthrough hydrolysate and at the same time reduce water and energy consumption.

In this study, a kinetic model for xylan hydrolysis in liquid hot water flowthrough pretreatment will be presented which utilizes a declining xylan hydrolysis rate constant with increasing conversion in combination with direct xylooligomer degradation. The model is able to describe experimental results obtained from flowthrough pretreatment of corn stover and triticale straw at various pretreatment temperatures, and will be applied to predict and compare the performance (xylooligomer concentration, xylan conversion, degradation product concentration) of xylan hydrolysis in countercurrent and cocurrent flowthrough pretreatments with various temperature gradients. Predictions on pretreatment performance at various water loadings and solids and liquid residence times will also be discussed.

Lignocellulose-derived inhibitors of enzymes and microorganisms

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Thermochemical pretreatment of lignocellulosic feedstocks generates by-products that inhibit hydrolytic enzymes and microorganisms used in subsequent fermentation steps. Actions taken to achieve higher product concentrations, such as the use of lignocellulose hydrolysates with high sugar concentrations or slurries with high dry-matter content, contribute to higher concentrations of inhibitors. Recirculation of process streams would also lead to higher concentrations of inhibitors. New findings in the area will be discussed, including connections between inhibition of enzymes and inhibition of microorganisms, and the possibility to perform chemical detoxification in situ in bioreactors [1].

[1] Alriksson B, Cavka A and Jönsson LJ (2011) Bioresour. Technol. 102, 1254-1263.

Recyclable enzymes for the hydrolysis of lignocelluloses

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Efficient recycling of enzymes can clearly reduce enzyme costs in total hydrolysis of lignocellulose. Enzyme costs are one of the major operation cost factors in the conversion of lignocellulosic raw materials to sugars and further to ethanol. One major possibility for reducing the cost would be efficient recycling of enzymes or the major components of the enzyme mixtures. Reduction of enzyme costs by providing enzymes suitable for recycling would accelerate the development of the approaching cellulose-to-ethanol industry. Presently, this is hindered by the lack of easily recyclable enzymes and consequently by technology for enzyme recycling.

After hydrolysis of the common lignocellulosic substrates, a significant part of the enzymes remains bound to cellulose and/or to lignin residues in the biomass and the enzymes are poorly desorbed by chemical or physical means without losing the activity. In this work, modifications were made to the cellulolytic enzymes and hydrolysis conditions. These modifications resulted in significantly higher amount of free enzymes after the hydrolysis. Thus, the amount of free enzymes in the liquid phase, possible to be recovered for recycling, was increased to about 60-90% of the enzyme protein applied. This makes it possible to develop enzyme mixtures with increased recyclability without compromising the hydrolysis efficiency.

Driving new advances in cellulosic enzymes for biorefinery development

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Efficient utilization of lignocellulosic materials in a biorefinery depends upon the advances in pretreatment, enzyme production, enzyme hydrolysis, as well as fermentation and conversion of those sugars to usable fuels and chemicals. In particular, efficient hydrolysis of lignocellulosic biomass requires the concerted action of both cellulase (i.e., endo-glucanase, cellobiohydrolase, and beta-glucosidase) and hemi-cellulase activities. Toward this end, Genencor has continued to lead the development of highly efficient yet cost effective multi-component enzymatic systems to provide an economical and environmentally-friendly solution to the biorefinery.

In this presentation, the performance of Genencor's latest "whole cellulase", Accellerase® TRIO, for biomass conversion will be shown. The sugar conversion (both glucan and xylan) during enzymatic saccharification of various substrates associated with different types of pretreatment will be presented. Furthermore, how the enzyme operates at industrially-relevant conditions to provide fermentable sugars at rates needed to supply an increasingly diverse range of manufacturing platform technologies will be discussed. How Genencor sees the market developing and how Accellerase® biomass enzymes help meet biorefinery's unmet needs will be examined.

Improving enzyme performance in biomass hydrolysis

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Further optimization of the hydrolytic capacity in commercial enzyme products for biomass conversion into fermentable sugars can increase process profitability. Improved performance in enzyme products can significantly affect important process cost factors such as enzyme dose, solids loading, sugar yield and process time. Recent advances in enzyme development will be presented and the significance of these in the process application will be discussed.

Peptide Pattern Recognition, a new alignment-independent method predicts the function of glycoside hydrolases with high precision

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Prediction of function from sequence has become even more urgently needed as the amounts of gene sequences are increasing dramatically. Similarly, screening of large metagenomic and metatranscriptomic libraries for occurrence of specific proteins could bring significant biological knowledge. New methodological approaches to increase discovery and understanding are needed.

We have invented a new principle for sequence analysis and implemented it as a new analytic tool called Peptide Pattern Recognition, PPR, by which protein function and function-related subfamily delineation can be predicted. PPR is alignment-independent method that compares multiple biological sequences at a time and finding the characteristic features. of the group.

As an example of the use of the grouping of longer biological sequences, we used PPR to predict the function of eukaryotic proteins classified as glycoside hydrolases family 5 (GH5). This group includes proteins with four different enzymatic functions that are difficult to predict by alignment. PPR provided a number of functionally relevant subgroups of the GH5 proteins and was able to predict the function of other eukaryotic GH5 proteins with 97 % accuracy. Furthermore, PPR could successfully analyze 24 glycoside hydrolases family 45 (GH45) proteins with the conserved domains at different positions in the proteins showing that the method is able to compare sequences with different domain structures.

Finally, we demonstrate how the list of n-mer short conserved sequences generated by PPR can be used to find new members of interesting protein families, by identifying conserved peptides in 467 proteins belonging to the glycoside hydrolase family 61 (GH61) of fungal proteins. These peptides were used to design degenerated primers for amplification of new GH61 proteins from 14 thermophilic fungi.

In conclusion, PPR is a new method for simultaneous comparison of multiple sequences. The method is different from previously described alignment-dependent and alignment-independent methods.

Sustainability and GHG criteria for lignocellulosic biofuels – the role of BioGrace

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In the European Union the Renewable Energy Directive and the Fuel Quality Directive set sustainability criteria for biofuels. This presentation will focus on the sustainability requirements for lignocellulosic (LC) biofuels, and in particular on the GHG emission criterion (35% reduction). The following four key messages will be addressed:

1. For biofuels which are produced from secondary wastes and residues, the GHG criterion is the only criterion to meet. Defining wastes and residues is a task for all 27 individual Member States, which might lead to differences in different MS.

2. The default values for GHG emission reduction from Annex V.B of the RED are high enough to meet the 35% criterion. So for LC biofuels listed in Annex V.B of the directive there is no need to calculate actual GHG emission reductions.

3. For LC biofuels not listed in Annex V.B of the RED, compliance to the GHG criterion must be shown by calculating actual GHG emissions. This can be done using the BioGrace GHG calculation tool. This tool is currently being evaluated by the Commission to be recognised as a voluntary scheme or "voluntary tool".

4. Other GHG calculation tools are around which unfortunately give different results. It will be explained why different tools give different results. BioGrace is approaching the owners of other tools as well as policy makers in the 27 MS in an attempt to define the key parameters unambiguously so that different tools give the same result, which will enhance the level playing field on the European biofuels market and will facilitate auditors to verify calculations.

Indirect Land Use Change – How Good are the Models?

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In the past several years there has been significant discussion regarding indirect land use change emissions (ILUC). These emissions, usually losses of soil and/or biomass carbon, are usually found some distance from where the actual crop is produced and result from changes in cropping patterns and practices as a result of the crop being used for biofuel production rather than for the traditional use of the crop.

Indirect land use emissions are required to be estimated in the US RFS2 program as part of the lifecycle emissions determination. The California Air Resources Board also requires that they be estimated as part of the Low Carbon Fuel Standard being implemented there. In Europe, there is an ongoing discussion about the inclusion of an ILUC factor in their Renewable Energy Directive.

The issue of indirect land use changes resulting from increased demand from biofuels is a very complex issue. Several different approaches have been used to try and quantify the impact but none of the approaches can yet produce consistent results that are not subject to criticism from the biofuels community.

Most of the approaches for estimating ILUC emissions involved some sort of econometric modelling combined with additional calculations. The econometric models that are being used were generally designed to investigate trade flows and the impact of changes in tariffs or other government policies. They were used to provide directional information or a comparison of two options, not to derive a single numerical value of a parameter to use for regulatory purposes.

A number of the models have similar issues and shortcomings and the presentation will address these issues and make recommendations for the improvements that must be made to the models if they are to be used for regulatory purposes.

Thermodynamic optimization of the use of biomass for energy services

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Biomass offer several options for displacing fossil resources to meet demands for energy and material services, and biomass is perceived as one of the main pillars of a future de-carbonized energy supply due to its potential contribution to climate change mitigation and to supply security. The IPCC assess that by 2050 80 % of the worlds energy demand can be covered by biomass. However, biomass, renewable as it is, is for any relevant, finite time horizon still to be considered a finite resource as it replenishes at a finite rate, and conscientious stewardship of this resource requires that measures are taken to optimize utility of the given resource.

Biomass do service through conversion from a current state to a state closer to or in thermodynamic equilibrium with the surrounding environment. Many studies show that combustion of biomass to generate heat returns a better energy balance than e.g. production of electricity or liquid fuels. An energy balance, however, may not be a proper tool for evaluating systems supporting different energy services. The 2nd law of thermodynamics provides a framework for analyzing the destruction of energy quality in the conversion of biomass to energy services. We present a 2nd law analysis of various options for utilizing cereal straw for energy services and quantify the destruction of energy quality in different process steps in the analyzed pathways. Furthermore we discuss the application of thermodynamics in the evaluation of sustainability.

Conference topic: Feedstock Production for Biorefineries – Linking Eco System Services with Energy Service Demands.

The Integration of a Sustainable Advanced Bioethanol Production in the Value Chain of a Pulp and Paper Biorefinery – European Perspectives based on an Austrian Case Study

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The share of biofuels in the Austrian transportation sector (350 PJ/a) is already 7%, so reaching the European target of 10% in 2020 will be possible with 4 - 5 PJ/a biofuels (150 - 200 kt/a) of 2nd generation like bioethanol from wood, as lingo-cellulosic bioethanol is counted with a factor of 2.5 according to the Renewable Energy Directive (RED). In Austria there are about 10 pulp and paper production plants that have a sustainable wood supply system and have excellent conditions for integrating a bioethanol production using synergies with the current production facilities, e.g. use of excess process heat, water, energy, raw material logistics, personal, co-fermentation of sulphite spent liquor. Based on differnet process technologies 12 different concepts for the integrated bioethanol production in a pulp and paper plant were developed and a sustainability assessment with regards to economic, environmental and social aspects was made based on the whole value chain. The key characteristics of these 12 concepts were the feedstock (e.g. sulphite liquor, wood, starch, maize), the by-products (e.g. feed, phenols, power) and the degree of integration. The optimal integration in the pulp and paper production leads to a very low energy demand, e.g. 0.5 kWh power and 5 kWh per t of bioethanol. Based on investment costs of up to 200 Mio € an annual revenue of up to 80 Mio € is possible and the bioethanol production costs are 0.4 - 0.5 €/l bioethanol. Based on life cycle analyses according to RED the greenhouse gas saving of the lingo-cellulosic bioethanol in these concepts is up to 80% compared to gasoline. In the Austrian pulp and paper industry a realisation of 2 - 3 integrated advanced bioethanol biorefineries until 2020 are feasible, this also reflects attractive perspectives for the European pulp and paper industry for these concepts.

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Robust microorganisms – the key to successful lignocellulose based ethanol production?

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Bioethanol production from lignocellulose raw materials bear a lot of promises, and leave a number of challenges before it can be commercialized with good economical perspectives. The development of the process is driven towards higher gravities and better process integration in order to optimize energy input and water usage. From a microbial point of view this leads to more stressful conditions, including high inhibitor concentrations, high ethanol concentrations and poor nutritional conditions in the hydrolysates to be fermented. Furthermore, at large scale, process hardiness will be expected, including varying environmental conditions during fermentations, due to long mixing times, unsterile conditions leading to contaminations, which calls for solutions that improve the robustness of the fermentation process.

During this presentation the concept of microbial robustness will be discussed and examples of strategies to the design of increased microbial robustness and increasing knowledge of the underlying mechanisms of limited robustness will be given.

Our work is part of Chalmers Energy Initiative and in additions supported in the EU-project NEMO, the Nordic Top level research Initiative project HG Biofuels, Vinnova and the Swedish Research Council.

Development of engineered *S. cerevisiae* strains enabling C5-sugar fermentation for cellulosic ethanol production in the framework of the KACELLE project

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The objective of the KACELLE project is to demonstrate on industrial scale the production of second generation bioethanol in a cost- and energy-effective way. To this direction, the use of lignocellulosic feedstocks, such as wheat straw, instead of starch-based plant material is considered to be of great economic and environmental significance. An important option to improve the economy in large-scale production processes is the complete and fast conversion of both C6- and C5-sugars derived from feedstocks into bioethanol. One of the main challenges for this approach is the efficient fermentation of xylose and arabinose by *Saccharomyces cerevisiae*, as these C5-sugars cannot be used by wild-type *S. cerevisiae* strains. DSM has developed industrial advanced yeast strains that have been genetically engineered to enable the simultaneous conversion of the most abundant biomass sugars (glucose, xylose, arabinose, galactose and mannose) at high yield to ethanol. Here we describe the work aimed at developing and selecting advanced yeast strains that can be applied for the conversion of C5-sugars liberated in the Inbicon process. In this presentation we will share the latest results generated in the framework of the Kacelle project with respect to the fermentation performance of DSM advanced yeast strains.

The research leading to these results has received funding from the European Community's Programme (FP7/2007-2013) under grant agreement n° 239379.

Rapid xylose and glucose fermentation by engineered *S. cerevisiae* for commercial producton of cellulosic ethanol

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Successful use of lignocellulosic material as a feedstock for bioethanol production requires fermentation of both hexose and pentose sugars into ethanol at a high yield and rate. The yeast *Saccharomyces cerevisiae* is an efficient and widely used ethanol producer, but does not naturally ferment xylose, which is the dominant pentose sugar in lignocellulosic materials. Xylose fermentation by *S. cerevisiae* has been achieved by introducing heterologous pathways converting xylose into xylulose, which enters the central carbon metabolism via the pentose phosphate pathway. However, pentose fermentation by *S. cerevisiae* is still slow compared with fermentation of hexose sugars.

By identifying and alleviating a previously unexplored bottleneck in the xylose metabolic pathway, Terranol has developed an industrial S. cerevisae strain for fermentation of xylose at increased rates. In addition to upregulation of several activities of the pentose phosphate pathway, and an efficient bacterial xylose isomerase, the strain expresses a xylose 1-epimerase. The epimerase catalyzes an otherwise slow conversion between the β- and α-epimers of D-xylose, of which only the α-epimer is a substrate of the xylose isomerase.

All inserted genes are stably integrated in the genome of the yeast and the strain has undergone extensive adaptation by evolutionary engineering. The resultant strain has high resistance towards biomass derived inhibitors, low formation of the byproduct xylitol and ferments hexose and xylose in liquid corn stover hydrolysate corresponding to 20 % total solids with an ethanol yield of more than 80 % of total monomeric sugars in as little as 48 hours.

Continuous Biofilm Reactors for Organic Acid Production: Influence of Shear Homogeneity on Biofilm Productivity

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The continuous and anaerobic production of L(+)-Lactic acid and Succinic acid was investigated in two separate studies, employing *Lactobacillus Rhamnosus* (ATCC 9595) and *Actinobacillus Succinogenes* respectively. Both organisms are prone to surface attachment and biofilms were grown in various reactor geometries to determine the influence of liquid shear and the shear distribution on the productivity. A tubular geometry resulted in the highest productivity per biofilm area for both reactions. The packed bed reactor (slightly ebulated to prevent clogging) gave an inferior performance with regards to immobilized area, hinting that shear variations and attrition plays a major role in biofilm productivity.

The investigation was extended on the Lactic acid tubular reactor to gain further understanding on the reactive properties of the biofilm. Proportionality between the area to volume ratio and productivity was obtained and small diameter tubes resulted in productivities as high as 70 g.l-1.hr-1 for a 40 g.l-1 glucose feed. Optimum productivity was obtained at a linear recycle velocities ranging from 0.15 and 0.3 m/s. The biofilm characteristics were devided into 3 zones (as a function of dilution rate) and a model was developed that accurately predicted the experimental data.

Biophysical factors in biomass recalcitrance

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Factors other than intrinsic enzyme activity affect biomass degradation rate. Environmental factors such as the states of water and water accessibility, the compositions of solvents and substrates and the solids content all affect the likelihood of a successful enzymatic hydrolysis. Furthermore, mechanical effects of mixing and re-arrangement of polymers during pretreatment and enzymatic hydrolysis, ensure disruption of the cell wall matrices and facilitate the successful contact between enzymes and substrate necessary for an effective liquefaction and hydrolysis. Lastly, the supra-molecular structure of the cell wall polymers such as misalignments (dislocations) of cellulose microfibrils as well as cell wall pores and tissue compositions in plant organs determine the amounts of easily digestible biomass, which could help explain the high initial conversion rates witnessed in hydrolysis experiments of heterogeneous substrates.

Investigations by our group have been undertaken, highlighting various aspects of biophysical factors affecting biomass recalcitrance, especially at high solids concentrations. These studies include: 1) The detrimental effects of enzyme adsorption onto lignin and the beneficial effects of calcium ions; 2) The states of water present before and during enzymatic hydrolysis as studied by low-field NMR; 3) the role of dislocations in plant cells during hydrolysis of biomass; 4) CLSM studies of possible pH differences between cell wall pores and the bulk solution; 5) practical application and testing of enzymes in small scale setups at high solids concentrations, allowing testing of novel thermostable enzymes in small amounts; 6) TEM analyses of the structural changes taking place particularly in the crystalline and most recalcitrant regions of cellulose due to oxidative cleavage by GH61 and 7) ATR-FTIR, AFM, SEM and CLSM analyses of cell wall surfaces and plant organs of wheat straw, separately pretreated and enzymatically hydrolysed in order to compare the enzymatic digestibility of leaves and stems in relation to available cell types and their composition. All these investigations elucidate the dynamics of a complex system of multiple factors affecting the conversion rates of enzymatic hydrolysis of lignocellulose.

Cellulose cleavage by a novel type of copper oxidases

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Recently, it has been shown that proteins hitherto (erroneously) classified as CBM33 and GH61 are enzymes which cleave recalcitrant polysaccharides via an oxidative mechanism involving molecular oxygen [1]. The activity of these enzymes, which comprise a new type of copper oxidases, was first discovered for a CBM33 from the bacterium *Serratia marcescens* which is called CBP21 and which acts on chitin [1,2]. A different CBM33 from *Streptomyces coelicolor* (CelS2) was later shown to degrade cellulose by the same mechanism [3].

These studies were followed up by several studies showing that structurally homologous fungal GH61 proteins occurring in species such as *Thermoascus aurantiacus*, *Phanerochaete chrosporium* and *Neurospora crassa* use a similar mechanism for the degradation of cellulose [4-6]. The CBM33 and GH61 enzymes are unique in that they work on crystalline surfaces, thus boosting the activity of classical hydrolytic enzymes that are dependent on gaining access to single ("de-crystallized") polysaccharide chains and chain ends [1]. These novel enzymes, which are abundant in biomass-converting microorganisms, represent a new paradigm for the saccharification of cellulose.

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Fundamentals and practices for efficient production of biofuel from lignocellulose

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This presentation will reveal some of the fundamentals related to lignocellulose recalcitrance and inhibitive phenomena to enzymatic hydrolysis of lignocelluloses. Specifically, I will discuss our understanding of the effects of cellulose accessibility, lignin inhibition, as well as biological properties of lignocelluloses on enzymatic hydrolysis. I will then discuss the consequences of these issues to practical biorefinery operations. Based on the fundamental understanding, I will outline potential solutions to address practical issues related to cellulose saccharification, process energy efficiency such as wood size reduction, water usage, and co-product potential. I will use the sulfite Pretreatment to Overcome Recalcitrance of Lignocelluloses (SPORL) developed in our laboratory to demonstrate these potential solutions.

Discovery of novel GH61 and attempt to correlate subfamily grouping with taxonomy and physiology of producing organism

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In the continuous search, for even more effective enzymes for biomass degradation the glycoside hydrolase (GH) family 61 has been shown to be an important auxiliary protein in fungal degradation of lignocellulosic biomass. There are 152 fungal entries for GH61 in the CAZY database and so far no subgroupings have been identified. A conserved domain search in the protein databases available at NCBI (http://NCBI.nlm.nih.gov.protein/) identifies more than 467 proteins from 51 different fungi, thus a single species can have the genetic capacity to produce several GH61 proteins. With the aim of understanding the biology behind the various genetic capacity of the GH61 genes in each fungus, we are analysing the gene expression pattern of the set of GH61 genes present in a single fungus, by challenging the fungus with different substrates and analysing the gene expression pattern by quantitative real time PCR.

We are also aiming at discovering new GH61 proteins from relevant biomass degrading fungi. Due to low sequence similarity a conventional degenerated primer approach based on sequence alignments has low success rate, thus the new tool for Peptide Pattern Recognition (PPR) was applied (Busk PK and Lange L, AAU patent). PPR divided the GH61 family into a number of comprehensive groups and identified the conserved motifs in each group and showed that one group of GH61 proteins was specifically present among the basidiomycetes. This allowed us to target the degenerated primer design for this specific subgroup and resulted in successfully identification of novel GH61 sequences from 5 basidiomycetous fungi. At present we are working on the heterologous expression and purification of the identified GH61 proteins.

Techno-economic evaluation of integrated ethanol production from sugarcane

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Sugarcane bagasse and leaves are valuable lignocellulosic residues for the development of 2nd generation ethanol production, due to availability, amount and composition. Today, bagasse is burned in sugar mills with low efficiency to provide energy for the plant self-sustainment. Alternatively, in the near future sugarcane residues can be used as a lignocellulosic feedstock to produce extra ethanol which can be integrated in several ways with the actual sugar ethanol production. However the rise in electricity selling price creates a competition for the residues supply to either electricity or 2nd generation ethanol production.

In the present work the process of 1st and 2nd generation bioethanol from sugarcane as a whole is modeled in the flowsheeting program AspenPlus. Bagasse and leaves are converted by catalyzed steam pretreatment followed by enzymatic hydrolysis and fermentation. Biogas is obtained from the stillage by anaerobic digestion and the combusted in the boiler to improve the energy recovery.

The aim of the study is to highlight the effects of different integration options on mass and energy balances as well as on operating and capital cost. Also the opportunity to improve, replace or add new stages is evaluated.

Results from the technical and economic evaluation will be presented for the most promising scenarios, discussing the marginal production costs from 2nd generation ethanol in relation to process configuration, energy use, electricity and overall ethanol production.

Development of a Biorefinery concept for integrated production of Biomedicals, Biochemicals, Food, Feed and Fuels from selected plant materials (BIOREF)

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The core of the project is development of a biorefinery concept using selected biomasses (alfalfa and compost) for an integrated production of biofuels, biochemicals, antibiotics, and additives to food and feedstocks. Production of high-value compounds of industrial importance is highly appealing in addition to biofuels even when this production makes use of the same substrate. This opportunity will be pursued along with development of new methods that effectively extract compounds from the biomass material for direct use as food and feed additives, and from the pretreated biomass for use as chemicals and drugs.

Alfalfa was selected as the primary biomass because it is not used directly for food, has a high content of protein, and can be harvested twice a year. Compost was selected because of its low value and high content of lignocellulose.

Through the project we have obtained different important results: We have developed a method for extraction of high quality proteins from alfalfa for use as feed. We have identified compounds in the plant material that have antimicrobial properties by blocking quorum sensing of pathogenic bacteria. We have identified several new biocatalysts for production of enzymes, biochemicals and biofuels. We have also discovered new thermophilic enzymes (cellulases) and thermophilic biofuel-producers, which will economise the processes in the biorefinery. Selected biocatalysts are being improved using genetic engineering.

Co-production of ethanol, hydrogen and gas in a second generation biorefinery based on agro-wastes. Conceptual design and NPV analysis in mid-size economies

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Economics of ethanol production have been widely studied under single-product production schemes. In the case of using agro-wastes as a feedstock, large-scale production costs of ethanol are mainly driven by raw materials (i.e. feedstock and enzymes) availability and prices. For medium and small-scale agro-industrial sectors, processing technology plays an important role and its associated costs may contribute on a similar basis as the raw materials do, to the final production costs. Under these conditions multipurpose production strategies, commonly employed in other sectors such as pharmacy and food, may benefit economics of ethanol making it feasible for medium and small-scale agro-industrial sectors.

This work presents the conceptual design of a multipurpose plant for the co-production of bioethanol, biohydrogen and biogas using a hypothetical raw material based on wheat straw for small-to-medium scale agro-industrial sectors. Production costs are assessed using standard NPV techniques. The studied biorefinery flowsheet is based on a previous proposal employed for bioethanol production (including 1) acid pretreatment, 2) enzymatic saccharification and fermentation, 3) bioethanol separation, 4) waste water treatment, 5) cogeneration) in which a dark fermentation stage was added for biohydrogen production and the waste water treatment stage was modified to consider the input from the biohydrogen process residues. Conversion rates and separation factors were obtained from the open literature and our own experimental data. The paper briefly presents the state-of-the art for the biogas and biohydrogen production and elaborates the rationale employed in choosing the process technologies as well as their integration to the previous production scheme. The process equipments were dimensioned based on the flowrates from mass balances and a standard conceptual design procedure was employed to calculate their costs. Total capital investment and production costs were calculate using the same procedure. Boundary values for polysaccharide concentrations and costs correspond to agro-wastes currently available in the domestic markets. Plant sizes were bounded between 100 and 2,100 ton/day thus making this study representative of small-to-medium-scale agricultural sectors.

The paper presents the results of the production costs analysis using NPV techniques with feedstock flowrate and polysaccharides concentrations (i.e. feedstock price) as parameters. Life plant and ROI were fixed at 15 years and 4% respectively. Boundary cases for biohydrogen and biogas production (0% and 100% of hidrolyzate to biohydrogen production) were included in the NPV model with fixed selling prices. Minimum ethanol price (0.43 USD/L) was achieved using 35% polysaccharides content and 2100 ton/day as feedstock flow rate. At the same conditions but with the single-production of ethanol, the price is 0.52 USD/L. Coproduction schemes in all cases showed an approximate 20% increase in total income compared with the single-production of bioethanol. However, ethanol production prices remain still high to compete with expected prices in the short term. The paper concludes comparing these results with others previously published and discussing advantages and disadvantages for the different scenarios considered.

Keywords: biorefinery, conceptual design, NPV, bioethanol, biohydrogen, biogas.

Introduction to HGBiofuels

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HGBiofules is a Nordic consortium of Chalmers University; University of Copenhagen; Statoil-Hydro; Inbicon - a DONG Energy subsidiary and SEKAB E-Technology. HGBiofuels is an abbreviation for High Gravity hydrolysis and fermentation of lignocellulosic material for production of Bio-fuels.

With the diminishing availability of oilbased fuels, new energy solutions are called for. There is a large interest of using bioethanol as a transportation fuel, however, using raw materials that compete with food production is not a sustainable and acceptable solution. Therefore we will work with the 2nd generation biofuels, making use of lignocellulosic raw materials. To meet the requirement of an economically feasible process, we will develop the process towards high gravity, i.e. operating at as high raw material concentrations as possible.

Biobutanol is an alternative to bioethanol that has received increasing attention in later years, due to that it has several advantages over bioethanol; however, its production via a fermentation pathway faces a number of challenges. In the project we will develop and compare ethanol produced via a high gravity path with butanol production using bacteria and yeast. Life cycle analsysis will be used to assess the environmental performances. The focus area will be to optimise the enzymatic hydrolysis and the fermentation process. i.e. how are these two process steps interacting and integrated in a high gravity process. In addition, the project aims at solving and understanding fundamental challenges that arise when operating at high gravity.

Innovative Biofuel-driven Biorefinery Concepts and their Assessment – An Outlook until 2025 in IEA Bioenergy Task 42 „Biorefinery“

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In IEA Bioenergy Task 42 „Biorefineries“ biorefinery is described: “Biorefining is the sustainable processing of biomass into a spectrum of bio-based products (food, feed, chemicals, materials) and bioenergy (biofuels, power and/or heat)”.

Both “energy-driven” biorefineries and “product-driven” biorefineries are distinguished:

- In energy-driven biorefineries the biomass is primarily used for the production of energy carriers (transportation biofuels, power and/or heat); process residues are sold (e.g. feed) or upgraded to added-value bio-based products, to optimize economics and environmental benefits.
- In product-driven biorefineries the biomass is fractionised into a portfolio of bio-based products with maximal added-value and overall environmental benefits, after which the process residues are used for power and/or heat production.

Based on the ongoing activities in the 13 participating countries (A, AUS, CA, DK, EU, FR, G, I, IR, NL, T, UK, US) the task identifies and assesses the current status and development potential of both energy-driven and product-driven biorefineries. These assessments are based on a “Full Value Chain approach”, covering raw materials issues, conversion processes and final product applications in an integrated approach.

The following most interesting energy-driven biorefinery concepts until 2025 and their value chains, including the integration and deployment options in existing industrial infrastructures are analysed, where these concepts are oriented on the most interesting transportation biofuels until 2010:

- A 1-platform (oil) biorefinery using oil based residues&oil crops for biodiesel
- A 1-platfrom (C6 sugar) biorefinery using sugar&starch crops for bioethanol
- A 2-platform (syngas, electricity.&heat) biorefinery using wood for FT-Biofuels
- A 2-platform (electr.&heat, syngas) biorefinery using straw for FT-Biofuels
- A 3-platform (electr.&heat, lignin, C6&C5 sugar) biorefinery using wood chips for bioethanol
- A 3-platform (C6&C5 sugar, electr.&heat, lignin) biorefinery using straw for bioethanol
- A 5-platform (biogas, biomethan, green pressate, fibres, electr.&heat) biorefinery using grasses for biomethan
- A 5-platform (biogas, biomethan, green pressate, fibres, electr.&heat) biorefinery using grasses for biomethan
- A 2-platform (electr.&heat, biomethan) biorefinery using wood chips for biomethan (SNG)
- A 4-platform (electr.&heat, hydrogen, biomethan, syngas) biorefinery using wood for biomethan
- A 3-platform (pulp, syngas, electr.&heat) biorefinery using wood for FT-Biofuels
- A 4-platform (C6&C5 sugar, lignin&C6 sugar, electr.&heat) biorefinery using saw mill residues and wood chips for bioethanol
- A 4-platform (biogas, biomethan, oil, el. &heat) biorefinery using algae for biodiesel,
- A 5-platfrom (C6-, C6&C5 sugar, lignin, syngas, electr.&heat) biorefinery using starch crops&straw for bioethanol

The Task 42 is assessing the sustainability assessment of these concepts by analysing economic, environmental and social aspects in comparison to conventional processes and products. For this comparative assessment of biorefineries and conventional systems the following framework is relevant:

- The biorefinery system and the conventional systems provide the same amount of products with the same service, e.g. same amount of transportation service, same amount of materials or chemicals
- The same amount and type of biomass is considered in both systems
- The same amount of agricultural and/or forestry area use is considered in both systems
- The whole (“value”) chain approach – from resource to products – is considered

Value Added Products from Biorefineries – Bio Based Chemicals

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Around the world significant steps are being taken to move from today's fossil based economy to a more sustainable economy based on biomass. The transition to a bio-based economy has multiple drivers;

- The need to develop an environmentally, economically and socially sustainable global economy,
- the anticipation that oil, gas, coal and phosphorus will reach peak production in the not too distant future and that prices will climb,
- the desire of many countries to reduce an over dependency on fossil fuel imports, so the need for countries to diversify their energy sources,
- the global issue of climate change and the need to reduce atmospheric green house gases (GHG) emissions,
- and the need to stimulate regional and rural development.

One of the key institutions to accommodate this transition is the IEA Bioenergy implementation agreement. Within IEA Bioenergy, Task 42 specifically focuses on Biorefineries; e.g. the co-production of fuels, chemicals, (combined heat &) power and materials from biomass. A key factor in the realisation of a successful bio-based economy will be the development of biorefinery systems allowing highly efficient and cost effective processing of biological feedstocks to a range of bio-based products, and successful integration into existing infrastructure. Although global bio-based chemical and polymer production is estimated to be around 50 million tonnes, the historic low price of fossil feedstocks together with optimized production processes has restricted commercial production of bio-based products. The recent climb in oil prices and consumer demand for environmentally friendly products has now opened new windows of opportunity for bio-based chemicals and polymers. Industry is increasingly viewing chemical and polymer production from renewable resources as an attractive area for investment. Within the bio-based economy and the operation of a biorefinery there are significant opportunities for the development of bio-based building blocks (chemicals and polymers) and materials (fibre products, starch derivatives, etc). In many cases this happens in conjunction with the production of bioenergy or biofuels. The production of bio-based products could generate US$ 10-15 billion of revenue for the global chemical industry.

The economic production of biofuels is often a challenge. The co-production of chemicals, materials food and feed can generate the necessary added value. This report highlights all bio-based chemicals with immediate potential as biorefinery 'value added products'. The selected products are either demonstrating strong market growth or have significant industry investment in development and demonstration programmes. The report introduces companies actively developing bio-based chemicals and polymers and provides Information on potential greenhouse gas emission savings and how the co-production of bio-based chemicals with biofuel can influence the economics of biofuel production.

Biorefinery Developments in Australia

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The current progress and developments of Biorefineries in Australia will be presented and analysed. The first part of the presentation will review the unique conditions under which a Biorefinery must operate in Australia. The second will go over the biomass sources available. Finally the current operating biorefineries and the main research and development activities will be presented.

Updates on the implementation of biofuels

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The International Energy Agency's Bioenergy Task 39 undertakes a biannual review of biofuel implementation agendas across the Task membership and in other key biofuel producing nations around the world. This presentation provides the result of our most recent update. Among the discussion points are relatively new policy developments in the US, where a 30-year excise tax exemption on biofuels has expired, along with tariff barriers that prevented trade with Brazilian ethanol producers. We also consider statements by military and by airlines which could potentially create new markets for biofuels, although not necessarily markets for low-energy density options such as ethanol. The current status of key issues affecting biofuels – particularly indirect land use change – is explored. One interesting conclusions is that policy supporting biofuels in the future needs to shift from a focus on fuel types (i.e. ethanol, biodiesel, 1st- or 2nd-generation) and focus more intensively on environmental metrics in order to respond to challenges from both environmentalists and other energy industries.

Liquid Biofuel Production in New Zealand: Opportunities and Challenges

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New Zealand is a geographically-isolated country which imports essentially all its transportation fuels, with small amounts of biofuels currently produced internally. Increased production of biofuels would lessen New Zealand's dependence on fossil fuels, improve it's balance of trade, reduce greenhouse gas emissions and also have a positive impact on regional employment. Biofuel substitutes for diesel and jet fuel have been identified as being more strategically important, as these are critical to the farming, transportation, construction and fishing sectors.

Only wood can be grown in sufficient quantities to make biofuels mainstream without impacting on existing uses for higher-value agricultural land. New Zealand currently has 1.8Mha of plantation forest, and 9.1Mha of hill country that is either marginal land or currently used for low to moderate productivity grazing. Our work indicates that 100% of New Zealand's road transport fuel requirements could be met by establishing forests purpose-grown for biofuel production on 2.5Mha of this marginal land. Forest residuals and wastes, while being environmentally beneficial if used for energy, are limited in their scale. New Zealand's current plantation forest estate is predominantly Pinus radiata and this is also likely to be an important species for these future forests due to its proven performance in New Zealand. Afforestation scenarios demonstrate that these new plantings would have environmental benefits from reduced erosion, reduced greenhouse gas emissions and increased carbon stock.

The industry-driven New Zealand Bioenergy Strategy builds on this thinking. The strategy aims to achieve economic growth and employment and realise greater value from New Zealand's existing forestry resource and new energy crops, and to lift bioenergy use to 25% of consumer energy by 2040, including supplying 30% of the country's transportation fuels. A recently-released economic impact assessment of this strategy will be briefly discussed, as will the challenges associated with implementing this strategy.

Biofuels Demoplants

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Throughout the past few years an increasing number of advanced biofuels projects are being pursued, promising that soon technology providers will demonstrate their ability to produce transportation biofuels from lignocellulosic raw materials. However, only few facilities in the demonstration scale are actually operating to date. The biofuels community is curious to find out who is pursuing these projects, which technologies are being developed, and by when lignocellulosic biofuels will finally become available in significant volumes.

IEA Bioenergy Task 39 "Commercializing Liquid Biofuels from Biomass" as a global network on biofuels is in the unique position to provide a global overview on lignocellulosic biofuels production facilities. A database on biofuels demoplants has been set up. This database feeds into an interactive map (available online at http://demoplants.bioenergy2020.eu) and into a report (available at the IEA Bioenergy Task 39 website www.task39.org). The presentation will include a tour around the globe on biofuels demoplants.

Status of Production of Advanced Liquid Biofuels in the United States

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A variety of biochemical, thermochemical and hybrid technology pathways exist to produce advanced liquid biofuels from lignocellulosic biomass and many of these routes are conceptually promising from technical, economic and environmental perspectives. While no processing approach has yet been proven at commercial scale and only modest volumes of advanced liquid biofuels are currently being produced in the United States, numerous projects are underway to demonstrate the ability to scale up the technologies and achieve cost-competitive production economics. Many of these projects are being cost-shared by the United States Department of Energy and some are also getting government-backed loan guarantees to reduce the financial risk associated with constructing first-of-a-kind demonstration facilities. This presentation will describe the range of projects active in the United States and discuss their current status. Many demonstration and commercial scale facilities in the United States are forecast to be producing multi-million gallon volumes of advanced liquid biofuels products within the next several years.

Biomass conversion into YXY (furan) building blocks for polyester applications

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Avantium Chemistry (www.avantium.com) explores novel furan chemistry, focused on efficient and low cost conversion of C6 sugars (i.e. glucose, mannose, galactose and fructose) and C5 sugars (i.e. xylose and arabinose) into derivatives of the promising chemical key intermediate hydroxymethyl furfural (HMF) in the presence of a homogeneous or solid acid catalyst.

By applying its advanced high-throughput R&D technology, Avantium develops a next generation biopolymers and biofuels, called "YXY Building Blocks", which can be produced on the basis of sugars and other, non-food, carbohydrates. Furanics are products derived from carbohydrates. The company is developing chemical, catalytic routes to produce Furanics for a range of biopolymer and biofuel applications with the following advantages: The use of biomass as feedstock, leading to green and sustainable products with a low carbon footprint and small Non-Renewable Energy Usage; a fast and cost-effective production process on the basis of catalytic technology; the excellent fit of the technology with existing chemical production assets, such as chemical plants and refineries; the broad range of applications representing high-value markets

Polyesters are a fast-growing group of plastic materials. The most important polyester is PET (polyethylene terephthalate) which is produced on the basis of purified terephthalic acid (PTA) and ethylene glycol (EG). Exchanging the EG building block in PET for another diol makes it possible to produce other polyesters, such as PBT (polybutylene terephthalate, a polyester based on PTA and butylene glycol) and PPT (polypropylene terephthalate, a polyester based on PTA and propylene glycol), each with its own specific properties, applications and volumes. Avantium aims to replace oil-based polyester (such as PET) with Furanics polyesters (such as PEF) in a wide range of applications including bottles and carpets.

The price-performance ratio of any new material is essential to successful market adoption. Unlike the current biomaterials which are mainly characterized by biodegradability (rather than durability) and poor processability at a high price obstructing their commercial roll-out, furan based polyesters have unique properties resulting in renewable, durable, high-quality materials at a competitive price.

In this paper the whole value chain from feedstock sourcing, upscaling of the technology as well as new results on the physical and chemical characteristics (Tg, Mn, viscosity, colour) of furan based polyesters as well as processability and behavior in films and bottles will be discussed.

Biorefining for high value food and feed ingredients

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Several current industrial processes utilizing plant material as the raw material is economically dependent on a single product.

Development of sustainable industrial plant products based processes aiming at utilization of residual products which currently is low value products has been a main research focus in the last decades. Key targets in this work has been the search for added quality of final products to ensure added value. Added value to all products is vital as increased industrial process steps normally is linked to increased process costs.

A semi-industrial scale process for biorefining of starch potatoes based on extractions using "Green Chemistry" has been developed and patented. The developed gentle environmental friendly process steps avoid use of any petrochemicals and it provides the basis for production of high quality products for use in food and feed applications.

This type of processing result in a yield of protein products, dietary fibres and fine chemicals giving the basis for a significant increase in the value of processed products compared to the traditional potato processing mainly relying on sales of potato starches.

Likewise, innovative processes designed for industrial processing of different legume and cruciferous crops has been developed with a significant potential for increased value and reduced environmental impact.

Low pH Fermentation to Succinic Acid, the Basis for Efficient Recovery

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Royal DSM N.V., the global Life Sciences and Materials Sciences company headquartered in the Netherlands, and the French starch and starch-derivatives company ROQUETTE have joined forces to implement and commercialize the fermentative production of bio-renewable succinic acid, which – amongst other applications - opens the possibility to produce bio-based performance materials. Succinic acid is a chemical currently produced as a derivative from crude oil and natural gas. It is commonly used directly in a variety of industry applications, such as pharmaceuticals, food and automotive and also as an intermediate for the production of several (high-performance) polymers.

For future economic success of its major applications it is important to realize the lowest possible cost price for succinic acid. We deliberately chose for a fermentation process at low pH, thereby directly producing the acid. In this way salt production in the recovery process is minimized and at the same time its carbon footprint will be lowered. In this paper we would like to stress the importance of certain key performance indicators (KPI's) and illustrate their effect on cost price.

To realize our ambitions we have engineered yeasts to produce succinic acid with high titer and yield. This was accomplished by introducing heterologous genes that were optimized for expression in the host. Strain performance was further improved in controlled fermentations towards a low pH. Currently, the process has been successfully scaled up to ~100 m^3 in our demonstration plant. In parallel, implementation for future bigger scale operation is in progress.

Pilot plant facility for the scale-up of continuous mode lactic acid fermentation

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Renewable feedstocks (e.g. crops, lignocellulosics, green biomass, residues) are being used as raw materials for the production of microbial lactic acid. Lactic acid, its salts and esters have a wide range of potential uses and are extensively used in diverse fields. The goal is to develop a fermentation process based on the substitution of expensive nutrient supplements by cheaper materials from biomass due to their main proportion of the whole process costs.

The scale-up to a technical scale of several processing steps have to be developed for transferable solutions of biotechnologies for renewables. For that purpose a multifunctional pilot plant was planned and built at the site of ATB to investigate different raw materials and products. First results of the continuous lactic acid fermentation in a 450-L-bioreactor will be presented. One of the usual ways to keep the biomass inside of the system for increasing the overall pro-ductivity is the cell retention with hollow fibre membranes. In comparison to the process with-out cell recycle (e.g. chemostat) there is a triple up to four time's higher productivity of lactic acid.

Depending on the further processing of the lactic acid the separation of impurities after fermen-tation is a major process cost too. Therefore an optimization is necessary to find a balance be-tween the substitution of expensive nutrients and the limitation of interfering or undesirable components of natural raw materials respectively. Exploitation of high quality L(+)- and D(-) lac-tic acid for the production of biopolymers is one of the recent applications. Conventional proc-esses for down-streaming are based on precipitation steps that generate large amounts of chemical effluents. Consequently the environmental impact of traditional processes can be re-duced by using alternative technologies, such as electrodialysis with monopolar and bipolar membranes.

Experience from the Operation of the Inbicon 2nd Generation Bioethanol Demonstration Plant

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The international biofuel industry has set its focus on the development of new technologies for producing ethanol from agricultural waste and energy crops. In Denmark, DONG Energy A/S biotech subsidiary Inbicon A/S has built a demonstration plant for the conversion of wheat straw to ethanol. The plant demonstrates the process developed by Inbicon and was inaugurated November 2009. The process steps were successively demonstrated and the first dehydrated ethanol was produced in March 2010. In October 2010, the 2nd Generation ethanol was introduced nation-wide on the Danish market by oil company Statoil. The plant capacity is 30,000 tonnes of wheat straw per year, which will produce 4.300 tonnes ethanol, 13,100 tonnes of lignin pellets and 11,250 tonnes of C5 molasses suitable for cattle feed or as a biogas booster. The investment is around €53 mill., of which €10 mill. is funded by Danish government grants, while demonstration and optimization is now supported by EU FP7 program by €9.1 mill. in the Kacelle project. In the 90s, Danish power companies started using biomass for power production, and now several power plants handle 500 tonnes pr. day of wheat straw. Based on the experience with the straw handling, a process was developed for the pretreatment of biomass with the intention of producing ethanol, and in 2002, a R&D project (”Co-production biofuels”) partly funded by the European Commission was initiated. In the course of the R&D project, several technological breakthroughs were achieved, and two pilot plants built in 2003 and 2005. After the completion of the R&D project, the subsidiary Inbicon was formed to focus on and accelerate the commercialization of the technology.

A Biorefinery approach to production of lignocellulosic ethanol and chemicals from low value biomass

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Recent development within the biofuel industry has shown that it is challenging to build a sound business case for advanced biofuels as a standalone operation, even with economic incentives from the national authorities. As a biorefinery operator, Borregaard is currently producing specialty cellulose, lignin chemicals, vanilla flavor and ethanol from various species of wood. The strategy has been particularly successful for lignin, which is considered a troublesome by product in many other contexts. We have adapted a similar strategy to the processing of energy crops, sugarcane bagasse and other agricultural waste products

The result is an operation that is economically sound with a very favorable carbon footprint. Borregaards new biorefinery pilot will turn low value biomass into bioethanol, lignin chemicals and various sugar based chemical products. We have shown that the wood based biorefinery can serve as a template for concepts based on agricultural waste products, and that lignin can be turned into valuable specialty chemicals. Examples are soil conditioners, concrete additives and pelletizing agents. The approach gives opportunities for process integration with existing bioethanol plants to reduce initial investment costs and optimize feedstock costs, simultaneously increasing revenue streams.

SEKAB experiences from scaling up and commercialization of CE-technology

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The presentation will give an overview of technical and commercialization status for SEKAB E-Technology´s integrated cellulose ethanol technology.

Since 2004 cellulose ethanol technology developed within Swedish national ethanol programmes since early 1990´s has been verified and further developed in a continuous demo plant in Örnsköldsvik. The Swedish Energy Agency was the major financer of the demo plant and has also to a large extent financed development projects. SEKAB E-Technology is the company responsible for both operations and technology development in the demo plant. SEKAB E-Technology´s business strategy is to commercialize the technology on an international market. Feedstock of interest are both forest based and agro based lignocellulose feedstock. Different species of softwood and hardwood has been run in the demo plant as well as sugarcane bagasse, wheat straw, corn stover and corn cobs.

The demo plant is operated 24 hours, 7 days per week and the efficient running time now exceeds 30 000 hours. The development, conducted in close collaboration with companies , universities and institutes, has generated extensive experiences and know-how for designing flexible, cost-efficient and robust process solutions. In addition to cellulose ethanol production the technology can be adapted to produce other green chemicals based on the intermediate sugar platform. Present status and results from the development work as well as challenges in taking the technology from demo to business scale will be discussed in the presentation. The ethanol production must be integrated with efficient utilization of dissolved components as biogas and solid residues for e.g energy production. The technology is ready to be implemented and verified in large-scale production. Based on data from the demo plant SEKAB conduct feasibility and pre-studies of production plants e.g. in combination with existing 1st generation ethanol plants

From Sugar Mills to Biorefineries – the sustainable future for the sugar industry

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Through a combination of organic growth and strategic acquisitions AB Sugar (previously known as British Sugar Group) are one of the largest sugar producers in the world. We have sugar cane and beet operations in 10 countries, employing over 42,000 people with a combined sugar production capacity of around 5 million tonnes.

Operations stretch from the UK and Spain in Europe, to southern Africa, to North and South China, encompassing climates suited to both beet and cane, and sited in countries at all stages of economic development.

Whilst sugar remains at the heart of what we do, we have developed and expanded the range of co-products we produce, which include chemicals, fuels, animal feed, soil conditioning and landscaping products, electricity and even tomatoes. The Group is also in seed coating and enhancement technology.

This approach has turned simple sugar mills into world class biorefineries and this presentation will show how the industry has developed and gives some clues to how it might sustainably develop in the future.

Silicon deposition in plant cell walls: transporters involved and implications for bioenergy production

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Incorporation of silicon (Si) in plant cell walls enhances their mechanical strength and contributes to alleviation of both biotic and abiotic stresses (Epstein, 1994). However, when plant residues are used for bioenergy puposes, Si is a problematic element which has negative impact on the quality of the biomass for biocatalytic and thermal conversion processes. Reduced Si content is therefore an important quality parameter and there is a strong interest in reducing the Si content in plant biomass (Gressel, 2008).

We have screened a panel of 20 wheat genotypes for natural variation in straw Si concentration. A 2-fold range in Si concentration was found, reflecting differences among both genotypes, nitrogen regime and growth locations. A micro-scaled enzymatic saccharification assay is carried out to investigate the effect of Si on straw sugar yield. The results obtained so far do not indicate that Si is a significant inhibitor of saccharification but the data analysis is still ongoing.

Si transport in plants is mediated by two aquaporins, Lsi1 and Lsi6. In addition, a secondary active transporter Lsi2 is involved. Lsi1 and Lsi2 are involved in Si uptake and transport through the roots while Lsi6 plays a role in the distribution of Si within the shoots (Ma et al., 2011). In order to find mutant plants defective in Si transport, we have screened a mutant population of the grass species *Brachypodium distachyon*. This species combines many desirable attributes such as a small plant size, short generation time, transformability and small genome (Garvin et al., 2008) and is increasingly used as a model for dedicated bioenergy crops. Phenotypic screening using germanium (Ge), which is a toxic analogue of Si, enabled us to identify mutants in Si transport since Ge is taken up via same pathway as Si but causes necrosis in the leaves. Based on selection of Ge tolerant plants, single mutants for each Si transporter were isolated together with a triple mutant which has mutations in all the Si transporters. After backcrossing, the mutant plants will be characterized with respect to Si accumulation and distribution in the cell walls. Further investigations of how Si interacts with and is integrated into the cell walls are in the pipeline.

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An exceptional biomass for feed, energy and bioremediation. Duckweeds: from characterization to utilization

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Duckweeds are a group of ubiquitous aquatic plants, which live in eutrophic freshwaters and have the highest growth rate of angiosperms. They can reduce the levels of polluting nitrogen, phosphorous and heavy metals, and weight for weight, more ethanol than from corn can be obtained from them without competing with food resources.

Duckweed biomass also has outstanding potential as animal feed since it possesses a nutrient value similar to soybean. However the cell wall compositions of duckweeds are poorly understood. Therefore we will characterize different species and ecotypes to elucidate cell wall composition and architecture and thus find the best candidates for the production of energy and biocomponents. We will use our high throughput microarray technology, which allows to process several hundreds of samples using low levels of reagents and materials.

Genome wide association study for conversion of barley straw into second generation biofuel

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Transformation of biomass into second generation biofuels is still affected by several issues. Among them the inherent recalcitrance of cell wall structures to be accessible by hydrolytic enzymes is a key factor. The recent technical advances in the field of genome scanning and sequencing permit now to explore the genetic biodiversity of crops for a better comprehension of a highly complex system as biomass synthesis is. Undoubtedly it involves thousands of genes spanned over the whole genomes which act also in response to environmental factors.

In this study a collection of 125 old and modern winter barley (Hordeum vulgare L.) varieties was grown in field trials in the north of Italy for 2 years as part of the ERA-PG EXBARDIV project. At the maturity stage plants were harvested and straw collected. Lines were genotyped with 9000 SNPs using the high throughput Illumina iSelect assay, a novel and highly flexible array technology capable of whole genome scanning for SNP polymorphism.

To characterize lignocellulosic biomass for genome wide association studies (GWAS) several components were taken into consideration: degree of recalcitrance to enzymatic hydrolysis, bioethanol yielded, cellulose and lignin content, ferulic and p-coumaric acid. Classic wet chemistry for biomass analysis is time consuming and labor intensive and generally considered as the bottleneck for GWAS. To speed up the phenotypization process we applied modern partial least square (PLS) techniques to create provisional models for biomass composition using near infrared (NIR) spectroscopy and ad hoc software. Association study allowed identifying QTLs involved in cell wall composition and recalcitrance to transformation. Synteny between Barley and Rice, Sorghum and Brachypodium Distachyon was explored to identify candidate genes positioned within the QTLs for further studies.

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Evaluating commercial hybrid poplar clones for bioethanol production by phenotyping analysis

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The expansion of the use of biomass as an energy source is seen by developed nations and many developing countries as a way to reduce the need for fossil fuels to secure national energy supply. Furthermore, this principle is considered a feasible approach to support sustainable development of rural economics based on agriculture and forestry.

The phenotyping analysis is used to support many of its own initiatives revolving around the biology of wood formation and the quality of the resource sector.

The main goal of this study was to suggest a preliminary lab-scale evaluation method for the feasibility of utilizing cellulosic biomass for bioethanol production using phenotyping analysis. Five trees of each of hybrid poplar clones were used in the experiments and analyses. Growth characterization of trees, morphological phenotyping of wood and fibers, and chemical analyses of wood were evaluated and compared to the results from subsequent experiments on saccharification of hybrid poplar samples for ethanol production.

The results from phenotyping analysis were acceptable as the preliminary evaluation method for the feasibility of utilizing hybrid poplar for bioethanol production. As the results, it is obvious that the phenotyping facility will function to characterize existing diverse cellulosic biomass resources for bioethanol production. The phenotyping facilities can provide several critical analyses on any given wood or plant samples. These analyses have been developed to accommodate small samples including Arabidopsis stems and young trees as well as the traditional wood resources.

Keywords: Hybrid poplar, clones, bioethanol production, phenotyping, cellulosic biomass, wood.

Second-generation ethanol production from olive tree pruning

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The use of second-generation bioethanol from lignocellulosic agricultural waste is a very interesting option to reduce dependence on fossil fuels. Biomass of olive tree pruning can be considered a suitable raw material for the production of ethanol due to its high content of potentially fermentable carbohydrates. In addition, it could be an interesting biomass for the production of other products of high added value, such as oligosaccharides and antioxidants, which can positively influence comprehensive waste utilization and reduction of ethanol production costs.

In the present study olive tree pruning biomass pretreated by steam explosion was tested as substrate for ethanol production by the Simultaneous Saccharification and Fermentation (SSF) bioconversion process. Previous to steam explosion pretreatment, a water extraction stage of olive tree pruning was carried out due to the high content in extractives compounds that can produce condensation reactions between extractives and acid insoluble lignin, hindering the enzymatic hydrolysis of the solid pretreated biomass. The steam explosion pretreatment conditions, 187 ºC and 30 min, were selected in a previous work. Pretreatment was carried out in a pilot unit 10 L reactor capacity. Simultaneous saccharification and fermentation (SSF) and presaccharification and simultaneous saccharification (PSSF) of slurry was tested in laboratory experiments at 20% (w/w) consistency using commercial cellulases and hemicellulases [20 mg/ g glucan of CellicCTec2 boosted with CellicHTec2 content (kindly provided by Novozymes A/S) in a ratio of 90:10 based on protein load], and *Saccharomyces cerevisiae* (Ethanol Red from Fermentis, France) under different reaction configurations. The highest ethanol concentration attained in PSSF media was 40 g/L, which corresponded to a yield of 71% of theoretical.

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Genetic analysis of biofuel-associated traits in the C4 plant sweet sorghum

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China, with its remarkable growing economy observed in the last three decades, has shifted from being a net energy exporter to being an importer since late 1990s and is currently one of the largest importers in the world. With its energy consumption doubled over the last 20 years, China is currently the second largest energy consumer (1). Search for alternative sources of energy have become a priority for the Chinese government who has designed a series of planning projects for energy development (2). The focus is on Biofuel, as it is believed to be the most promising substitute for fossil energy and China is now the third largest bioethanol producer (3).

After 2007, the Chinese Government declared that use of cereals as feedstock for bioethanol production is prohibited from expanding due to the impacts on the global food prices and the threat on national and global food security (4-5). After domestic studies and experimentations, three main plant species appear to be potential candidates for biofuel feedstock in China; including cassava, sweet sorghum and sweet potatoes. Sweet Sorghum is believed to be the most promising feedstock source for bioethanol in China because it has a series of advantages.

Sweet sorghum belonging to the Poaceae family is a herbaceous C4 plant with rich sugar content in the stalk directly fermentable into ethanol. A rich germplasm resource is available, high biomass yield, clean and low production cost and the capacity of being grown in "marginal lands" making sweet sorghum the perfect crop for bioethanol production. However the genetic basis for the accumulation of sugar in the stem is poorly understood. Previous research demonstrated that three major components, independents of each others, are necessary in a proper combination to obtain the sweet stem, the texture "porous VS compact", the juice volume "dry stem VS juicy stem" and the sugar content "low VS high".

For this study genome-wide high density molecular markers including SNPs, Indels, PAVs and CNVs were developed (6). Furthermore, 500 sorghum lines were obtained from USA, Africa, Europe and India showing important variations in biofuel-associated traits, multiple segregation populations constructed using parental lines with high density molecular markers and contrasting phenotypes. To characterize and understand the genetic control of these traits quantitative genetic tools will be used for mapping the loci responsible of the stem texture, the juice volume and the brix content (sugar content). Genes involved in the sweet stem will be cloned to develop a marker-assisted stacking of biofuel-associated traits in sweet sorghum.

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The future design of bio-energy crops: Glycan microarrays as a novel tool for the identification of the biosynthetic machinery involved in construction of the cell wall components

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The industrial and therapeutic applications of complex carbohydrates are becoming increasingly important. Efforts to produce energy-efficient crops for bio-fuel production are also underpinned by a detailed knowledge of cell wall biosynthesis, and especially the activities of the glycosyltransferase (GT) enzymes.

One approach for identifying and characterization the in planta activities of GTs is to produce knock-out plants. However, screening large populations of mutants is time consuming and complex because knocking out a single GT often has multiple pleotropic effects. Biochemical characterization of GTs involves heterologous expression of the enzymes followed by in vitro activity assays. This process is complicated by the fact that the appropriate acceptors and donors substrates are often unknown. In the work presented, we describe how carbohydrate microarrays of cell wall related glycans can be used in the GT assays, thereby allowing high-throughput multiplex analysis that will serve as a tool for the identification and characterization of novel cell wall related GTs.

2nd Generation Biofuel Production from Selected Agricultural Waste Resources in Ghana

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The distinctive resources of a tropical, developing country introduces new and complex issues within the conversion of biomasses into energy carriers. In order to assess these, the knowledge gained in the developed countries within the last decades in this area must be transferred, adjusted and further developed.

The main focus in our project will be on development of conversion technologies within both pretreatment of the different biomasses and the biological transformation of pretreated material into energy carriers. Ghana is used as a the model country. The initial part of the project has included characterisation of a wide array of the most common Ghanaian bioresources namely: yam peelings, cassava peelings, cassava stalks, plantain peelings, plantain trunks, plantain leaves, cocoa husks, cocoa pods, maize cobs, maize stalks, rice straw, groundnut straw and oil palm empty fruit bunches.

In this contribution the emphasis will be on the compositional analysis of plantain peelings, trunks and leaves which shows quite different results even though it is closely related biomass residues. The clear differences between the residues will imply alternating optimal uses within bioenergy.

Assessment of second generation biofuel residuals as soil amendment for soil fertility improvement

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A remarkable breakthrough in the search for alternative energy sources to reduce the negative economic and environmental impacts of fossil fuel was the discovery of First Generation Biofuels. Unfortunately, it is realised that their use can contribute to increasing food prices and land use change. Promising alternative raw materials is conversion of non-food crops and agricultural and industrial residues – collectively termed "Second Generation Biofuels". These include Jathropha and lignocellulosic materials such as baggasse, straw and cob residues, wild grasses, saw dust, household waste, green waste and domestic waste among others.

After conversion a modified residue is available as fertilizer, like e.g. anaerobic digestate (biogas), as well as soil improver, like e.g. composted bagasse from bioethanol fermentation. A strong demand and higher prices for energy and primary commodities are expected and it is crucial to utilize these alternative fertilizer resources.

These residues therefore, need to be characterized and managed for additional environmental and socio-economic benefits in crop production. In this study, the nutrient composition of feedstocks, their potential use in direct application to soil to improve soil fertility in rural communities, including microeconomic considerations, will be investigated.

The study will determine i) the chemical characteristics of potential feedstocks and biofuel residues in Ghana; ii) residual nutrient release patterns and greenhouse gas emissions; iii) residual influence on soil biology and fertility including soil physical properties such as hydrophoby, moisture retention and aggregate stability). Appropriate experimental procedures will be adopted to generate the requisite data for statistical analysis and interpretation. The study will focus on biofuel residuals obtained from Jathropha cake, biogas from household, municipal and industrial waste.

Enhancing the availability of carbohydrates in fibre hemp and maize by anaerobic preservation

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Lignocellulosic materials have already reclaimed their place as replacing the first generation biofuel substrates. The questions, which materials would be the most valuable to convert to which energy carrier or which are the best energy crops in different climates if energy crops are acceptable among agricultural residues, are exciting. What is the right pretreatment for different raw-material and what is the effect on storing the crop materials are also of great interest?

The main emphasis in this study was on the role of anaerobic preservation prior the methane process and the enzymatic hydrolysis. Two potential biomass substrates, fiber hemp and the whole crop maize, cultivated in the boreal climate were stored in anaerobic conditions. The increased availability of carbohydrates and the higher conversion of monosaccharides in preserved hemp were observed thus increasing the methane yields by 36% of dry matter. The methane yields of maize silage increased as well however, the prolonged preservation subverted the beneficial effect. The positive effect of preservation in hemp was observed also in enzymatic hydrolysis by the additional dosage of pectin. The total increase in all hydrolysis products, including monosaccharides and galacturonic acid was 54% and 64% (from theoretical carbohydrates) for acidic- and alkaline preserved materials, respectively, whereas the increase in fresh hemp was clearly lower at 26% of the theoretical carbohydrates. The results show that the anaerobic preservation of fresh materials is a potential pre-treatment method for methane production. Improvements in enzymatic hydrolysis were also promising although to obtain a high conversion, further treatments are required

Pretreatment as the crucial step for a cellulosic ethanol biorefinery: Tuning wet explosion process parameters to accomodate conversion of high dry matter concentrations into sugars

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The choice of a suitable pretreatment method and the adjustment of the pretreatment parameters are crucial for the efficiency of the subsequent conversion of any biomass in a biorefinery concept. In this study, the optimal pretreatment parameters for wet oxidation and dilute acid wet explosion were investigated for the conversion of Cocksfoot grass in a biorefinery for 2nd generation bioethanol as the primary product (BornBiofuels). The pretreatment was performed at high dry matter concentration (25%) with no dilution before enzymatic hydrolysis for reducing volume flows through the biorefinery. Pretreatment conditions were 160°C, 170°C, 180°C, 15 min, 87 psi O_2 for wet oxidation and 170°C, 180°C, 190°C, 210°C, 15 min, 0.2%, 0.5% H_2SO_4 for dilute acid wet explosion.

The efficiency of the two pretreatment methods was assessed in terms of cellulose and hemicellulose recovery in the solid and liquid fraction, respectively, their conversion into C6 and C5 sugar monomers (C6 sugars after enzymatic hydrolysis) and finally ethanol fermentation of the C6 sugars by *Saccharomyces cerevisiae* and of C5 sugars by Pichia stipitis CBS 6054. Results show high cellulose and hemicellulose recoveries of up to 99% and 97%, respectively, higher than previously reported for high dry matter concentrations. The enzymatic hydrolysis of cellulose from the solid fraction was generally significantly improved after pretreatment. The highest conversion into C6 sugars was achieved for the dilute acid wet explosion at 180°C, 15 min, and 0.2% H_2SO_4. The formation of degradation products (furfural, hydroxymethylfurfural (HMF), and carboxylic acids) increased with the pretreatment severity with the highest levels of 5.7-7.8 g/100gDM for carboxylic acids, 2.4-4.5 g/100gDM for furfural, and 0.5-1.7 g/100gDM for HMF at pretreatment conditions of 180°C, 15 min, 87 psi O_2 and 210°C, 15 min, 0.5% H_2SO_4, respectively. Simultaneous saccharification and fermentation (SSF) by *Saccharomyces cerevisiae* resulted in ethanol yields from the cellulose fraction of up to 251 mL/Kg-DM (98% of theoretical) for the dilute acid pretreatment at 180°C, 15 min, and 0.2% H_2SO_4. The resulting ethanol yield from C5 monomers in the liquid fraction from the pretreatment was up to 158 mL/Kg-DM (92.2% of theoretical) for wet oxidation at 160°C, 15 min, 87 psi O_2. Ethanol yields from C5 monomers in the liquid fraction decreased, however, drastically for more severe conditions (180°C, 15 min, 87 psi O_2/190°C, 15 min, 0.2% H_2SO_4/210°C, 15 min, 0.5% H_2SO_4) due to high degradation of C5 monomers in the liquid hydrolyzate. Consequently, pretreatment with a higher severity would be the choice for achieving highest cellulose conversion while less severe pretreatment would be the method of choice to achieve highest total ethanol yields from both C6 and C5 sugars.

Optimization of microwave pretreatment on wheat straw

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Lignocellulosic materials represent an abundant, inexpensive feedstocks for fuel ethanol production. Recalcitrance of lignocellulosics to degradation is one of the main processing problems. Pretreatment of lignocellulosic raw material is necessary to open up the structure and to increase accessibility to enzymatic attack, thereby improving the overall biological conversion to ethanol. A lot of attempts have been done in this area including physical, biological and chemical technologies.

Our research aimed to use microwave pretreatment of wheat straw in order to enhance bioethanol production. Based on our earlier results a statistical design (orthogonal design - $L_9(3^4)$) was used to optimize the process, where the effect of four factors including the ratio of biomass to NaOH solution, pretreatment time, microwave power, and the concentration of NaOH solution with three different levels on the enzymatic and ethanol convertibility of wheat straw. After pretreatments the slurry was always filtered and both liquid and solid fractions were analyzed. Solid fraction was further tested for convertibility in enzymatic hydrolysis at 50°C for 72h at pH4.8 applying commercial enzyme preparations and for ethanol convertibility in Simultaneous Saccharifcation and Fermentation (SSF) process by baker´s yeats.

According to the orthogonal analysis, pretreatment with the ratio of biomass to liquid at 80 g kg^{-1}, the NaOH concentration of 10 kgm^{-3}, the microwave power of 1000Wfor 15 min was confirmed to be the optimal condition. The ethanol yield was 148.93 g kg^{-1} wheat straw at this condition, much higher than that from the untreated material, which was only 26.78 g kg^{-1}.

Compositional changes in high yielding energy grass ensiled with different inoculants and at different dry matter concentrations

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The biological storage method of ensiling shows promise as a mild, cost-effective method that could potentially reduce severe hydrothermal pre-treatments for lignocellulosic biomasses, such as perennial grasses and agricultural residues. Here, we correlate changes in biomass structure under different ensiling conditions, including different dry matter (DM) concentrations and silage inoculants.

Festulolium Hykor, a crossbreed of perennial rye grass and tall fescue grass developed and produced by DLF TRIFOLIUM was harvested and ensiled in vacuum bags at DM concentrations of 22, 32, and 42 percent, - and treated with two commercial lactic acid bacteria (LAB) inoculants, LACTISIL CCM and LACTISIL Grass Plus from the company Chr. Hansen. Controls without LAB inoculation were also ensiled for each of the DM concentrations. At 47 days the silaged grasses were analysed for their biochemical composition and compared with dried grass.

Analysis of the chemical composition showed that both cellulose and hemicellulose were stored without any losses. Free sugars and oligomer carbohydrates, were consumed by LABs producing different mixes of organic acids as well as mannitol. Low DM concentrations at ensiling gave rise to the highest production of organic acids, and also the largest differences between inoculants treatment. At higher DM concentrations compositions of produced organic acids showed high similarity. The results showed the importance of having a controlled and efficient ensiling process, when storing biomass for bioethanol fermentation, and thereby reduce the loss of free sugars.

Laccase detoxification of steam-exploded wheat straw for ethanol production by SSF processes with the thermotolerant yeast *Kluyveromyces marxianus*

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Among the strategies for energetic diversification in the transportation sector, the use of liquid biofuels from vegetable biomass, such bioethanol or biodiesel, is a simple, efficient and realistic choice in short-medium term. In this context, biochemical ethanol production from lignocellulosic biomass is one of the most promising options. However, the recalcitrant structure of lignocellulosic material limits the accessibility of cellulose to cellulolytic enzymes, doing necessary a pretreatment step. Hydrothermal processes, such as steam explosion, are the most effective technologies for the pretreatment. During this step, lignin is redistributed and hemicellulose is partially hydrolyzed and solubilized, making cellulose more accessible to enzymes. In contrast, this pretreatment generates some soluble inhibitory compounds, derived from a partial sugars and lignin degradation, which can affect enzymatic hydrolysis as well as fermentation steps. Thus, a detoxification step is necessary for the use of the whole slurry obtained after pretreatment.

This work is aimed to study the use of laccase for the detoxification of steam exploded wheat straw. The detoxified material was later used in a simultaneous saccharification and fermentation process using the thermotolerant yeast *Kluyveromyces marxianus* CECT 10875. Laccase treatment removed selectively phenolic compounds, been enough to reduce greatly lag phase of the microorganism and enhance ethanol production and yields.

Integrated storage and pretreatment of biomass for more efficient biofuel production

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Preservation of biomass and its pre-treatment represent major inputs of energy and costs in the biofuel production process. Especially thermochemical pre-treatment of lignocellulose is a major bottleneck in commercialising ethanol production from lignocellulosic biomass, since it takes about 20% of the total process energy and produces inhibitors for the subsequent fermentation. Drying of biomass for preservation is an energy demanding method, which can take up to 60% of total energy consumption during production of cereal grain and is not exactly determined for lignocellulose.

We investigated ethanol production from moist stored wheat or wheat straw. For wheat grain, more than 10% increased ethanol yields were obtained compared to dry grain. Biopreservation with the biocontrol yeast Hansenula anomala efficiently inhibited mould growth on the grain, but had no impact on ethanol production, compared to non-inoculated controls that were not infected by moulds. On moist wheat straw, the yeasts H. anomala and Scheffersomyces stipitis were most efficient in inhibiting moulds, which was the most critical problem in moist storage. After a non-optimised laboratory scale pretreatment an increase in ethanol yield of 10-40% was observed, with slightly higher values for S. stipitis.

Thus, we developed an Integrated Storage and Pretreatment (ISP) method for an efficient conversion of biomass to ethanol. Further aspects, like decreasing the input in thermochemical pretreatment after ISP are currently under investigation in a project financed by the thematic research program MicroDrive and The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Formas).

Ionic Liquid based biorefinery – the Ionosolv pretreatment

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Ionic liquids are low-melting organic salts and can be used as virtually non-volatile polar alternatives to established molecular solvents. They have shown potential in the processing of cellulose and lignocellulosic biomass and as they can solubilise the entire wood composite or selectively hemicellulose and lignin.

Since 2007 our research group has investigated the application of ionic liquids in biomass pretreatment and contributed to understand the role of ionic liquid composition, studied the effect of moisture introduced by biomass and solvent, discovered a moisture-tolerant ionic liquid pretreatment, demonstrated energy-efficient particle-size reduction in the presence of ionic liquids and investigated the impact on biomass.

The opportunities and challenges of ionic liquid pretreatment are discussed and an outline for a potential biorefinery concept involving ionic liquids presented.

Enzyme hydrolysis of sugar cane bagasse pre-treated for fermentable sugar obtainment

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The sugar cane bagasse is a residue produced in a large volume in the ethanol production process. In this year, Brazilin industries of sugar and ethanol produced around 168Mi ton of bagasse. The high carbohydrate content of this residue became it an important substrate to obtainment of fermentable sugar for ethanol production.

The present work evaluated the use of microwave for pre-treatment of sugar cane bagasse in presence of glycerol, acids and alkali for 5 min at 180 ºC, ultra-sound and ozone. The assays were carried out in factorial designs. The highest level of reducing sugar and phenolic compounds was obtained in treatment with microwave and glycerol, followed by those in presence of NaOH. The treatment with ozone and ultra-sound did not provide a significant increase in the reducing sugar liberation compared to water only. The subsequent enzymatic hydrolysis showed that pre-treated bagasse with microwave in presence of glycerol afforded the highest reducing sugar releasing. HPAEC-PAD analysis revealed the presence of arabinose, galactose, glucose, xylose, xylobiose and cellobiose.

Key words: sugar cane bagasse, pre-treatment, enzyme hydrolysis.

Studies on cellobiohydrolases from *Aspergillus terreus*

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Cooperative action of a versatile mixture of cellulolytic enzymes is necessary to break down cellulose in plant cell walls. The main players in this enzymatic system are: endoglucanases (breaking internal cellulose bonds), cellobiohydrolases (breaking cellulose from reducing and non – reducing ends exposed by endoglucanases) and β-glucosidases (releasing glucose from cellulose). A combination of cellulolytic enzymes is exactly what biorefineries use to convert plant biomass into different biobased products such as biofuels and bioenergy. Filamentous fungi, utilizing carbon available in plants, can naturally produce a variety of enzymes: cellulases, hemicellulases, pectinases, esterases, oxidoreductases and proteases.

In this project, different fungal species (*Aspergillus* sp., *Trichoderma* sp. and *Penicillium* sp.) were screened for cellulase production by growing them on different carbon sources. *Aspergillus terreus*, producing high levels of endoglucanases, was also capable to secrete the highest levels of efficient cellobiohydrolases. Being one of the most promising enzyme producers, it was selected for targeted gene finding followed by expression studies in order to identify highly abundant cellobiohydrolases. Recently, A. terreus sequencing project annotated genes encoding cellobiohydrolases from glycosyl hydrolase (GH) family 6 and 7. This facilitated amplification of whole cellobiohydrolases genes in our *A. terreus* strain using PCR with gene specific primers. In addition, rtPCR are used to estimate cellobiohydrolases cDNA levels in 2, 4, 6, 8 and 10 day cultures of A. terreus growing on different carbon sources. The future potential of this research could be to supplement other fungal species lacking efficient cellobiohydrolase activity with cellobiohydrolase encoding genes from *A. terreus*.

Comparison of novel thermostable cellobiohydrolases for enhanced enzymatic hydrolysis

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Thermostable hydrolases are interesting enzymes for conversion of lignocellulosic feedstocks into fermentable sugars. They have several benefits including high specific activity, high stability, and flexibility for different process configurations. They have expectedly also longer life times. Thermostable enzymes make the use of high substrate concentrations possible because the hydrolysis can be performed at elevated temperatures thus decreasing the initial substrate viscosity.

In this study, novel thermostable GH7 family cellobiohydrolases (CBH) were compared for their ability to hydrolyze two different lignocellulosic substrates: steam pretreated giant reed and thermochemically pretreated spruce. The process concept used was based on a short high temperature (60°C or 70°C) prehydrolysis followed by SSF. The CBHs were added at the prehydrolysis step as part of a mixture containing thermostable hydrolases: endoglucanase II, xylanase and β-glucosidase (5 mg/g DW). The SSF conditions were mimicked using a decreased hydrolysis temperature at 35°C. At this stage a commercial mixture of mesophilic enzymes Celluclast 1.5L and Novozym 188 were added (5 mg/g DW). Two of the tested CBH enzymes showed superior performance compared with *Trichoderma* Cel7A at the elevated temperatures but they also performed remarkably well at the SSF temperature in synergy with the other enzymes. Thus, an increasing number of functionally interesting enzymes will be available for high temperature process concepts, and will also contribute to decreasing costs of enzymatic hydrolysis.

Enzymatic hydrolysis and fermentation of pretreated spruce

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One of the main challenges for industrial implementation of enzyme-based cellulosic ethanol production is to combine chemical pretreatment with enzymatic treatment, which produces a high yield of fermentable sugars at a low cost. A comparison was carried out between the cellulase enzyme products Cellic® CTec and Cellic® CTec2, supplied by Novozymes. As a substrate, spruce slurry produced on demo scale by SEKAB E-Technology, was used. The comparison, based on ten trials of enzymatic decomposition during 48 hours, turned out to be favorable for Cellic® CTec2, which with 0.034 and 0.043 ml enzyme/g WIS produced 17 % more glucose than Cellic® CTec. With SSF methodology, using spruce slurry with 12.5 % WIS and 0.043 ml Cellic® CTec2/g WIS, 31 g/l ethanol was produced during 117 hours.

Power consumption and torque profiles during high solid saccharification of pre-treated lignocellulosic materials

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A common trend in the research on 2nd generation bioethanol is the focus on intensifying the process and increasing the concentration of water insoluble solids (WIS) throughout the process. A number of economical benefits are foreseen if this is accomplished while still retaining a reasonable yield, e.g. lower capital costs and reduced distillation costs. However, increasing the WIS content is not without problems. For example, the viscosity of pretreated lignocellulosic materials is known to increase drastically at increasing WIS contents. At elevated viscosities problems related to poor mixing of the material, such as poor distribution of the enzymes and/or difficulties with temperature and pH control, arise, resulting in possible yield reduction. Achieving good mixing to deal with this is unfortunately not without consequences, since the power requirements needed to operate the impeller at high viscosities can be substantial. This highly important scale-up problem can easily be overlooked.

In this work, we monitor the impeller torque (and hence power input) in a stirred tank reactor throughout high solid enzymatic hydrolysis (>20% WIS) of steam-pretreated Arundo Donax and Spruce. It is shown that the impeller torque decreases very rapid during hydrolysis of pretreated Arundo Donax (i.e. it loses its fibre network strength) whereas the strength is retained for a longer time within the spruce material. This results in a substantially larger stirring power demand for the spruce material. Furthermore it is shown that the power input greatly affects the hydrolysis yield of pretreated spruce.

The precipitation of *Trichoderma reesei* commercial cellulase preparations under standard enzymatic hydrolysis conditions for lignocelluloses

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Commercial *Trichoderma reesei* cellulase preparations were assessed for the degree of precipitation that occurs during enzymatic hydrolysis of lignocelluloses. Comparative studies between preparations show, depending on the preparation and loading, total protein precipitation can be as high as 30% under standard hydrolysis conditions (50°C, pH 4.8). ATR-IR and SDS-PAGE data suggest precipitates are not only protein related but contain key cell wall hydrolyzing enzymes.

Precipitation was shown to increase considerably with incubation temperature; roughly 50 to 150 % increase from 40°C to 50°C and 800 % greater at 60°C. In addition, supplementation of the non-ionic surfactant PEG 6000 resulted in an 80 % reduction in 24 h precipitation levels. Observations on basic protein precipitation in this context may help explain the rapid reduction from initial conversion rates, which are traditionally observed and often related to plant cell wall recalcitrance issues. Overall the results suggest that protein precipitation is a significant factor to be taken into account by researchers studying lignocellulosic conversion processes and options for enzyme recycling.

Pre-hydrolysis and SSF at high solids using commercial and thermostable cellulases

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Hydrolysis and fermentation at high solids concentration (>20% DM) has several advantages economically. Less water is required meaning less waste water produced, and the final ethanol yield increases requiring less energy for distillation. Unfortunately, using high solids also has its disadvantages in the process such as product inhibition due to rapid accumulation of glucose. It is therefore preferably to run the process as SSF, but due to the high consistency it is challenging to mix the biomass to a homogenous slurry. To avoid problems with mixing, a liquefaction step (pre-hydrolysis) is added before SSF, with the purpose of reducing the viscosity of the biomass to an extent where it is pumpable. The liquefaction step should be as short and efficient as possible, which can be done with the right combination of cellulases. Thermostable enzymes have several benefits as liquefying cellulases. When increasing temperature, the reaction rate increases and the liquefaction time can be shortened.

In this study, the hydrolysis yield at high DM (25%) using mesophilic, commercial enzyme mixtures and novel thermophilic monocomponents were investigated. The synergy and hydrolysis yield of mesophilic and thermophilic cellulases were measured. Enzyme mixtures were tested at optimum temperature for the mesophilic enzymes and optimum temperature for the thermophilic enzymes. During liquefaction and hydrolysis, monosaccharides and reducing ends were measured.

Life Cycle Assessment of 2nd generation biofuels production using high-gravity hydrolysis and fermentation

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The search for sustainable production of 2nd generation biofuels is an ongoing effort. This production is not only aiming at substituting fossil-based transportation fuels with their renewable counterparts. It is also aiming at using renewable resources that will not affect food production, an issue that the 1st generation biofuels (e.g. ethanol from corn) have been criticized for. As well, 1st generation biofuels have been shown to be not as advantageous from an environmental perspective as was first expected, for instance due to the increased use of fertilizer. Lignocellulosic materials such as wood and straw are resources that can be used for the production of these 2nd generation biofuels. However, it has been particularly difficult to develop economically feasible processes for the production of these fuels.

High gravity processes, i.e. processes with high raw material concentration, have the potential of meet the economic requirements. In order to assess the environmental impact of such a process, Life Cycle Assessment (LCA) is applied in order to account for these impacts along the value chain of these fuels. This process technology development project focuses on the production of ethanol and 1-butanol using high gravity hydrolysis and fermentation. However, parts of this technology are still in the early stage of development and have not even reached the pilot scale stage. Therefore, lab scale data and information are used to assess the technology under development and process simulation is needed to generate data about the industrial-scale process. This implies that scale-up issues need to be taken into account in the LCA. Issues specific to biofuel production such as land use and land use change, and water use need to be addressed as well. The outcomes of the LCA can then be used to identify the weak and strong points of the process and this information can then be used in the development of the technology.

This poster presents a short review of the literature on the relevant issues regarding biofuel production and the use of LCA in technology development. Furthermore, a project plan for the environmental assessment of the technology under study and the use of these assessments in the technology development process is presented. LCA will thus be used during the technology development and will potentially have a significant influence on this development, and therefore on the sustainability of 2nd generation biofuels that are produced with a high gravity production process.

Sustainability assessment of large-scale combined heat and power production based on willow in Denmark

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The renewability of a bioenergy production system providing combined heat and power (CHP) based on intensive willow production is assessed. Material, energy, labour and service inputs in the production, transport and combustion of willow in a large CHP plant were identified. Two assessment methods were applied, eMergy accounting that indicates "the available solar energy used up directly and indirectly to make a service or product" (Odum, 1996) and Gross Energy Requirement (GER) that indicates the embodied fossil energy of inputs. Four indicators of energy production efficiency and sustainability were calculated. The eMergy analysis showed that production of 1 J of CHP requires 191,000 solar energy equivalent Joules (sej) in total environmental support – mainly from the applied manure.

The main difference between the two analyses is that eMergy accounting allocates all the direct and indirect energy inputs in pig farming to the manure and hence to the bioenergy produced with it while GER usually considers manure as a waste and thus does not allocate any fossil energy inputs to its production. The GER analysis showed that the energy output to fossil fuel input ratio is 25 with diesel use in agricultural activities and biomass transport as main inputs. The eMergy analysis found bioenergy from willow to be only 16% renewable when renewability is based on whether the energy sources used to make the inputs available are renewable. Based on the eMergy analysis it was concluded that willow-based heat and power should not be considered a primary energy source because it is highly dependent on support in the form of energy and materials and therefore delivers a poor net energy contribution to society. The study suggests that the renewability of bioenergy should be equal to the share of inputs based on renewable energy. Furthermore, it is suggested that GER analyses consider the embodied fossil energy use of all inputs, including wastes such as manure.

Keywords: sustainability, bioenergy, willow, combined heat and power generation (CHP), emergy, energy balance.

Efficient energy utilization in an integrated 1st and 2nd generation ethanol plant

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The demand for renewable and sustainable energy sources is increasing due to the ascending greenhouse gas emissions and the prospect of scarcity of fossil fuel in a near future. Only in Europe the transport sector needs to find alternative feedstocks for fuel production covering 10% of the used transport fuel in order to meet EUs set target for fuel coming from renewable sources by 2020. To supply this demand the possibility to utilize agricultural sources in a more efficient way has to be investigated more thoroughly. One of the most abundant crops in Europe today is wheat, which yields major residual material in form of straw. Ethanol production from the wheat kernel has the drawback that an external source of energy, preferably renewable, must be supplied in order to run the energy demanding processes for converting starch to ethanol. One such potential energy source is wheat straw, which could be collected during harvest. However, since the wheat straw is a lignocellulosic material the cellulose and hemicellulose can be utilized to produce ethanol and biogas, before the lignin residue is burnt to supply energy to a combined processing plant.

Integration of 1st and 2nd generation plants will account for higher crop utilization and could therefore be beneficial from an energy point of view. However, this integration could be done in a variety of different process configurations, depending on the ingoing material and desired co-products. The process flowsheet could, for example, be modeled as two stand-alone plants supplied with the surplus energy from the 2nd generation process, or as a combination of the two processes that could be integrated in for example the distillation or fermentation steps. Depending on the configurations there will also be different energy demanding steps and possibility to integrate different heat sources or sinks in the process.

Therefore a study is conducted in order to locate and improve the energy utilization steps in the combined 1st and 2nd generation processes using the flowsheet simulation program Aspen Plus utilizing results from laboratory trials.

Analysis of Feedstock Options and Socio-Economic Sustainability of Biofuel Production in Ghana using Integrated Assessment Methods

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In Ghana there is an expressed political interest in introducing new biofuel technologies for transforming energy crops and hitherto unsaved biomass (waste) into new energy commodities with expected economic, social and environmental benefits. But whereas residues are hypothetically regarded to be largely unused and discarded, this may not necessarily be the case. Clearly, there could be multiple uses of residues and it is important to consider that competing uses for biomass and lignocellulosic residues exist, which may impact the availability and opportunity costs for the material. In some localities, forestry and agricultural residues are traditionally used for energy production as fuel for residential cooking and heating. Removal of crop residues, such as leaves and stalks can negatively impact on soil structure and create erosion. Also, large-scale production of biofuels in Ghana will create social and economic changes at local to national scales as it may involve competition between biofuel production and other economic activities (including food production) for scarce resources such as capital, labour, land, water, and biomass. Biofuels production furthermore potentially involves externalities that may reduce or increase social and economic sustainability. Finally, the biofuel value chain development will often involve external interventions, supported by foreign donors or companies, with implications for value chain configurations and sustainability impacts.

The aim of the study is to perform an integrated assessment of biofuel production in Ghana, considering agricultural residues and other lignocellulosic materials for 2nd generation biofuels production. The assessment will cover an analysis of feedstock options and key socio-economic impacts of biofuels production, evaluate the trade-offs between impacts, and discuss technological and system-level strategies for mitigating adverse impacts, increase net private and social benefits, and make explicit and reconcile conflicting – environmental and economic – objectives. The assessment will focus on the level of feedstock production, and, when feasible, also consider activities further upstream in the value chain, including transport, pre-treatment and conversion.

Influence of cultivation procedure for *Saccharomyces cerevisiae* used as pitching agent in industrial spent sulphite liquor fermentations

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The cell viability and fermentation performance often deteriorates in fermentations of spent sulphite liquor (SSL). This investigation therefore addresses the question how different cultivation conditions for yeast cells influence their ability to survive and boost the ethanol production capacity in an SSL-based fermentation process. The strains used as pitching agents were an industrially harvested *Saccharomyces cerevisiae* and commercial dry baker's yeast.

This study therefore suggests that exposure to SSL in combination with nutrients, prior to the fermentation step is crucial for the performance of the yeast. Supplying 0.5 g/l fresh yeast cultivated under appropriate cultivation conditions may increase ethanol concentration more than 200 %.

Screening and heterologously expressing of high activity xylose isomerase in *Saccharomyces cerevisiae* for xylose metabolic engineering

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Heterologous expression of xylose isomerase (XI) (EC 5.3.1.5) is considered as an effective strategy to establish the xylose metabolic pathway in *Saccharomyces cerevisiae*, since the converting of xylose to xylulose catalyzed by XI is cofactor-independent. However, only few XI genes (*xyl*A) were functionally expressed in *S. cerevisiae* at 30 °C and their catalyzation efficiency need to be further improved. Therefore, a large scale screening method depending on cell growth on the plate, which xylose is the sole carbon source, was set up to collect function expressing XIs in our group. Genetic engineering operations, which were expression of genes encoding xylose reduactase (XR), xylitol dehydrogenase (XDH), overexpression of xylolukinase (XK) gene and four genes in non-oxidative part of the pentose phosphate pathway (PPP), deletion of *GRE3* gene, deletion of *COX4* to eliminate the respiration, were performed. Then, the respiration deficiency strain was adaptively evoluted in the medium using xylose as the sole carbon source for over 1000 hours until until the biomass doubling time (*T*) did not show significant shortening. Finally, the plasmid containing *XYL1*, *XYL2* gene encoding XR and XDH respectively were lost in non-selective culture medium, resulting the strain BSPX042 (XK, PPP, *gre3*Δ, *cox4*Δ, AE). Unlike the native *S. cerevisiae* strain with weak growth background, the BSPX042 strain cannot grow completely on xylose plate. Furthermore, the strain shows obvious growth much more than the unmodified strain on xylose when effective pathway to convert xylose to xylulose was built. Various sources *xyl*A were transferred to the BSPX042 strain. A transformant, which showed exciting growth on xylose plate, was selected. The xylose isomerase gene contained in this transformant was obtained from a bovine rumen metagenomic library and was name as Ru-*xyl*A. Strain BSPX042 (Ru-*xyl*A) showed 1.3U g^{-1} protein specific XI activity and could finish the fermentation of 20 g l^{-1} xylose in 36 hours. The ethanol yield was 0.44 g g^{-1} xylose.

Key words: Xylose isomerae; adaptive evolution; Rumen metagenomic library; Saccharomyces cerevisiae.

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C5/C6 *Saccharomyces cerevisiae* strains for industrially viable bioconversion of lignocellulose

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Lignocellulose raw materials are composed of lignin, cellulose and hemicellulose and require pre-treatment at elevated temperatures with acid or base to make cellulose and hemicellulose accessible to subsequent acid or enzymatic hydrolysis. Cellulose and hemicellulose are hydrolyzed to fermentable monomer C6 and C5 sugars (glucose, mannose, galactose, xylose and arabinose), while lignin may be recovered as fuel. During pre-treatment and hydrolysis fermentation inhibitors – phenol and furan derivatives and low molecular weight fatty acids - are also released. Hydrolyzed lignocellulose raw materials thus comprise a mixed-sugar substrate in an inhibiting matrix. The yeast *Saccharomyces cerevisiae* has been used throughout recorded human history to produce bread, beer, and wine and is, due to its robustness, currently the prime choice for industrial ethanol production.

Genetic engineering allows construction of new yeast strains that can effectively consume not only the C6 sugars in lignocellulose hydrolysates, but also the C5 sugars xylose and arabinose. Various metabolic engineering strategies as well as random methods such as breeding, adaptation/evolutionary engineering and mutagenesis are generally used to produce such strains. We have constructed rationally designed and engineered yeast strains with effective C5 fermentation performance. Improved strains utilizing the pentose (C5) sugars xylose and arabinose have been developed, as well as strains with improved inhibitor tolerance to be exploited in the future biofuels and biorefinery industries based on renewable lignocellulose raw materials. Performance of these strains in fermentation of lignocellulose hydrolysates will be presented.

Selective suppresssion of bacterial contaminants by process conditions during lignocellulose based yeast fermentations

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Contamination of bacteria in large scale yeast fermentations is a serious problem and threat to the development of successful biofuel production plants. Huge research efforts have been spent in order to solve this problem, but additional ways must still be found to keep bacterial contaminants from thriving in these environments. The aim of this project was to develop process conditions that would inhibit bacterial growth while giving yeast a competitive advantage.

Lactic acid bacteria are usually considered to be the most common contaminants in industrial yeast fermentations. Our observations support this view but also suggest that acetic acid bacteria, although not so numerous, could be a much more problematic obstacle to overcome. Acetic acid bacteria showed a capacity to drastically reduce the viability of yeast. In addition, they consumed the previously formed ethanol. Lactic acid bacteria did not show this detrimental effect on yeast viability. It was possible to combat both types of bacteria by a combined addition of NaCl and ethanol to the wood hydrolysate medium used. As a result of NaCl + ethanol additions the amount of viable bacteria decreased and yeast viability was enhanced concomitantly with an increase in ethanol concentration. The successful result obtained via addition of NaCl and ethanol was also confirmed in a real industrial ethanol production plant with its natural inherent yeast/bacterial community.

Production of Fuel Ethanol from Softwood at High Dry Matter Content

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Bio-ethanol can be produced from cellulose-rich material such as softwood by enzymatic hydrolysis and fermentation. To perform the enzymatic hydrolysis and the fermentation in one single step, so called "simultaneous saccharification and fermentation" (SSF), has proven to result in higher product yields and lower production costs than performing the two steps separately. The raw material is pretreated with steam at high temperature and pressure to break down the hemicellulose and make the cellulose more accessible to the enzymes in the hydrolysis.

Previous studies have shown high ethanol yields for SSF of steam pretreated spruce run at up to around 10% WIS (water insoluble solids). It is however important to run SSF at higher dry matter contents in order to achieve higher ethanol concentrations to lower the energy demand in the distillation needed in the post treatment and to reduce the production cost. Previous studies of SSF with higher dry matter contents have shown a decrease in ethanol yield due to poorer mass transfer and increased inhibition by toxic compounds present in the pretreated material.

High solid SSF with prehydrolysis has shown to result in high ethanol concentration without compromising the total ethanol yield. This process option was studied for high solid steam pretreated spruce and the influence of different process parameters was investigated. The results from this study will be presented at the conference.

Insights on high solids biomass hydrolysis and fermentation: A succesful industrial application of 30% dm of wheat straw loading

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The future of second generation bioethanol is to become an economically viable renewable biofuel. The key to overcome this barrier is the founding of a technology able to obtain an efficient breakdown of lignocellulosic biomass, working at very high solids concentration in order to improve product concentrations, plant productivity, and decrease energy input and final costs. Most of available data in literature regarding the use of high solids concentrations refer to 10-15% of water insoluble solids (WIS). In this work, solids concentrations up to 40% of WIS are applied.

At lower solids concentrations (below 15% DM), the enzymatic hydrolysis and fermentation can be started simultaneously (SSF) and is operated at 30-35°C. However, at increasing solids concentration like in industrial bioethanol plants, the material will be difficult to mix and a pre-hydrolysis at optimum temperature of the enzymes (50°C) is needed in order to ensure a fast liquefaction. After a few hours, the hydrolysate is cooled down to the desired fermentation temperature. The length of pre-hydrolysis is related to the biomass concentration, the efficiency of the enzymes, the enzyme loading and the process equipment.

In this work the length of pre-hydrolysis was investigated in order to find the optimal conversion of cellulose to ethanol at 85% of maximum theoretical using the wheat straw at 30,35 and 40% of WIS content. The results also indicate that the reactor system plays a crucial role on the length of pre-hydrolysis, thus several equipments and their efficiency in mixing were investigated: Roller Bottle Reactor (RBR), horizontal oriented mixed reactor, RBR-with antisense internal impeller (RBR-AI), Terrafors® bioreactor, and Belach MemmaLys® fermentor.

A novel process configuration of Simultaneous Saccharification and Fermentation for bioethanol production at high solid loadings

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Simultaneous saccharification and fermentation (SSF) is a process option for lignocellulosic bioethanol production that has proven to have several advantages compared to separate hydrolysis and fermentation. The economical viability and commercialization of cellulose-to-ethanol demands the process to work under high-solid loadings to result in high sugar yield and final ethanol titer in *S. cerevisiae* based SSF process.

In a conventional batch SSF process practical limitations to high-solid loadings include, poor mixing and accessibility of enzymes to substrates and high inhibitors concentration that reduces the yeast viability and metabolism. In order to overcome these limitations, we propose a novel SSF process configuration involving feeding of substrate, enzyme and yeast. It is possible to overcome mixing issues associated with a batch SSF at high-solid loadings by a feed of substrate, enzyme and yeast. The feed of freshly cultivated yeast throughout the fermentation process ensures active metabolic state of yeast. In addition, the substrate feed ensures low inhibitors concentration at any given time point increasing the survival ability of yeast compared to a batch SSF. The enzyme feed ensures slow release of glucose providing an opportunity for xylose consuming yeast strain to co-consume xylose together with glucose. The aim of the current work is to understand how different combinations of feeding strategies influence the outcome of the SSF process. In the longer perspective, we aim at deducing an optimized SSF process that can handle very high-solid loadings with efficient hydrolysis and fermentation process at low enzyme and yeast loadings, respectively.

Keywords: Bioethanol, SSF, high-solid loadings.

High Gravity Fermentation: Process optimization with regard to physiological requirements of yeast

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High gravity fermentation processes are considered as an attractive solution in order to decrease the overall cost of biofuels production by making the isolation of the end-product easier and cheaper. However, the use of high concentrations of pretreated (unwashed) lignocellulosic biomass (slurries) represents a challenge for successful SSF process. Under these conditions, a significant inhibition of both enzymes and yeast could occur due to the presence of furans, lignin derived or other inhibitors present in the medium. These inhibitory effects could become more severe as the initial substrate concentration increase. Other issues such as nutrients requirements and yeast cells viability are also connected with the special conditions during fermentations at high solids. Overcoming these limitations is crucial for a large scale implementation as well as for the economical efficiency of the process.

Different detoxification methods were evaluated with regard to the fermentability of different lignocellulosic feedstocks by Saccharomyces serevisiae. The experimental plan focused on improving the inherent ability of yeast cells to adapt in such toxic environments under specified conditions. The complementation of the culture broth with additional nutrients was also studied with regard to yeast toxicity tolerance and adaptation towards high gravity conditions. Technical limitations connected to high gravity conditions were also subject to process engineering during the optimization procedure of the fermentation process.

Bioethanol production from wheat straw using simultaneous saccharification and fermentation process-effect of stirring speed

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The simultaneous saccharification and fermentation (SSF) process of lignocellulosic materials requires the utilization of microorganisms capable of working at high temperatures. The selection of *Saccharomyces cerevisiae* strains able to ferment sugars obtained from lignocellulosic material at temperatures above 37 ºC with high ethanol yield has become a necessity. In this work, a flocculant *S. cerevisiae* was screened for their ability to grow and ferment glucose in a temperature range of 40 - 45 ºC. SSF was performed on the autohydrolysis pretreated solids at 45 ºC. Moreover, in order to evaluate the effect of stirring on ethanol yield four stirring speeds were studied. When the SSF was conducted at 150 rpm, 11.33 g/L was obtained after 96 h. When the SSF was conducted at higher stirring speed (250 rpm), 15.09 g/L of ethanol was obtained. This corresponds to an overall ethanol yield of 63.5 % and 84.49 %, respectively. In terms of ethanol yield there were statistical significant ($p < 0.05$) between the different stirring speeds. The results show that the combining high temperatures and stirring speed can be an alternative for achieved high levels of ethanol yield in SSF.

Comparison of *Saccharomyces cerevisiae* TMB3400 and two selected mutants for glucose and xylose co-fermentation in wheat straw hydrolysate

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Bioethanol is today produced from sugar- or starch-rich raw materials but further expansion has to be based on lignocellulose. Commercialization of second generation bioethanol based on softwood or wheat straw for instance, has still not been realized due to several factors, including poor biomass utilization and high production cost.

Two of the most important parameters to reduce the production cost are the ethanol yield and the concentration in the fermentation broth. High ethanol concentration requires high water insoluble solids in simultaneous saccharification and fermentation or in enzymatic hydrolysis in separate hydrolysis and fermentation. This usually results in lower ethanol yield due to the increased inhibition and poorer mass transfer. However, the ethanol yield and concentration can also be improved by co-fermentation of glucose and xylose; especially from agricultural by-products, such as wheat straw, due to the high amount of hemicelluloses in these materials.

Saccharomyces cerevisiae TMB3400 has been improved and selected for better xylose fermentation, inhibitor- and thermotolerance. Glucose and xylose co-fermentation ability has been investigated using the original and the two selected mutants in batch fermentation of steam pretreated wheat straw hydrolysate (non-diluted, 1.5 and 2 times diluted). Experiments with one of the selected strains showed improved xylose uptake compared with the original strain, and resulted in increased ethanol yield, although xylitol formation also slightly increased. The study has now been continued with assessment of the strains performance in fed-batch fermentation. The results of these experiments will also be presented.

Development of a photosynthesis microbial fuel cells with *Spirulina platensis*

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In the development of solar cells, the ultimate goal is to search for the way that turned light energy to electrical energy. Photo-synthetic organisms are used as the catalyst for microbial fuel cells. However, in the literature, the studies mainly focused on improving the efficiency of the battery in the microbial fuel cells or photosynthetic microbial fuel cells. To achieve this purpose, the proton membranes and mediators had to be applied in the process, leading to the death of organisms and thus reducing the fuel cell's performance. In this study, a membrane-less and mediator-less photosynthetic microbial fuel cell was designed. The effects of biomass of algae, electrode distance, and electric quantity, on the cell performance were investigated.

Spirulina platensis was used as the biocatalyst of the photosynthetic microbial fuel cells on the anode. The anodic electrode being a gilding gold membrane and the cathode is a carbon fiber membrane. It is noted that the chlorophyll concentrations on the anode actually varied the open circuit voltage (OCV). When the electrode distance is 4cm and the concentration of the chlorophyll is 0.5 mg, it has a maximal OCV of 0.48V. When the external resistance is 1kΩ, the cell has a maximum power density of 10mW/m^2. Besides, a cultivation of the used algae was carried out. The result displayed that the cultivated algae can provide the same OCV of 0.48V like the original algae after a 15-hour culture.

Keywords: photosynthetic microbial fuel cells; microbial fuel cells; mediator less; membrane less.

Photo-biohydrogen production potential of *Rhodobacter capsulatus-PK* from Wheat Straw

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Cellulosic material is the largest biomass reservoir with great turnover rate on the planet earth. Its biotechnological exploitation is promising for sustainable and environmentally sound energy provision strategies. Wheat straw (WS) may represent majority of crops' origin agroindustrial wastes. Further, WS is one of widely available, inexpensive and renewable lignocellulosic biomass comprising of 75-80% cellulosic and hemicellulosic contents. The substrate can be hydrolyzed into monomeric sugars by chemical and/or biological methods. This study examined comparative potential of dilute acid digested and ammonia pretreated following with enzyme hydrolyzed WS for hydrogen production by using purple non sulfur bacterium *Rhodobacter capsulatus-PK*. In each case, 1.5g of the hydrolyzed substrate was supplemented with 0.03g of yeast extract. The gas production became noticeable after 14hrs of inoculation in WS pretreated with 4% acid and subsequently detoxified (overliming; pH 7.0±0.2). The detoxified liquid hydrolyzate (DLH) attained 540ml/l of the culture after 36h under illuminated conditions at 30±2.0ºC, whereas the non-detoxified acid pretreated hydrolyzate (NDLH) wheat straw could yield only upto 341m/l after 36 hrs post inoculation. Further, the production started 2hrs late as compared to the situation observed for the DLH. This reduction in yield is attributable to relatively higher prevalence of furfural, HMF and acetic acid in case of non-detoxified process.

Evolution of H_2 production became observable just after 10±2.0h of inoculation by employing 48h inoculum age in case of 20% ammonia pretreated WS hydrolyzed by employing cellulase 84FPU/ml and β-glucosidase 442 CBU/ml at 60ºC with continuous shaking for 24h. This experiment showed highest level of hydrogen production upto 763ml/l of culture raised in otherwise comparable conditions. This 57% and 41% higher hydrogen yields than the DLH and NDLH substrates, respectively is explainable on the basis of significantly higher monomeric sugars contents of the enzymes' hydrolysed substrate and lesser amounts of toxic derivatives including pH reducing agents. The surface response methodology was employed to study the effects of major process parameters of hydrogen production in order to establish a model for the prediction of hydrogen production from WS. Results of this study are suggestive for designing commercial level hydrogen production programmes addressing biotechnological exploitation of abundantly available and low-cost cellulosic substrates.

High throughput platform for evaluating plant cell wall deconstruction

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A lot of research has been done during the past decades in order to improve the conversion rate for deconstruction of lignocellulosic biomass to soluble sugars for biofuel production. However, most of the research has focused on catalyst improvement, technology optimization and industrial process development. Understanding the recalcitrant nature of cell wall structures may provide a cost and labour efficient route to improve the conversion. High throughput system is capable of processing large population of plant materials to help understanding the recalcitrance.

We have developed a new grinding and dispensing robotic system that integrates solid and liquid handling platforms which can be applied to evaluate substrate digestibility, compare different enzymes, or other types of characterization. This robotic system is highlighted for its flexibility which provides many options in output type, process mode, sample mapping mode and other system parameters. Plant material can be automatically grinded, weighed and dispensed to many types of plates or GC vials for downstream experiments. A 96-well aluminium microtiter plate system is applied most frequently for evaluating the digestibility of plant material. The plant material is heat treated up to 190 degree C in the aluminium plate, thereby mimicking the large scale pretreatment technologies. Desired enzyme mixture is then added to each well by the liquid dispensing station followed by enzymatic hydrolysis. The released sugar monomers, mainly glucose and xylose, are determined by commercial glucose and xylose assays.

We will be presenting results from testing large populations of plant materials, e.g. raw wheat straw, barley, pretreated wheat straw to overview the accuracy, reliability and repeatability of the high throughput system and also to characterize recalcitrant nature of different types of plant materials.

A mechanical approach to fibre attrition during enzymatic hydrolysis

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It was recently shown that irregular regions in thick walled cells such as tracheids and fibres are the likely locations of segmentation during enzymatic hydrolysis under industrial-like conditions [1]. This result implies that these structures are important for liquefaction of biomass during high solids hydrolysis. The irregular zones are known as dislocations, nodes or slip planes and occur naturally in the stems of many plant species, for example wheat, flax, hemp and nettle as well as in wood. In cotton seed hairs they are, however, absent [2]. In the present study we subjected flax fibres and cotton seed hairs to enzymatic hydrolysis during gravimetric mixing. Flax and cotton are both long and slender textile fibres but only flax contains dislocations, so by selecting these two substrates we aimed at highlighting the role of dislocations for fibre attrition during hydrolysis. Prior to hydrolysis an attempt was made to cut both fibre types into 5 mm segments. Somewhat broader fibre segment length distributions were achieved, especially for cotton.

From a mechanical standpoint dislocations are weak points known to be the likely locations of crack initiation during tensile loading of individual fibre cells [3-5]. If dislocations also are the weak spots when fibres are loaded beyond their bending strength during gravimetric mixing, one would assume that fibres with dislocations more easily break into segments. Furthermore, one can speculate that the lever principle would play a larger role for the development in the segment length distribution during hydrolysis in the absence of dislocations. In other words, as the largest stresses are expected halfway between the two fibre ends, a homogenous fibre segment will most likely break into two segments with nearly the same lengths. In the case where dislocations are present this picture will presumably be less clear as fibres now have length-wise variations in their material properties.

The results from FiberTester measurements confirmed that more short segments were formed during the hydrolysis of flax than during the hydrolysis of cotton. Actual length distributions at various time points during hydrolysis were compared to modelled distributions based on segments being cut either randomly or in the middle.

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Multivariate analysis of cellulase activity as monitored by low-field NMR

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During the bioconversion process of biomass to ethanol, physical and chemical changes to the substrate take place and influence the interactions between water and the cell wall matrix. Water present inside respectively outside the cell wall of cellulose fibers presumably play different roles during cellulose hydrolysis, as cellulases break down biomass only when adsorbed to the substrate surface. Using the low-field NMR technique, different states of water (bound vs. free) during hydrolysis of filter paper at high solids loadings (33 wt %) were detected based on different T2 (spin-spin) relaxation times. To monitor the changes between bound and free water caused by commercially available cellulases, heavy water (D2O) was used to replace either type of water as deuterium is not detectable by LF-NMR. Multivariate analysis of the LF-NMR data acquired during hydrolysis by Principal Component Analysis (PCA) showed a clear distinction between samples treated by active enzymes, inactive protein (BSA), and blanks (water only). The model could also differentiate between samples depending on whether they contained bound H2O to begin with and had free D2O added at the onset of hydrolysis or vice versa. Partial Least Squares (PLS) regression was also used to explore possible correlations between the amount of sugar released and the spin-spin relaxation time distributions at various time points during hydrolysis.

Oxidative cleavage of cellulose by a CBM33 from *Streptomyces coelicolor*

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Enzymatic degradation of recalcitrant polysaccharides is efficiently achieved through the synergistic action of a diverse set of secreted carbohydrate active enzymes (CAZymes). The effectiveness of this process has been thought to be mainly governed by the complementary activities of exo- (processive) and endo- (non-processive) glycoside hydrolases. In 2010, it was discovered that proteins belonging to the families CBM33 and GH61 of CAZymes are metal-dependent oxidative enzymes that act synergistically with classical hydrolytic enzymes in the conversion of recalcitrant polysaccharides such as chitin and cellulose [1-2]. CBM33 proteins were originally thought to act on chitin only [1,3]. We describe the first example of a CBM33 that cleaves cellulose, CelS2 from *Streptomyces coelicolor* [4].

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Water constraint and the potential limitations cell wall component dynamics impart on the enzymatic saccharification of lignocelluloses

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A recent essay in Science Magazine by Himmel and co-workers strongly reiterated and strengthened the importance of the phrase "biomass recalcitrance" to describe the resistances limiting the degradation of lignocellulosic structural carbohydrate by biological systems. This reiteration has served as inspiration for increased investigation, funding, and enthusiasm with respect to improving understanding of what has been known thirty years now; that is, lignin, hemicellulose, and crystalline cellulose all contribute to lignocellulose recalcitrance, but it depends on how you approach the problem. The majority of research to date has been from the perspective of the physical association of components within the cell wall complex and the mostly steric hindrances they exert on enzymatic systems. Despite this, it is important to consider the chemical nature of the different cell wall components, how they interact with each other, how they interact with the aqueous environment, and more critically the associated enzymatic degradation systems.

In this study, to approach this we utilize low field nuclear magnetic resonance (NMR) spectroscopy to observe how the constraint and distribution of water in systems of saturated of plant cell wall isolates varies across substrate types. In general, hemicellulose, lignin, silicate and pectin rich isolates were more constraining to water in the system than purified celluloses. Amongst purified cellulose preparations the constraint and distribution of water in saturated mixtures varied considerably, and, interestingly, this variation appears to correlate to the saccharifying potential of each substrate. Furthermore we noted that the greater the disparity in the constraint imparted by the cellulose on water in the system versus that of the alternate cell wall isolates the more probable it is that the isolate will have a negative impact on cellulose conversion itself. Overall, this short work raises questions on the true nature of biomass recalcitrance with respect to enzymatic conversion processes. From a plant perspective, the battle against degradative forces is a war and it seems only logical that mechanisms more elegant than just simply putting up walls may be at play. In the end, a complete understanding of the molecular dynamics between different cell wall components and degrading enzymes may inevitably be the last frontier of research needed to allow an infant biofuels industry to make breakthroughs towards truly cost effective processes.

A novel high-throughput method for enzyme screening based on glycan microarrays and glycan specific probes

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Advances in bioinformatics and genome sequencing have lead to the identification of large numbers of putative plant cell wall degrading enzymes including glycoside hydrolases (GHs). GHs have many commercial applications in the food, feed and biorefinery sectors, for example during the saccharification steps of biofuel production. However, there is a lack of high-throughput technology for the rapid screening of the activities of these enzymes on individual polysaccharide substrates and cell wall materials. There is therefore a pressing need for the development of new HTP technology for GHs screening and here we present approaches based on combining the high-throughput capacity of carbohydrate microarrays with the specificity of monoclonal antibodies (mAbs).

Combined ethanol and biogas production from steam pretreated corn stover

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Lignocellulosic biomass as raw material such as corn stover has a good potential to be converted to ethanol by enzymatic hydrolysis of the carbohydrates, i.e. cellulose and to some extent hemicellulose. For an effective conversion of lignocellulosic biomass to soluble sugars and later ethanol, the pretreatment step is crucial as it affects all other process steps. In this study steam pretreatment of Chinese corn stover, with and without dilute acid as catalyst, was investigated for various residence times (5-10 min) and temperatures (180-220°C). Two acids were studied; one weak organic acid (acetic acid at 1%) which is of interest as it can later be degraded to biogas and sulfuric acid at 0.2% and 0.5%. The assessment of the pretreatment was performed by analysis of the hydrolysates and solid material after pretreatment, regarding carbohydrates and inhibitor as well as by enzymatic hydrolysis of the pretreated material. The enzymatic hydrolysis was performed at the same experimental conditions and enzyme loadings using both washed and unwashed pretreated material.

The pretreatment conditions resulting in highest sugar yields were selected to be subjected to simultaneous saccharification and fermentation to ethanol as well as using the remaining organic compounds for biogas production.

Keywords: Steam pretreatment, corn stover, ethanol, biogas.

Process-simulation-aided experiments to develop corn-fiber-based biorefinery concept

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Corn fiber, which is derived from the corn wet-milling process, is a promising raw material for biorefining. It contains approximately 21% starch, 15% cellulose, 37% hemicellulose and less than 3% lignin related to the complete dry mass. In a preliminary study process simulation of a corn-fiber-based biorefinery plant was performed using Aspen Plus V7.1 (Aspen Technology, Inc., Cambridge, MA) flowsheeting program. In the simulated process the pretreatment method consists of a destarching step followed by weak acid treatment (120°C, 1% sulfuric acid) to separate pentose sugars, mainly arabinose and xylose. The conversion yields of these two steps originate from the publication of Kálmán et.al. [1]: both steps are carried out at 10% dry matter (DM) content. However, the process simulations elucidate that energy efficiency of the process increases by applying higher DM concentration. In the model, 30% and 22% were assumed in the destarching step and in the weak acid treatment, respectively. Laboratory experiments are performed to investigate the effect of increased DM concentration for the conversion yields. After both steps of the pretreatment the solid and the liquid fractions are separated by filtration. Examinations are carried out to verify the required washing liquid volume calculated by the process model. The model includes the simulation of xylitol fermentation and recovery based on several up-to-date publications [2-6], and also contains some assumptions. From the separated pentose-rich liquid fraction xylitol is fermented in two steps: first from xylose by Candida yeast, and then from arabinose by recombinant Escherichia coli strain. This is due to the lack of microorganism with the capability to effectively form xylitol from both arabinose and xylose. Other option is to separate the high-value arabinose before the xylitol production. To achieve this goal experiments are performed to investigate the opportunities of the selective arabinose separation from the destarched corn fiber by different pretreatment strategies (hot water, weak acid, soaking in aqueous ammonia, enzymatic treatments and some of these combinations).

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Techno-economic analysis of a corn-fibrebased biorefinery

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Corn fibre, a co-product of corn wet milling, can be a suitable raw material of a biorefinery producing value-added products such as bioethanol, biomethane and xylitol. Corn fibre contributes approximately 10% to the dry matter of corn grain, and it has high carbohydrate and low lignin contents (starch: 21% of grain dry matter, cellulose: 15%, hemicellulose: 37%, lignin <3%). A corn-fibre-based biorefinery can be a standalone process, however, it can also be integrated into a corn-wet-milling plant. In the Hungarian wet-milling plant, in Szabadegyhaza, approximately 100 000 dry tonnes of corn fibre are produced yearly, which could be a proper basis for an industrial-scale biorefinery.

Process simulation was performed using Aspen Plus V7.1 (Aspen Technology, Inc., Cambridge, MA). The first step of the designed process is starch liquefaction, which is carried out with alfa-amylase enzymes at 85°C. The water-soluble liquefied starch is separated in a filtration step, and then it is saccharified using gluco-amylase enzymes. The solid lignocellulosic residue is pretreated at 120°C in 1% sulphuric acid so that the hemicellulose fraction is solubilised. From the separated pentose-rich liquid fraction xylitol is fermented in two steps: first from xylose, and then from arabinose. Xylitol is purified via evaporation, clarification applying activated carbon and crystallisation. The cellulose in the pretreated solid fraction is broken down in enzymatic hydrolysis. The hexose streams from starch and cellulose hydrolyses are mixed and then ethanol is fermented by ordinary baker's yeast propagated on-site. Ethanol is recovered by distillation. Biogas is produced in anaerobic digestion of the stillage and other organic-matter-rich streams, and part of the biogas is upgraded into biomethane. The effluent of anaerobic digestion is treated aerobically. Combined heat and power production is carried out by incinerating part of the biogas, the pressed aerobic sludge and the pressed cell mass of xylitol production. Capital investment was either estimated in Aspen Process Economic Analyzer V7.1 (Aspen Technology, Inc., Cambridge, MA) or it was based on vendor quotation.

Various process scenarios regarding process configurations, types and amounts of products are compared in terms of energy efficiency and annual cash flow (capital, operational expenses and product revenues). Both standalone and annex processes are investigated and discussed. Sensitivity analyses are also performed, in which the effect of product prices on annual cash flow is studied.

Keywords: Process design, Economic analysis, Corn fibre, Bioethanol, Biomethane, Xylitol.

Biofuel by-products for poultry diets

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The valorization of by-products from biofuel industry will promote the application of the 2010 (2003/30/EC) and 2020 European Directives, stipulating the inclusion of biofuels in transport sector. Replacement of fossil energy for sustainable energy by cereal utilization in bioethanol production increases the competition for starch between monogastric organisms and first generation biofuels. Very wide and interesting opportunities toward Sustainable Development are opened by by-product valorization. Biofuel industry produces enormous quantity of lignocellulose by-products. This biomass constitutes a broad resource for animal feed and the approach pursued in this work is the utilization of fibrolytic rumen enzymes to valorize by-products in the digestive tract of monogastric animals.

A methodology of enzyme production from rumen was first elaborated to demonstrate the potentialities of in vitro cellulolysis (40 to 60% of cellulose hydrolysis depending on by-products used). Hydrolysis was done in physico-chemical conditions simulating digestive tract of poultry. Cellulolytic bacteria was then isolated from bovine rumen and cultivated on specific medium to stimulate the biomass and the cellulase production in an ex vivo system (witch reproduces ruminal anaerobic condition). The cellular biomass reached 109 bacteria/mL in 24 hours (in 10L bioreactor) and 2.5 to 15 Units/mL in C/N =30 medium with cellulose as the predominant source of carbohydrates (0.2% of glucose).

Production of fibrolytic enzymes in fermentors by bacteria culture stimulation permitted to obtain the rate of activity needed to make in vivo experimentation with monogastric animal (poultry digestibility assay).

Process Design of cellulose based bioenergy combines : Microbial biofuel production integrated with CHP type infrastructure

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Commercial cellulosebased microbial biofuel production is underway but questions regarding biomass pretreatment, process design, biofuel yield, waste management and overall cost remain and specifically microbial bioethanol production must resolve issues on pretreatmant inhibitor production and pentose processing. Here we summarise our previously published results on combined ethanol fermentation and biogas digestion of oat straw and also describe our current aproach in processdesign of robust and modular bioenergy combines with Swedish CHP-type infrastructure. Especially we investigate modelsystems with recirkulation of nutrients and low capital investments. The research is performed within the Microdrive program at the Swedish University of Agricultural Sciences.

MicroDrivE – a thematic research program on sustainable biofuel production

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MicroDrivE is a thematic research program on sustainable biofuel production, integrating research on bio-preservation, enzymatic pre-treatment, ethanol fermentation, bioprocessing of distillers waste, biogas fermentation, and the re-circulation of plant nutrients in biogas digestate as bio-manure. Initially, the focus is on cereal grains, sugar beets and straw as the feedstock, but wood and other cellulosic biomass sources are also explored. *MicroDrivE* is located at the Faculty for Natural Resources and Agriculture (NL), Swedish University of Agricultural Sciences (SLU). The program is run in co-operation with partners from industry and sectorial organisations. *MicroDrivE* started in 2007 and will continue until 2013, The program involves 16 scientists, several post-docs and PhD students, with specialist competence in microbiology, molecular biology/enzymology and chemistry.The scientists supervise MSc-thesis students doing 20-week projects, often in joint projects with our industrial partners.

Implementation of 2nd Generation Bioethanol Production in Ghana

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Abstract of PhD Synopsis

The production of bioethanol from food crops is seen as a potential threat to food production in Ghana. With the development of local technology and capacity in second generation (2G) ethanol, the threat of using arable land for the cultivation of energy crops for bioethanol production will be curtailed. Household waste, as well as residues from agriculture and agro-based industries, that are usually disposed inappropriately can serve as sustainable feedstock for bioethanol production. This PhD study will focus on the development of sustainable local technologies and production systems for the conversion of identified waste into bioethanol. Emphasis will be placed on the development of low tech processing methods, characterization of feedstocks, development of pretreatment methods which are low cost and well-adapted to the socio-economic conditions of Ghana, and development of effective process integration. This PhD is being undertaken with financial support from DANIDA, and under the collaboration between DTU, KNUST, and Zoomlion Ghana Limited. Over 75 % of the work will be carried out in Ghana. Research findings will be published in scientific and technical journals, and also presented at international conferences and seminars. The development of local technology in 2G bioethanol will support national programmes along the core benefits of bioethanol utilization: availability of clean-burning fuel, reduction in the use of petroleum-based fuel, net greenhouse gas emissions reduction, reduced threat to food crop cultivation, and job creation especially for the youth.

The use of of lignocellulosic materials for biogas, biodiesel and bioethenol production in Ghana

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The use of lignocellulosic materials from waste stream production of bioenergy is worth considering especially in an era where almost all the generated waste is disposed off at dumpsite. Considering the value of the waste there is the need to carefully plan a suitable use that will not only mitigate the negative environmental consequences, but add value as well to the waste. Any conversion approach will have to take into consideration some critical conditions such as the rate of waste generation, characteristics of the waste, economic viability of any expected output, environmental friendly by-products, among others. This study aims at assessing the suitability of biofuel production to allow for implementation by end users for the waste biomass of lignocellulosic materials in selected areas of Ghana while considering the by-products in agricultural activities. In doing this, the study will map out the biomass needs, quantify and characterize them to ascertain their sustainability for use as raw materials; investigate existing biofuels(Biodiesel, bioethanol, biogas, etc.) production technologies, compost production plants, and other waste treatment facilities in Ghana to determine their output, by-products, raw material needs; the performance of the existing biofuels plants will be assessed using economics of scale, environmental compatibility and at the same time compare to similar facilities in advance countries to identify any lapses; by-products from biofuels installations will be subjected to laboratory analysis to determine their suitability for use as a fertilizer; the by-products will be tested on degraded agricultural and mining lands and compared the results to same product on fertile agricultural lands. It is expected that the study will give a guide for implementation of the needed biofuel technology and also provide a concept for degraded soil amendment.

Key words: biodiesel, bioethanol, biofuel, biogas, biomass.

Stablising oil or biodiesel by the natural antioxidant lignin – a spectroscopic study

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As the fossil fuel reserves steadily decline society is faced with a need to replace these with renewable fuel sources. Some of these fuel sources such as biodiesel may have relatively poor storage stability due to autooxidation.This is a very complex free radical initiated process which is presently not fully understood. Many factors promote this and of these the most ubiquitous is the presence of dissolved molecular oxygen. The presence of oxygen is impractical to prevent and its detrimental effects are therefore usually counteracted by adding substances which slow down or delay autooxidation. These substances – antioxidants – are usually H atom donating dissolvable compounds such as phenolics.

In the present work we examine the possible antioxidant effect of lignin rich plant cell wall particles in relation to mineral oil and biodiesel autooxidation. For this purpose spectroscopic analysis is used – most notably ATR-FTIR spectroscopy using a heated ATR crystal to follow *in situ* oil autooxidation. The results are discussed in relation to basic autooxidation mechanisms of the oil and diesel samples, and the structure of the cell wall polymer lignin.

Obtaining nanofibers from sugarcane bagasse and curauá fibers using enzymatic hydrolysis followed by sonication.

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The objective of this study was to prepare cellulosic nanoparticles using enzymes and sonication, without using mechanical treatments such as disc refining, high pressure homogenizer or microfluidizer processors. Sugarcane bagasse and curauá fibers from Brazil were initially treated with a sodium hydroxide solution followed by sodium chlorite bleaching. The fibers were then treated with appropriate enzymes to convert polysaccharides such as hemicelluloses, amorphous cellulose and pectins into soluble sugars. Since these were initial trials, a variety of combinations and concentrations of Novozymes' FiberCare® R and Viscozyme® L were used to produce nanofibers and whiskers using only sonication after enzymatic reactions. The fibers obtained were investigated by Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and X-ray diffraction (XRD).

It was observed by SEM that bagasse fibers surface had changed after bleaching, and showed small holes. After enzymatic reactions, the fibers showed significant surface modification, with production of thin films and peeling on the surface, but without changes in the fiber dimensions. Curauá fibers SEM results showed that bleaching separates the twisted fibrils, with some changes on the surface fibers. Enzymatic reactions promoted the same effects observed in sugarcane bagasse fibers. TEM results showed that the sonication procedure after enzymatic reactions produced nanofibers from bagasse and curauá fibers, defibrillating the microfibrils, and exposing their nanometric units. XRD results showed that cellulose crystallinity index increased after bleaching and decreased after enzymatic reactions to both fibers. Results of this study will be presented. The ultimate aim, which we are still to achieve, is to keep the crystalline structure of cellulose intact and to remove other polysaccharides while minimizing the enzyme concentrations and time of hydrolysis. Dewaxed and bleached fibers can be used to produce both nanofibers and whiskers using only sonication after enzymatic treatment.

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