First European workshop on biotechnology for lignocellulose biorefineries

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Content

Design and development of an enzyme product for cellulosic biomass conversion

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Many companies have begun process development and scale-up of their cellulosic biomass conversion technologies. It was Genencor's desire to quickly launch a product to support the needs of these process developers.

This paper will discuss many of the steps to launching this first commercial product specifically for this market, including the design of the enzyme system, performance testing and validation, confirming reproducible production quality and delivering on needed production cost reductions. Performance comparisons will be shown between AccelleraseTM 1000 and SpezymeR CP, a previous benchmark cellulase product from Genencor.

Quantification of the CBD-FITC conjugates surface coating on cellulose fibres

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Cellulose-Binding Domains (CBD) are modules present in most celulases, being responsible for their high affinity to cellulose crystalline surfaces. The CBD used in this work, produced by limited proteolysis, belongs to cellobiohydrolase I (CBHI) of *Trichoderma reesei*, as shown in a previous work. Three tyrosine residues define a flat surface, which may be responsible for the affinity to cellulose. This protein has a single amine, the N-terminal of the linker region, which allows a specific reaction with fluorescein isothiocyanate (FITC).

Several recombinant CBDs, fused to different proteins, have been produced, as recently reviewed by Shoseyov et al. Cellulose-Binding Domains (CBD) have been used to target functional molecules to cellulose-containing materials, to improve pulp properties or as an additive for paper recycling. Bearing in mind that these applications are related to surface effects, in this work we attempted to quantify the CBD surface coverage of cellulose fibres, using the approach based on the use of CBD-FITC previously developed. The conjugation with FITC does not affect the CBD interaction with cellulose, since the N-terminal is isolated from the cellulose interacting part of the protein. Indeed, the conjugation of FITC does not modify the CBD adsorption isotherms. Since there is only one amine group present in the CBD, the stoichiometry of the conjugation reaction is 1:1.

In this work, our aim was to quantify the protein adsorbed on cellulose fibres and, more specifically, the surface concentration of CBD. This value could, alternatively, be estimated by measuring the specific surface area, by means of the BET isotherm. However, the BET approach is not ideal for porous materials. The presence of CBDs in the interior of the fibres was also investigated. Cellulose Binding Domains (CBD) were conjugated with fluorescein isothiocyanate (FITC). The surface concentration of the Binding Domains adsorbed on cellulose fibres was determined by fluorescence image analysis. For a CBD-FITC concentration of 60 mg/L, a coating fraction of 78% and 110% was estimated for *Portucel* and Whatman fibres, respectively. For a saturating CBD concentration, using Whatman CF11 fibres, a surface concentration of 25.2×10^{-13} mol/mm^2 was estimated, the equivalent to 4 protein monolayers. This result does not imply the existence of several adsorbed protein layers. It was verified that CBDs were able to penetrate the fibres, according to confocal microscopy and TEM-immunolabelling analysis. The surface concentration of adsorbed CBDs was greater on amorphous fibres (phosphoric acid swollen) than on more crystalline ones (Whatman CF11 and Sigmacell 20).

1. Pinto, R., Amaral, A.L., Carvalho, J., Ferreira, E.C., Mota, M., Gama, F.M. Development of a method using image analysis and CBD-FITC conjugates for the measurement of CBDs adsorbed onto cellulose fibers. **Biotechnology Progress** (in press).
2. Pinto, R., Amaral, A.L., Ferreira, E.C., Mota, M., Vilanova, M., Ruel, K., Gama, F.M. Quantification of the CBD-FITC conjugates surface coating on cellulose fibres. **BMC Biotechnology** (accepted)

Elucidating pathways for efficient saccharification of lignocellulosic biomass: understanding the role of hemicellulolytic enzyme activities

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The recalcitrant nature of lignocellulosic biomass with respect to the production of sugars for fermentation to biofuels and products is directly related to the ability of cell wall degrading enzymes to gain access to their respective substrates. The close spatial and chemical relationships between cellulose, hemicellulose, and lignin within the cell wall matrix are thought to be the primary hindrance to access. Numerous chemical pretreatments have traditionally been employed to either remove and/or alter the hemicellulosic and/or lignin cell wall fractions in order to enable enzymes to reach their respective substrates. To date, most of these pretreatment schemes have been optimized with respect to a few commercial cellulase preparations, typically a fermentation product of *Trichoderma reesei*. In order to achieve optimal enzymatic saccharification with these cellulolytic systems, effective pretreatments have often utilized severe chemical and/or thermal conditions that typically require costly chemical recycling or post-treatment conditioning steps.

This presentation will highlight our work over recent years, which has focused on developing a deeper understanding of the roles that select non-cellulolytic enzymes play in the deconstruction of the plant cell wall. In doing so, we hope to reduce the severity of thermal/chemical pretreatment required to achieve a cost-effective saccharification. We have taken the approach of studying the effects of individually purified hemicellulolytic enzymes and the synergistic interactions they display with other enzyme components, both hemicellulolytic and cellulolytic. Purifying these enzymes from a number of in-house as well as commercially available sources, we are continuously expanding our library of component activities. The NREL collection currently includes a selection of cellulase, xylanase, beta-xylosidase, ferulic acid esterase, acetyl xylan esterase, arabinofuranosidase, and xyloglucanse activities. Using corn stover as our model substrate, we have acquired numerous samples of solids and hydrolysates produced from a wide range of different pretreatment chemistries and severities. By studying the interactions between individual activities on these substrates, we intend to more appropriately match key enzymatic activities and synergies to specific pretreatment pathways. We envision that information gained from such studies will lead to more in-depth investigation of alternate pretreatment pathways, the consideration of other enzymatic systems, and the development of new or modified cellulase preparations for saccharifying lignocellulosic biomass.

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Hemicellulases in biomass hydrolysis

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Recent advances in the development of yeast strains with improved ability of pentose utilization have increased the relevance of high hemicellulose recovery (1). Research efforts in production of ethanol from lignocellulosic biomass have therefore also put a higher focus on the role of hemicellulose within raw material sources.

Hemicellulose is a major constituent of many potential sources of biomass and is considered an important contribution to the economical viable production of bioethanol. There are, however, challenges in hemicellulose utilization.

In high severity pretreatments hemicellulose sugars are often converted into inhibitors which have a negative impact on the fermentation (2). In order to decrease the formation of inhibitors, increase the yield of fermentable sugars and to reduce cost of pretreatment, milder methods are desirable. Milder pretreatment conditions often result in a higher content of polymeric and oligomeric hemicellulose residues. The remaining polymeric hemicellulose can potentially also decrease the substrate accessibility of cellulose degrading enzymes (3) and the heterogeneity of hemicellulose oligomers might necessitate the use of a mixtures of hemicellulase activities.

Finding efficient hemicellulases are therefore highly important in order to produce high yields of fermentable sugars in future biomass to ethanol processes.

In this paper, Novozymes will include progress in enzyme development, understanding important enzyme activities, synergism and examples of application of enzymatic hydrolysis of hemicellulose.

References:

(1) Hahn-Hagerdal B.; Karhumaa K.; Fonseca C.; Spencer-Martins I.; Gorwa-Grauslund M.F., Applied Microbiology and Biotechnology, 2007, 74, (5), 937-953

(2) Klinke H.B.; Thomsen A.B.; Ahring B.K., Applied Microbiology and Biotechnology, 2004, 66, (1), 10-26

(3) Öhgren K.; Bura R.; Saddler J. and Zacchi G., Bioresource Technology. 2007, 98, (13), 2503-2510

Enzymatic hydrolysis of pre-treated lignocellulosics at high solids concentrations

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Due to the water-absorbing properties of plant cell walls, enzymatic hydrolysis of pre-treated lignocellulosics for the production of bioethanol has proven difficult at substrate levels above 10-15 % dry matter (w/w), especially due to mixing problems. Being able to increase the substrate concentration would significantly increase the economic viability of the production. The most important advantages of high-substrate concentration operations are: higher final ethanol concentration and thus more efficient distillation; higher production capacity due to less bulk and lower energy demands for heating; less waste water.

Recently, we have demonstrated that with proper mixing it is possible to hydrolyse pretreated wheat straw at up to 40 % dry matter (w/w) [1]. However, hydrolysis yields seem to decrease linearly with increasing solids level. Results have shown that the increased product inhibition is not solely responsible for the lower yields. Other factors such as lack of free water for hydrolysis, enzyme shear, oxidative stress and poor mixing have been investigated. Experiments indicate that proper mixing at the molecular level and/or transport of enzymes between access sites on the cell wall polymers is crucial in maintaining acceptable yields at high solids hydrolysis.

[1] Jørgensen H, Vibe-Pedersen J, Larsen J, Felby C. Liquefaction of lignocellulose at high solids concentrations. Biotechnol Bioeng. 2007; 96: 862-870.

Evaluation of Neurospora crassa as an enzyme factory for the bioconversion of brewer's spent grain

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Brewer's spent grain (BG), the most abundant brewing by-product, was used in the present study as a low-cost feedstock for the production of ethanol by the mesophilic fungus *Neurospora crassa* using a consolidated bioconversion process. Furthermore, the *N. crassa* crude enzyme preparation was tested for the release of ferulic acid from BG.

The production of required cellulolytic and hemicellulolytic enzymes was optimized under solid-state cultivation (SSC) concerning carbon source and initial moisture. The optimal medium contained BG and wheat straw (WS) in a ratio 1:1 while the optimal initial moisture is 61.5% (w/w). SSC in a laboratory horizontal bioreactor using the optimized medium allowed for the large-scale production of a multienzymic system. Yields as high as 1073, 56, 4.2, 1.6, 3.1, 5.7 and 0.52 U g^{-1} carbon source of xylanase, endoglucanase, cellobiohydrolase, β-glucosidase, α-L-arabinofuranosidase, acetyl esterase and feruloyl esterase, respectively, were obtained.

Chromogenic (fluorogenic) 4-methylumbelliferyl substrates were used to partially characterize the extracellular proteome of the microbe after the separation by isoelectric focusing (IEF) electrophoresis. Alkali pretreatment of brewer's spent grain and different aeration levels were studied for the optimization of the ethanol production by *N. crassa* in a consecutive submerged fermentation. A yield about 74 g ethanol kg^{-1} dry BG (5,6 g L^{-1}) was obtained under optimum conditions (aeration 0.1 vvm, pretreatment with 1 g NaOH 10 g^{-1} dry BG), corresponding to 35 % of the theoretical yield based on total glucose and xylose composition of BG.

An economic ferulic acid recovery from biomass via biological methods is of interest for a number of reasons. Ferulic acid is a precursor to vanillin synthesis. It is also a known antioxidant with potential food and medical applications. Despite its universal presence in all plant cell wall material, the complex structure of the plant cell wall makes ferulic acid recovery from biomass a challenging bioprocess. The *N. crassa* enzyme multienzyme preparation was successfully used for the partial degradation of cell wall components and the liberation of ferulic acid. A yield of 1.4 g kg^{-1} dry matter BG was obtained corresponding to a released level of 90 %.

Glucuronoyl esterases - novel family of carbohydrate esterases involved in plant cell wall degradation

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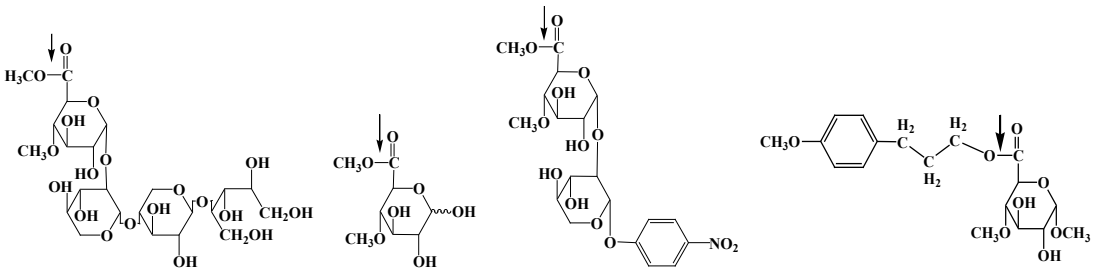
The enzyme system of the wood-rotting fungus *Schizophyllum commune* produced during growth on cellulose contains an esterase that hydrolyzes methyl and alkylaryl esters of 4-O-methyl-D-glucuronic acid [1,2] (see the formulas and linkages attacked below).



Substrate specificity of the purified enzyme, called glucuronoyl esterase (GE), was distinct from that of other carbohydrate esterases (CEs), such as acetylxylan esterase, feruloyl esterase and pectin methylesterase. The GE attacked the esters of 4-O-methyl-D-glucuronic acid exclusively, and the esters of 4-O-methyl-D-glucuronic acid were not hydrolysed by other CEs. Partial amino acid sequence of *Sc*GE was used for the search of homologous sequences in known microbial genomes.

The genes of putative GE were found in several genomes and in all cases they corresponded to genes of unknown function. In several fungi containing the GE gene, the presence of the enzyme was confirmed in cellulose-containing growth media using synthetic glucuronoyl esterase substrates. The genes of the fungus *Trichoderma reesei* [3], *Phanerochaete chrysosporium* and of the bacterium *Ruminococcus flavefaciens* were isolated, expressed homologously or in GE-free hosts. The corresponding gene products were purified to homogeneity and shown to exhibit the expected enzyme activity. Some of the enzymes contained a cellulose-binding module. Based on these results a newCE family, number 15, has been established (CAZY). The target of the enzyme in plant cell walls could be the ester linkage between 4-O-methyl-D-glucuronic acid residues of glucuronoxylan and lignin alcohols [4], shown to be one of the covalent linkages connecting the two cell wall polymers. This implicates an important biotechnological potential of these enzymes.

[1] Špániková, S., Biely, P. (2006) Glucuronoyl esterase – Novel carbohydrate esterase produced by *Schizophyllum commune*. FEBS Letters 580, 4597-4601.

[2] Špániková, S., Poláková, M., Joniak, D., Hirsch, J., Biely, P. (2007) Synthetic esters recognized by glucuronoyl esterase from *Schizophyllum commune*. Arch. Microbiol. 188, 185-189.

[3] Li X.-L., Špániková S., deVries R.P., Biely P. (2007) Identification of genes encoding microbial glucuronoyl esterases. FEBS Lett., 581, 4029-4035.

[4] Jeffries, T. (1990) Biodegradation of lignin-carbohydrate complexes. Biodegradation 1, 163-176.

On the roles of enzyme processivity and accessory proteins in the degradation of chitin and other recalcitrant polysaccharides

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Enzymatic degradation of recalcitrant structural polysaccharides in plant biomass, in particular cellulose, is a key issue in the development of second generation bioethanol. One important factor determining the efficiency of this process is the ability of enzymes to gain access to single polymer chains that they can guide into their catalytic centres.

Recently, studies of the enzymatic degradation of the non-plant cellulose analogue chitin have created new insight into the issues of substrate accessibility and enzyme efficiency. Firstly, it was shown that a processive mechanism, which is generally considered favourable because it improves substrate accessibility, in some cases may slow down enzymes [1]. While non-processive variants of a chitobiohydrolase showed an expected reduction in efficiency when degrading insoluble chitin, they displayed a remarkable increase in activity towards the soluble polymeric chitin-derivative chitosan [1]. Secondly, it was found that chitinolytic organisms produce non-hydrolytic accessory proteins that increase enzyme efficiency, presumably by disrupting the substrate [2-4]. These proteins interact with the crystalline substrate through a complex network of highly specific polar interactions [2,3]. Together with recent results from the cellulase field [5,6], these results point out new directions for optimizing enzymatic conversion of recalcitrant biomass [7].

[1] Horn, S.J. *et al.* (2006) Costs and benefits of processivity in enzymatic degradation of recalcitrant polysaccharides. *Proc. Natl. Acad. Sci. USA* 103, 18089-18094

[2] Vaaje-Kolstad, G. *et al.* (2005) Crystal structure and binding properties of the *Serratia marcescens* chitin-binding protein CBP21. *J. Biol. Chem.* 280, 11313-11319

[3] Vaaje-Kolstad, G. *et al.* (2005) The non-catalytic chitin-binding protein CBP21 from *Serratia marcescens is* essential for chitin degradation. *J. Biol. Chem.* 280, 28492-28497

[4] Vaaje-Kolstad, G. *et al.* (2008) Characterisation of the *Lactococcus lactis* ssp. *lactis* chitinase and its accessory chitin-binding protein that promotes chitin degradation. *Submitted for publication*

[5] Li, Y. *et al.* (2007) Processivity, substrate binding, and mechanism of cellulose hydrolysis by *Thermobifida fusca* Cel9A. *Appl. Environm. Microbiol.* 73, 3165-3172

[6] Cosgrove, D.J. and Tanada, T. (2007) Use of gr2 proteins to modify cellulosic materials and to enhance enzymatic and chemical modification of cellulose. USPTO 20070166805

[7] Eijsink, V.G.H. *et al.* (2008) From chitin to biofuels – degrading recalcitrant polysaccharides. *Submitted for publication*

Oral session: Novel enzymes

Tailor-made oxidoreductases for improved industrial utilization of cellulose and lignin polymers

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The general objective of this collaborative work is to obtain a new generation of enzymes, tailor-made oxidoreductases, as an environmental technology to substitute harsh chemicals in the industrial processing of plant polymers, as well as to develop new enzyme-based bioprocesses and bioproducts from these renewable feedstocks.

Plant polymers are the main source of renewable materials on Earth. The use of biotechnology will permit a development of new routes to cellulose and lignin-based added-value products, including speciality paper products and lignin-based chemicals. The industrial utilization of cellulose includes pulps for the paper industry. However, its characteristics permit the use of cellulose for speciality products whose potential is still to be fully investigated. Lignin is a heterogeneous aromatic polymer, highly recalcitrant towards degradation. Most industrial uses of cellulose require the previous removal of lignin, which is generally burnt at the mill. However, the chemical nature of lignin makes this polymer an interesting source of aromatic chemicals for lignocellulose biorefineries.

Oxidoreductases are involved in both lignin biosynthesis and biodegradation. Therefore, they have the highest potential for modification of lignocellulosics and lignins. However, the natural enzymes are far from optimal performance under industrial conditions. Some oxidoreductases have been extensively investigated in terms of structure-function relationships. This will allow a new approach based on tuning their catalytic and operational properties using protein engineering tools (such as forced evolution and site-directed mutagenesis) to obtain industrial biocatalysts.

During the first year of this collaborative work, a selection of lignocellulosic starting materials has been characterized using modern analytical techniques. Simultaneously, both microbial genomes currently available and large collections of microbial strains have been screened using high-throughput methods. As a result of these activities, the FOLy database of lignolytic oxidoreductase gene sequences has been implemented. Expression of the most interesting genes is in progress using hosts and vectors designed for this purpose, and a first shipment of selected oxidoreductases has been distributed to partners by Novozymes for the first application trials. The applications of tailor-made enzymes include among others: i) increase of strength and other properties of cellulose fibres, and improve refining; ii) production of lignin-free cellulose for high-quality products; and iii) production of lignin-based surfactants, adhesives and other chemicals. In this way, the results obtained will strengthen the EU leading position in the market of industrial biotechnology. Moreover, the use of the biocatalysts obtained will contribute to transform a part of the EU chemical sector to more sustainable and eco-friendly manufacturing processes.

Design of optimized Saccharomyces cerevisiae for ethanol production from pentose sugars

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Fermentation of lignocellulosic biomass from sustainable forestry or agriculture would be a favourable option for the production of bioethanol. Forestry and agricultural waste represent widely differentiated feedstocks with a low degree of competition for arable land with food and feed crops.

Baker's yeast *Saccharomyces cerevisiae* is the prime choice for the fermentative production of bioethanol. First, it can rapidly convert hexose sugars to ethanol with high yield and high productivity. In addition, it displays a good tolerance to several fermentation-inhibiting compounds present in lignocellulosic derived media. However, *S. cerevisiae* is not naturally able to utilize pentose sugars, which may constitute a significant portion of the lignocellulosic feedstock.

Since optimal process economy demands exhaustive substrate utilization, the feasibility of the forthcoming switch from oil- to biomass-derived raw materials for the fabrication of fuels and chemicals is strongly dependent on the development of a fermenting micro-organism with a broad range of substrates. Our research group is working on key aspects of the design of efficient lignocellulose-fermenting yeast strains, including xylose and arabinose pathways optimization, combination of multiple pathways and improvement of strain robustness for optimal performance in lignocellulosic hydrolysates.

Bacterial arabinose utilization pathway has previously been combined with the fungal xylose utilisation pathway, based on xylose reductase and xylitol dehydrogenase, both in laboratory and industrial strains of *S. cerevisiae*. Here we report a comparative evaluation of different sets of strains, in which different pentose sugars utilization pathways were combined to achieve optimal mixed sugars fermentation performance.

Simultaneous thermophilic fermentation of glucose, xylose and arabinose from corn fibre

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Price effective production of bioethanol from lignocellulosic biomass is limited by the lack of technology for two processes in particular: 1) The enzymatic conversion of the highly complex lignocellulose fibres into sugar monomers and 2) The efficient and simultaneous conversion of hexose and pentose sugars into ethanol. Corn fibre, a side product from the production of ethanol from corn kernels, is produced in greater and greater amounts as the production of ethanol increases. The corn fibre is very rich in the pentoses xylose and arabinose and ethanol production from these sugars is therefore instrumental if the fibre is to be used efficiently for ethanol production.

Thermoanaerobacter BG1 has been shown to be a very efficient ethanol producer in wheat straw hydrolysates containing mostly glucose and xylose, with ethanol yields of up to 0.42 g/g from glucose and xylose combined. The strain is able to grow in non-detoxified wheat straw hydrolysates and to produce ethanol from a wide range of sugars in continuous reactor systems. The growth temperature of 70 °C efficiently prevents contamination and thereby reactor downtime due to cleaning and sterilization. Recently we have shown that BG1 mutants are able to efficiently and simultaneously convert glucose, xylose and arabinose from corn fibre efficiently into ethanol in continuous reactor systems. Moreover, a significant conversion of sugar oligomers also takes place in the reactors. The efficient fermentation of the corn fibre sugars opens the possibility of generating more ethanol from corn kernels and at the same time maintaining a by-product rich in protein.

Mixed sugar fermentation in ethanol fermentation with Saccharomyces cerevisiae

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Industrial bioethanol production from lignocellulose fermentation with *S. cerevisiae* is intended to be performed from a mixture of sugars. Improvement in the performance of the process faces several bottlenecks since different substrates are assimilated in a different fashion.

Arabinose and xylose are pentose sugars, which are not naturally fermented by *S. cerevisiae* even though considerable amounts of them are present in industrial substrates. In this work, development of an industrial fermenting strain with a xylose fungal and arabinose bacterial pathways by genetic and evolutionary engineering is presented and analyzed in mixed sugar fermentation. Galactose is another sugar that has a delay in the uptake and assimilation due to glucose repression and which also has a slower rate of consumption. To circumvent this, up-regulation of the enzyme phosphoglucomutase that was previously identified as a target for genetic engineering (1) to increase galactose assimilation was tested in this work. Evaluation of this genetic modification was analyzed in laboratory strains with galactose as the sole carbon source but also in mixed sugar fermentation.

References

(1) Bro C., Knudsen S., Regenberg B., Olsson L., Nielsen J. (2005) Improvement of galactose uptake in *Saccharomyces cerevisiae* through overexpression of phosphoglucomutase: Example of transcript analysis as a tool in inverse metabolic engineering. Applied and Environmental Microbiology 71:6465-6472.

Biomechanical pulping of softwood with enzymes compared with the extracellular enzymatic machinery of the biopulping fungus Physisporinus rivulosus

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Manufacturing of mechanical pulp is a highly energy-consuming process. Application of enzymes to wood chips is an attractive alternative in order to decrease the energy demand in the refining process and to introduce novel functional properties in fibres. A variety of enzymes were applied to plug screw compressed chips in order to improve enzyme access onto wood fibres. Consumption of refining energy was examined with a laboratory low-intensity refiner after 6-hour enzyme treatments with manganese peroxidase (MnP), laccase-mediator system, pectinase, or a cellulase mixture. The results were compared to biopulping with the white-rot fungus *Physisporinus rivulosus*, a fungus that expresses MnP as the main lignin-degrading enzyme in wood chips.

Specific energy consumption resembling the first-stage refining was measured, and chemical modifications of the fibres were evaluated. The specific energy consumption in the refining of Scots Pine wood chips treated with MnP decreased by 11 % when compared to the untreated reference chips, and in the refining of Norway Spruce by a somewhat smallerdecrease, namely 6 %. Fungal pretreatment resulted in energy savings on spruce similar to that of MnP treatment. Hydrolytic enzyme and MnP treatments on pine resulted in similar energy savings on average, though the hydrolytic enzyme treatments at their best reached up to 15 % energy savings. Polyelectrolyte titration indicated increased surface charge in the case of MnP treated pine pulps, which were refined to low freeness values (CSF 85-130 ml). Most laboratory handsheet properties, i.e. strength, light scattering and opacity, were improved at given specific refining energy. Only brightness was slightly decreased. The MnP treatment seems to be a promising concept for energy savings and fibre surface modifications.

Evaluating the effectiveness of laccase natural mediators in bleaching of Sisal pulp

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In the present paper, Sisal pulp delignification and bleaching using laccase-mediator systems are shown for the first time. The aim of the study was to explore the effectiveness of four plant phenols, namely sinapic acid (SINAC), ferulic acid (FERAC), coniferyl aldehyde (COALD) and sinapil aldehyde (SALD), as laccase mediators and compare it to the one exhibited by 1-hydroxybenzotriazole (HBT) in terms of brightness, kappa number and viscosity modifications determined on the pulp treated.

The enzymatic stage (L stage) was followed by a peroxide treatment (P stage) in order to attain higher brightness. HBT resulted in a 15 % decrease of final kappa number and a similar increase of brightness. Natural mediators produced a minor decrease of final kappa number (7-8 %), and among them sinapilaldehyde was the only one enabling a small increase in brightness (4 %). The best improvement of brightness, obtained by using HBT, corresponded to a marked drop in viscosity after the L stage. Pulps treated with natural mediators did not experiment any decrease in this property. Pulps treated with FERAC, SINAC and HBT showed a similar decrease (12 %) in viscosity after being treated with peroxide.

After the L stage, all natural mediators produced an increase in kappa number, which denotes a possible partial condensation of the mediator in the phenoxy radical form on the pulp. Samples proceeding from this stage were extensively washed with acetone in a Soxhlet apparatus in order to eliminate the contribution to kappa number from phenolic compounds physically adsorbed on the pulp and better evaluate their tendency to condensate. A reduction in kappa number was produced by the acetone washing in the samples treated in the L stage; nevertheless, washed samples still showed a superior kappa number compared to the control, indicating that a radical condensation effectively occurred.

Enzymatic functionalisation of lignocellulosic fibres

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Improving the properties of wood fibres is a constant interest of pulp, paper and board manufacturing industry. The presence of surface lignin in pulp fibres offers possibilities to enhance or even to create completely new and innovative paper and board products by functionalisation. In the functionalisation method, the enzymatic radical formation is exploited in fibre activation and further functionalisation, *i.e.* bonding of new, desired chemical components to the fibre material.

Oxidative enzymes such as laccases can be used to activate the surface lignin of lignin-rich pulps by radicalisation. The primary reaction of laccase catalysed oxidation is the formation of phenolic radicals to the substrate. Due to the high reactivity of these radicals (either with each other or with a secondary substrate), reactions such as polymerisation, depolymerisation, co-polymerisation and grafting can occur. The size of laccases limits the action of the enzyme on the fibre surface, which can be considered both as a limitation or an opportunity when applying laccases in fibre applications.

In this work, the mechanisms and factors affecting the laccase catalysed functionalisation of lignin-rich fibres have further been clarified. The potential of this chemo-enzymatic functionalisation method developed for lignin-rich pulps will be discussed in the presentation.

Enabling a lignocellulosic biorefinery using pine as a feedstock

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The New Zealand government has recently set in place a goal to become one of the world's first carbon neutral economies. As part of this it has established a biofuels sales obligation of 3.4 % transport fuels by 2012. By integrating industrial biotechnology at a national scale with effective utilisation of softwood forestry plantations and primary processing infrastructure, a lignocellulosic bioethanol future is on its way.

As part of a feasibility study, we have analysed the competitive drivers for utilisation of biomass (pulp versus bioethanol versus thermal energy) and explored the technical challenges in processing softwoods into bioethanol. Significant insights have been gained into approaches to enabling enzymatic saccharification of softwoods suggesting that there are no major barriers to using softwoods as feedstocks. Some of the other challenges/opportunities in establishing a viable biorefinery using forestry residues or purpose grown trees are also discussed.

Towards a Beechwood Biorefinery

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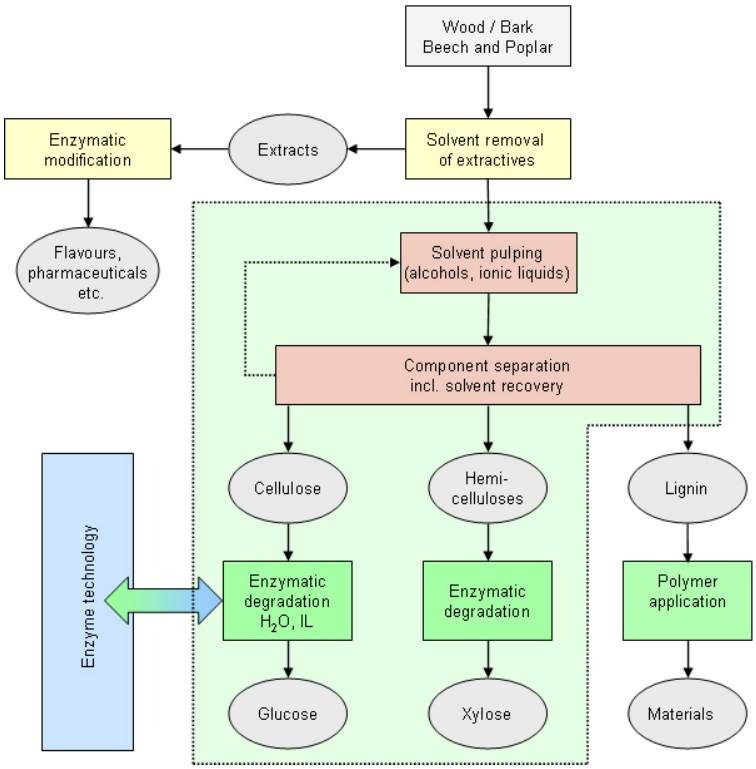
The principles of alcohol-water pulping are applied for the separation of beechwood into its components, namely extractives, cellulose, xylan and lignin. This process also serves as a pretreatment to make the beechwood polysaccharides accessible to hydrolytic enzymes. The component separation includes the positive effect that cellulose and xylan can be separately hydrolyzed into glucose and xylose to be used as platform chemicals afterwards (Fig. 1).

In this contribution, the efficiency of commercially available cellulases towards beechwood cellulose fractions dependent on residual xylan and lignin will be reported. Over a wide range, the accessibility of cellulases towards their substrate can be improved by increasing temperature and/or extension of time of the pulping process. When small amounts of acids or other ionic substances during component separation are added, a clear optimum of the pretreatment conditions with regard to cellulose accessibility can be identified.

The recovered beechwood xylan is partly fragmented. However, it is still intensively decorated by acetyl- and 4-O-methyl-glucuronic acid substituents. Accordingly, besides xylanases and ß-xylosidases, acetylxylanesterases and α-glucuronidases must be added for total xylan breakdown.



A systematic micro-dissection of cereal by-products

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Two major low-value co-products of the food processing industry are the bran of wheat (WB) after milling, and brewers' spent grain (BSG) from barley, the main residue from brewing. Currently, these are generally used for the production of low-value composts, livestock feed, or disposed of in landfill as waste. Cost-effective deconstruction of these co-products into their polymeric, oligomeric and individual components through mechanical and/or (bio)chemical means combined with a reduction in biomass, could provide valuable streams for exploitation in a number of different applications.

As part of a strategy to develop such an approach, it is becoming increasingly evident that an understanding of the make-up of such bulk residues in relation to their biological, post-harvest and processing history is crucial. A detailed microscopic and chemical analysis of WB and BSG reveals a wealth of structural and molecular components. Cereal grains comprise distinct layers of cells that are derived from the ripened ovary wall along with some of the outer layers of the seed, including the pericarp, the inner pericarp, the seed coat and the aleurone layers. These layers consist mainly of polysaccharides, including arabinoxylans, xyloglucans and cellulose, and many of these are cross-linked by phenolic acids and lignin. This phenolic-polymer-cross-linking is likely to influence the rate of bio-degradation of lignocellulosic fibre from cereal residues and will have potentially important consequences in relation to the functional properties and exploitation of such materials. A comparison between WB and BSG highlights the differences in compositional complexity between these different cereal residues. Microscopy of BSG is thus presented in greater detail. Comparison with chemical analysis confirms the presence of high proportions of useful components such as feruloylated arabinoxylan and protein.

Acknowledgements
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The role of surfactants in the reaction pathway of peroxidases

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Oxidative enzymes play a key role in the development of biorefinery processes based on the production of new materials. In this context, the tailoring of the reaction pathway in oxidative enzymes is relevant in view of the possible development of regioselective polymerisation processes. Dehydrogenative polymers (DHPs) are obtained by polymerising coniferyl alcohol using oxidative enzymes. The DHPs constitute interesting lignin models extensively used in the attempt to elucidate lignin biosynthesis and structure. However, by varying the polymerization experimental conditions, different structures of the DHPs are obtained.

Nuclear magnetic resonance techniques constitute an invaluable tool for the characterization of lignins. More specifically, HSQC experiments allow to unambigously identify the interunit bonding patterns, while ^{31}P-NMR of suitably derivatized samples is a fundamental tool for the quantitative detection of all labile H groups in lignins. Mass spectrometry, thanks to the introduction of soft ionization techniques, has been widely and successfully used in the study of a large number of natural and synthetic polymers, nevertheless only few works up to now refer to the use of soft ionization mass spectrometric techniques in the structure elucidation of lignin.

We report here the characterisation by HSQC, ^{31}P-NMR and ESI mass spectrometry of two different DHPs. More specifically, a classical DHP was compared with a new lignin model obtained in a reaction medium containing a surfactant able to maintain in solution the incoming polymer.

When coupled, the NMR and mass spectrometric data allowed to elucidate and identify different reaction mechanisms occurring during the polymerization processes, yielding the formation of DHPs with different structures.

Enzymatic tailoring of arabinoxylans

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Xylans are widely distributed plant cell wall polysaccharides. They are built up by 1,4-linked β-D-xylopyranosyl (Xyl*p*) units. Many xylans, especially from cereals, are highly substituted by 1,2- and/or 1,3-linked α-L-arabinofuranosyl (Ara*f*) residues. The degree of substitution affects the solubility and enzymatic degradation of arabinoxylans (AX). Highly substituted AX are generally water-soluble and form viscose solutions. Degradation of the AX backbone decreases the viscosity. The role of Ara*f* substituents on the viscosity is less well-known. Viscosity and water-holding capacity are important properties for example in applications where AX are used as hydrocolloids. Viscosity is also one key health-promoting property of water-soluble dietary fibre. Xylans are potential raw material for biodegradable films, too, and are lately reported to produce strong transparent self-supporting films with good oxygen barrier properties.

Recently, two different types of α-L-arabinofuranosidases (EC 3.2.1.55, AXH) acting on polymeric AX have been identified. AXH-m liberates 1,2- and 1,3-linked Ara*f* units from monosubstituted Xyl*p* residues whereas AXH-d3 acts solely on 1,3-linked Ara*f* residues in disubstituted Xyl*p* residues. The present paper will discuss the action of these two types of AXH on AX. Role of Ara*f* residues on the viscosity and on the morphology and mechanical properties of films was further investigated using AXH-m and AXH-d3. Removal of Ara*f* residues by AXH-m resulted in precipitation of less substituted AX. On the other hand, more specific action of AXH-d3 resulted in still water-soluble AX. The viscosity of AX solution decreased after the AXH-d3 treatment. The films from AXH-m treated AX showed changes in crystallinity and oxygen permeability. Controlled enzymatic treatments generated optimal properties of AX films.

Disassembly of Cereal By-Products by Multi-Enzymatic Mixtures

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Agro-industrial food waste produced across Europe amounts to millions of tonnes each year. Traditionally this has either gone to animal feed or composted. As industrial processes become centralised and enlarged due to consumer demands, more of this material ends up as landfill. This causes environmental impact. This material is rich in high value compounds and until it leaves the food production factory, can be considered food ingredient quality. EU FP6 projects, such as "REPRO" and "Healthgrain" had aims to develop technologies to fractionate and utilise cereal by-products.

As part of this approach, commercial food grade enzyme preparations have been examined for their ability to liquefy cereal-derived material, such as brewers' spent grain (BSG). The importance of carbohydrases, feruloyl esterases and proteases, as well as physical factors, in opening up the structure of BSG and its subsequent hydrolysis has been examined. We have screened enzyme mixtures for key activities and how these activities behave under different hydrolytic conditions. Minimal enzyme preparations based on these studies have been prepared, showing similar solubilisation to the whole mixture. Selective inhibition studies have also been performed. Results show, for example, protease activity is required to enhance carbohydrate-cleaving activities, but other non-catalytic factors can be important to facilitate hydrolysis under certain conditions. This has allowed us to understand key points required for enzymatic disassembly of BSG and what enzyme mixtures to use under different treatment conditions.

Acknowledgements
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Cloning of lichenase Cel12A from a new isolate of *Stachybotrys atra* and expression in *Aspergillus niger*

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A new fungal strain with a powerful cellulase system has been isolated from a rotten rag. Morphological characterization and rDNA sequence allowed the identification of the strain as belonging to the species Stachybotrys atra. The strain shows high cellulase secretion when grown in rice straw-supplemented media. Crude cellulase secreted by the strain shows maximum activity at 70ºC and pH 5, and remains stable at 60ºC. Zymographic analysis shows that Stachybotrys atra BP-A produce a complex cellulase system, comprising several endoglucanases. One of the cellulases of the strain has been cloned and characterized. The enzyme was cloned by PCR amplification with degenerate primers deduced from cellulase CelA from Memmoniella echinata, a taxonomically related fungus. The cloned gene, cel12A, shows an open reading frame of 848 bp interrupted by two introns of 73 and 58 bp, respectively. The structural gene devoid of introns was amplified by fusion PCR and cloned in Aspergillus niger under control of promotor contained in plasmid pRAXdes2. The cloned enzyme was purified and characterized from culture supernatants of the Aspergillus niger recombinant strain. The enzyme was highly active on mixed β(1→3)-(1→4) glucans (barley β-glucan and lichenan) while its specific activity on carboxymethyl-cellulose was much lower, and it was not active on crystalline celluloses. The specific activity of the cloned enzyme indicates it is a lichenase (β-(1→3)-(1→4)- β-D-glucan 4-glucanohydrolase, EC 3.2.1.73). The enzyme shows a molecular weight of 26 kDa and maximum activity at 45ºC and pH 6. Thin layer chromatography analysis of hydrolysis products released from lichenan and barley glucan shows the enzyme liberates a mixture of products from these substrates, including cellooligosaccharides of 2-4 units and products of intermediate mobility suggesting the presence of β 1-3 bonds.

Cloning, expression and characterization of a type C feruloyl esterase from *Fusarium oxysporum*: A potential enzyme tool for the release of phenolic compounds from agro-industrial by-products

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A hypothetical protein FOXG_12213.2 of *Fusarium oxysporum* was found to have high amino sequence identity with known type C feruloyl esterases (FAEs) containing a 13-amino acid conserved region flanking the characteristic G-X-S-X-G motif of a serine esterase. The putative FAE from the genomic DNA was successfully cloned in frame with the *Saccharomyces cerevisiae* α-factor secretion signal under the transcriptional control of the alcohol oxidase (*AOX1*) promoter and intergrated in *Pichia pastoris* X-33 to confirm that the enzyme exhibits FAE activity. In shake-flask culture induced with methanol, the FAE content was about 830 U/Lt. The molecular weigh of 62 kDa was in agreement with the theoretical calculated molecular mass indicating the correct process of the secretion signal in *P. pastoris*. The recombinant FAE was purified to its homogeneity and subsequently characterized using a series of model substrates including methyl esters of hydroxycinnamates and alkyl ferulates. The substrate specificity profiling reveals that the enzyme is a type C FAE showing broad hydrolytic activity against the four methyl esters of hydroxycinnamic acids and strong preference for the hydrolysis of *n*-propyl ferulate. Ferulic acid (FA) was efficiently released from destarched wheat bran (DSWB) and brewer's spent grain (BSG) when the esterase was incubated together with xylanase from *Trichoderma longibrachiatum*. A maximum of 65% and 18% of total FA was released after 1 h incubation, while after 24h incubation the release of FA reached 74% and 66% from DSWB and BSG, respectively. Ferulic acid is used as an antioxidant and flavor precursor in the food and pharmaceutical industries. The esterase showed broad pH stability make it important candidate for many biotechnological applications that require acidic or alkaline conditions (such as alkaline pulping).

Enzymatic components for the in vitro synthesis of estolides

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Industrial activities derived from the use of vegetal biomass generate each year high amounts of low price subproducts whose elimination has become an economic, energetic and environmental problem. However, certain enzymatic systems could help to increase the productivity of raw materials, could contribute to reduce the residues generated, and could lead to the production of added value products. In this context, a new class of oligoesters of hydroxyfatty acids (estolides) could be synthesized from vegetal subproducts by means of enzymatic reactions. Estolides are naturally found in some plants and show important applications as emulsifiers in food industry, lubricants, painting or cosmetics.

Strain *Pseudomonas* 42A2, isolated from an agroindustrial oil sample, can produce several varieties of estolides when growing on lipidic substrates. The synthesis of estolides in *Pseudomonas* 42A2 involves at least a lipooxygenase, a hydroxylase and a lipase (1, 2, 3). In a first step, oleic acid would be tranformed into hydroxylated derivatives (10-hydroxy-8E-octadecenoic acid) by means of a lipooxygenase plus a hydroxylase (4). The hydroxylated acid derivatives obtained would then be polymerized into estolides by the strain's extracellular lipases (2). According to this hypothesis, isolation and production of the set of enzymes used by the strain in this transformation, would allow the synthesis of estolides *in vitro*, thus contributing to provide an increased value to the agroindustrial byproducts and to set up a more economic and environmentally friendly system to remove them.

Among the enzymes responsible for the synthesis of estolides in *Pseudomonas* 42A2, lipases play a key role in the polymerization of hydroxylated acid derivatives. Thus, we analyzed the lipolytic system of the strain and found that it codes for at least two lipases, LipA and LipC. These lipases are located appart from each other at the strain's chromosome and result differentially expressed. However, both are dependent on a foldase (LipH), located at the same operon as LipA. At present, both lipases have been cloned, purified and characterized, and further experiments will soon be performed to test their estolide polymerizing capacity *in vitro*.

References

1. Guerrero, A. Casals, I., Busquets, M., León, Y., Manresa, A. (1997) Oxidation of oleic acid to (E)-10-hydroperoxy-8-octadecenoic and (E) -10-hydroperoxy-8-octadecenoic acids by *Pseudomonas* sp. 42A2. Biochim. Biophys. Acta 1347:75-81.
2. Pelaez, M.; Orellana, C.; Busquets, M.; Marqués, A. Guerrero, A. Manresa, A. (2003) Natural estolides produced by *Pseudomonas* sp. 42A2 grown on oleic acid: production and characterization. JAOCS 80: 859-866.
3. Busquets, M.; Deroncele, V.; Vidal-Mas, J.; Rodriguez, E.; Guerrero, A.; Manresa, A. (2004) Isolation and characterization of a lipoxygenase from *Pseudomonas* sp. 42A2 responsible of the biotransformation of oleic acid into (S)-(E)-10- hydroxy-8-octadecenoic acid. Antonie van Leeuwenhoek 85:129-139.
4. Vidal-Mas, J., Busquets, M., Manresa, A. (2005). Cloning and expression of a lipoxygenase from *Pseudomonas aeruginosa* 42A2. Antonie van Leeuwenhoek 87:245-251.

Recent development in enzymes for biomass hydrolysis

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Significant research efforts have been invested in evaluating and understanding the enzymatic hydrolysis of lignocellulosic substrates by cellulases produced by the fungus *Trichoderma reesei* (*1*). Commercial products of various *T. reesei* isolates have been available for a long time in cereal foods applications, the brewing industry, fruit and vegetable processing and have also been widely evaluated and applied in relation to bioethanol production processes. *T. reesei* secretes high amounts of enzymes, up to 100 g/L (*2*). These enzymes comprise a battery of activities that catalyze the degradation of the cellulose and hemicellulose network of the plant cell wall (*3*).

Prior to enzymatic hydrolysis, most lignocellulosic substrates undergo some sort of pretreatment to increase the accessibility of the substrate to enzymatic attack. The pretreatment should preferably result in solubilisation of hemicellulose, removal of lignin and an increase in the available surface area and the substrate porosity (*4*).

However, despite pretreatment of the lignocellulosic substrates, the respective activities in *T. reesei* cellulase products appear not always to be present in optimal ratios for degradation of lignocellulose (*5*).

This paper describes Novozymes' recent development of biomass hydrolytic enzymes and applications therein including lignocellulose and hemicellulose hydrolysis.

References:

1: Palonen, H; Tjerneld, F; Zacchi, G. J. Biotech. 2004, 107, 65-72.

2: Vinzant, T. B; Adney, W. S; Decker, S. R.; baker, J. O; Kinter, M. T; Sherman, N. E; Fox, J. W; Himmel, M, E. Appl. Biochem. Biotech. 2001, 91/93, 99-107.

3: Zhang, Y. –H. P; Lynd, L. R. Biotech. Bioeng. 2004, 88, 797-824.

4: Laureano-Perez, L; Teymouri, F; Alizadeh, H; Dale, B. 2005. Appl. Biochem. Biotech. 121-124, 1081-1099.

5: Berlin, A; Maximenko, V; Gilkes, N; Saddler, J. 2006. Biotech. Bioeng. 97, 2, 287-296.

Thermophilic fungal CBH enzymes for hydrolysis of lignocellulosic materials

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Enzymatic hydrolysis is currently considered the primary option to produce sugars from biomass for microbial fermentation to various chemicals, including ethanol. One of the major techno-economical challenges for large scale industrial processing is the cost and efficiency of the enzymes required. Use of thermostable cellulases could improve the overall efficiency of enzymatic hydrolysis of lignocellulosic materials, due to potentially higher specific activities and increased hydrolysis rates. In addition, higher thermal activity can provide flexibility in selection of process options. Even though the enzymatic degradation of cellulosic biomass to soluble sugars is performed by a consortium of enzymes acting in synergy, the key cellulases participating in the total hydrolysis are cellobiohydrolases. In current commercial cellulase products, these comprise mainly family-7 enzymes (Cel 7A) derived from fungi. In order to find new improved cellulases and optimal enzyme mixtures for the total hydrolysis of cellulose, comparison of the key components is required, followed by evaluation of these enzymes in hydrolysis experiments using pre-treated substrates.

In this work, thermostable CBH type enzymes were characterized and selected in order to develop new superior enzyme products for lignocellulose hydrolysis. The kinetic data of several thermostable fungal Cel 7A enzymes, analysis of their performance in hydrolysis of pre-treated lignocellulosic raw materials and as components in thermophilic enzyme mixtures were compared. The properties and performance of these cellobiohydrolases were compared to those of the CBHI (Cel7A) of *T. reesei*, which is one of the most thoroughly studied fungal cellobiohydrolases. For the work, several thermostable CBH's were purified and cloned and their enzymatic properties were characterized. Enzyme constructs containing only the catalytic core modules or the entire two-module proteins (composed of the catalytic and the cellulose-binding modules) were also compared. Interesting substrate-specific differences in the hydrolysis performance were detected. The selected CBH enzymes were studied as major components in mixtures with other thermostable enzymes. These mixtures could be successfully used in the total hydrolysis of pre-treated materials at elevated temperatures.

Acknowledgements
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NADH- vs. NADPH-coupled reduction of 5-hydroxymethylfurfural (HMF) and its implications on product distribution in xylose-fermenting Saccharomyces cerevisiae strains

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Baker's yeast *Saccharomyces cerevisiae* is notably considered as the organism of choice for the production of bioethanol from lignocellulosic feedstocks. Efficient ethanol production by *S. cerevisiae* requires generating yeast strains that (i) can efficiently ferment all sugars that are present in the lignocellulosic hydrolysate, i.e. both hexose and pentose sugars and (ii) can tolerate the inhibitors (furaldehydes, acids and phenolics) that are released during the hydrolysis steps.

We have previously generated *S. cerevisiae* strains that are capable of fermenting xylose to ethanol by introducing the fungal xylose pathway consisting of NAD(P)H-dependent xylose reductase (XR) and NAD^+-dependent xylitol dehydrogenase (XDH). While the level of the by-product xylitol that originates from the difference in co-factor usage between XR and XDH is high in defined mineral medium, it is low when using undetoxified lignocellulosic hydrolysate. It is suspected that the concomitant reduction of the furaldehydes 5-hydroxymethylfurfural (HMF) and furfural by endogenous yeast reductases changes the intracellular cofactor balance in favor of ethanol formation.

Recently, ADH6p and mutated ADH1p (mut-ADH1p) were identified as NADPH- and NADH-dependent enzymes responsible for HMF conversion in *S. cerevisiae* (Petersson *et al.*, 2006, Yeast.23:455-464; Laadan *et al.*, 2007, Yeast. In press). In the present work, we constructed xylose-fermenting *S. cerevisiae* strains in which either the *ADH6* or *mut-ADH1* gene was overexpressed. The strains were physiologically characterized under anaerobic conditions to analyze HMF uptake, xylose consumption and product distribution. The effects of NADPH or NADH-dependent HMF reduction on by-product formation are discussed.

Differences in mode of action of microbial endoxylanases

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Endo-β-1,4-xylanases (EC 3.2.1.8, EXs) are glycoside hydrolases (GHs) that catalyze the degradation of xylan, the main component of plant hemicelluloses. Microbial xylanases have been classified mainly into two GH families, family 10 and 11. Recent studies indicate a much greater heterogeneity of these enzymes, and on the basis of amino acid sequence similarities EXs have also been classified in GH families 5, 7, 8 and 43 [1].

The mode of action of endo-β-1,4-xylanases of three glycoside hydrolase families, GH10, GH11 and GH5, was examined in 4-*O*-methyl-D-glucuronoxylan and in a series of defined aldouronic acids. The structure of hydrolysis products was determined by combination of MS and enzymatic treatment.

EXs of GH10 liberated from glucuronoxylan aldotetraouronic acid ($MeGlcA^3Xyl_3$) and EXs of GH11 aldopentaouronic acid ($MeGlcA^3Xyl_4$) as the shortest acidic fragments [2]. Depending on distribution of MeGlcA residues on the glucuronoxylan main chain, EX of GH5 from *Erwinia chrysanthemi* (XynA) generated series of shorter and longer aldouronic acids of backbone polymerization degree 3-14, in which the MeGlcA is linked exclusively to the second xylopyranosyl residue from the reducing end. Aldotetraouronic acid $MeGlcA^3Xyl_3$ and aldopentaouronic acid $MeGlcA^3Xyl_4$ were resistant to the action of EXs of GH10 and GH11. EX of GH5 attacked aldotetraouronic and aldopentaouronic acids at the first glycosidic linkage from the reducing end [3].

The main differences in the mode of action of EXs of the above three families were observed with aldohexaouronic acid $MeGlcA^3Xyl_5$. The hydrolysis of this substrate by EXs of GH10 afforded Xyl_2 and $MeGlcA^3Xyl_3$. The degradation of aldohexaouronic acid by EXs of GH11 did not correspond to simple hydrolysis and involved a glycosyl transfer reaction. EX of GH5 attacked the substrate at the reducing end to give xylose and $MeGlcA^2Xyl_4$.

[1] Collins T., Gerday C. and Feller G. *FEMS Microbiol Rev* (2005), 29, 3-23

[2] Biely P., Vršanská M., Tenkanen M. and Kluepfel D. *J Biotechnol* (1997), 57, 151-166

[3] Vršanská M., Kolenová K., Puchart V. and Biely P. *FEBS J* (2007) 274, 1666-1677

Kinetic modelling of enzymatic hydrolysis of agricultural residues

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Conversion of cellulose from lignocellulosic biomass into fermentable sugars can be performed by chemical and enzymatic catalysis. The enzymatic catalysis has advantages such as relatively mild conditions and smaller loss in monosaccharides due to their degradation, resulting in higher yields. However, high enzyme loading is required to obtain a high degree of cellulose conversion, which increases the cost of the process.

In our experiments, enzymatic conversion of cellulose by commercial (Celluclast 1.5L, Novozymes) and "in-house" fermented enzymes has been studied. The soft-rot fungus *Trichoderma reesei* (also known as *Hypocrea jecorina*), one of the most studied cellulolytic organisms, was used as a cellulase producer. Various agricultural by-products were tested as carbon sources in the fermentation and as substrates in the hydrolysis. Wheat straw and corn stover (collected in Hungary) had been chosen among potential raw materials for 2nd generation bioethanol. Both agricultural residues are renewable, cheap and widely available (4-5 million and 8-15 million tons annual production in Hungary, respectively). In order to enhance accessibility of cellulolytic enzymes, lignocellulosic raw material was pretreated by steam-explosion in the presence of catalytic amount of sulphur dioxide at Lund University, Sweden and ENEA, Italy.

Glucose yield from the enzymatic hydrolysis of cellulose was investigated as a function of cellulase enzyme loading and solid concentration. The hydrolysis experiments were carried out at 50°C, 2 % dry matter content in 0.05 M acetate buffer solution (pH 4.8). Cellulase enzyme dosage was set to 10, 20 and 30 FPU per g cellulose. In order to avoid accumulation of cellobiose, β-glucosidase was supplemented to 10, 20 and 30 BGL U per g cellulose with a commercial enzyme preparation, Novozym 188 (Novozymes). In the case of corn stover, substrate concentration of 2, 4, 6, 8 and 10 % DM were investigated at 30 FPU cellulase and 30 U β-glucosidase per gram cellulose enzyme dosages. Samples were withdrawn at the start of the hydrolysis and after 6, 24, 48 hours. Hydrolytic process was monitored by high performance liquid chromatographic (HPLC) analysis of the supernatant for various sugars produced (cellobiose, glucose, xylose, arabinose). Each set point was run in triplicates.

Computational simulation based on a kinetic model was used to predict the hydrolysis reaction at various enzyme and substrate concentrations. Because of the complexity of the insoluble lignocellulosic substrate, synergistic actions of cellulase components and product inhibition, mathematical modelling of this hydrolysis process is difficult. The applied model was an empirical three-parameter rate equation, which was integrated numerically (too complex to solve analytically) applying the Euler method. The calculation was performed using the software Berkeley Madonna.

A multifunction process for the separation of cellulose fibres

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This work is developed in cooperation with the team at the Biotechnology Reseach Center, using our lab and simple tools to classify our ideas.

A multifunction process is described for the separation of cellulose fibres from the other constituents of lignocelluloses biomass as found in trees, grasses, agricultural waste and waste paper with application in the preparation of feedstocks for use in the manufacture of paper, plastics, ethanol and other chemicals. This process minimizes waste disposal problems since it uses only steam, water, and oxygen at elevated temperatures in the range that we limited through a little time "minutes" plus a quantity of chemical reagents to maintain pH in the range 8 to 13.

An energy recuperation function is important to the economic viability of the process. We applied biological research focused on two related themes: organism development for consolidated bio-processing and the fundamentals of microbial cellulose utilization. In the course of pursuing these two themes, we are also active in development of laboratory methods that enable us to pursue our goals.

Therefore, today's challenge is to improve and assemble the various process options of lignocellulose conversion into viable commercial ventures.

Hemicellulases as helper enzymes in the degradation of lignocellulosic substrates

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One promising option to get over the setback of upcoming energy crisis could be the utilization of biomass for fuel production (i.e. biodiesel and bioethanol). Beside starch, which is easy to convert to ethanol, lignocellulosics, such as soft and hard wood, agricultural, paper and pulp industrial wastes could be economically more feasible raw materials for bioethanol production. However, conversion of lignocelluloses to ethanol is not a simple process. Pretreatment followed by hydrolysis of raw material to monomeric sugars, and subsequently fermentation of monosaccharides to ethanol are the key steps of processing.

Hydrolysis of cell wall polysaccharides of plants requires synergistic action of several enzymes. Among them cellulases represent the primary enzyme family needed. Cellulolytic enzymes comprise cellobiohydrolases (CBH, EC 3.2.1.91), endo-1,4-β-D-glucanases (EG, EC 3.2.1.4), and 1,4-β-D-glucosidases (EC 3.2.1.21). In cases where there are remaining hemicellulose and lignin moieties on the cellulose surface or within the fibre structures, application of accessory, helper enzymes, such as hemicellulases, feruloyl esterases or lignases could be beneficial to improve the hydrolysis efficiency [[i], [ii], [iii]].

In this work several lignocellulosic substrates (softwood, agricultural byproducts) pretreated with different methods applying various conditions were hydrolyzed by the combined action of purified selected cellulases and hemicellulases. These target enzymes used in the controlled hydrolysis experiments were endoglucanases, cellobiohydrolases, β-glucosidase, xylanase and xyloglucanase. The selected hemicellulases had a generally improving effect on the hydrolysis of lignocellulosic biomass.

[i] Sørensen, H.R., Pedersen, S., Viksø-Nielsen, A., Meyer, A. S. (2005) Efficiencies of designed enzyme combinations in releasing arabinose and xylose from wheat arabinoxylan in an industrial ethanol fermentation residue, *Enzyme and Microbial Technology*, **36**, 773-784.

[ii] Tabka, M.G., Herpoël-Gimbert, I., Monod, F., Asther, M., Sigoillot, J.C. (2006) Enzymatic saccharification of wheat straw for bioethanol production by a combined cellulase xylanase and feruloyl esterase treatment, *Enzyme and Microbial Technology*, **39**, 897-902.

[iii] Berlin, A., Gilkes, N., Kilburn, D., Bura, R., Markov, A., Skomarovsky, A., Okunev, O., Gusakov, A., Maximenko, V., Gregg, D., Sinitsyn, A., Saddler., J. (2005) Evaluation of novel fungal cellulase preparations for ability to hydrolyse softwood substrates - evidence for the role of accessory enzymes, *Enzyme and Microbial Technology*, **37**, 175-184.

Strategies for cellulose degradation in basidiomycetes

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Cellulose is the main polymeric component of the plant cell wall, the most abundant polysaccharide on earth and an important renewable resource. As such, it is in a focus with respect to its use as a source of added-value products including monosaccharides or bioethanol. Basidiomycete fungi belong to the most potent degraders since many species grow on dead wood or litter, in an environment rich in cellulose and their cellulose-degrading capabilities might potentially be useful in biotechnology. For the conversion of cellulose in the low-molecular-mass products, basidiomycetes utilize a set of hydrolytic enzymes typically composed of endoglucanase, cellobiohydrolase and β-glucosidase. In addition, systems producing hydroxyl radicals based on cellobiose dehydrogenase, quinone redox cycling or glycopeptide-based Fenton reaction are involved in cellulose hydrolysis by some species. The cellulolytic system is typically composed of several of the above mechanisms that jointly contribute to the utilization of cellulose as a source of carbon or energy or degrade it to ensure fast substrate colonization. The efficiency and regulation of cellulose degradation differs among wood-rotting, litter-decomposing, mycorrhizal or plant pathogenic fungi and yeasts due to the different roles of cellulose degradation in the physiology and ecology of the individual groups. While the involvement of different cellulolytic mechanisms in wood degradation is already known to some extent and their enzymes have been tested in biotechnological applications, the cellulolytic systems of soil and litter-inhabiting basidiomycetes attracted so far only limited attention.

Analysis of structures limiting the enzymatic hydrolysis of lignocellulosic residues

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Fuels from lignocellulose biomass have a high potential to reduce green house gas emissions, and hence are important means to fulfil the road transport CO_2 emissions targets. Advanced conversion technologies are, however, needed to produce biofuels, such as ethanol, from a wider range of resources, including lignocellulosic biomass. The major obstacles in the enzymatic hydrolysis of lignocellulose into sugars are related to the recalcitrance and complex structure of the raw material itself, posing a scientific challenge and opportunity for biotechnical development.

The role of hemicellulose and lignin in the complete hydrolysis of lignocellulosic substrates is still not fully understood. To reduce the overall amount and costs of enzymes, the potential bottlenecks decreasing the enzymatic hydrolysis rate should be overcome. Various hemicellulases or other polysaccharide and lignin modifying enzymes can be used to enhance the conversion by hydrolysing or modifying the residual polymers in the matrix. The main aim is to develop improved enzymes for the complete conversion of lignocellulose to sugars based on the structural analysis and hydrolysis tests.

In order to understand the disassembling mechanisms of lignocellulose components, the limiting factors in the conversion of carbohydrate polymers into sugars were studied by characterization of the substrates. After enzymatic hydrolysis with the well characterized cellulolytic system of the fungus *T. reesei,* modifications in the chemical composition and structures of the hydrolysis residue were followed. These analyses included various chemical and spectroscopic methods, combined with enzymatic and chemical treatments. The results are used to identify the enzyme activities required to improve the hydrolysis of lignocellulose raw materials.

Factor affecting radical formation on lignicellulosic fibres after laccase treatment and related lignin chemical changes

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In the field of packaging, materials with high barrier and mechanical properties are generally required. Wood fibres can achieve these properties, after proper modification. Radical active centres can be produced on lignin at the fibre surface. As an example, the reaction of wood fibres obtained from thermomechanical pulp (TMP) with molecular oxygen and laccase as catalyst, was demonstrated to produce the radical activation of the lignin phenols surface through the formation of phenoxy radicals.

In this paper, the oxidation of different lignocellulosic pulp and fibres is compared using different laccases as catalyst with molecular oxygen or air as oxidant, electron paramagnetic resonance (EPR) spectroscopy made it possible to reveal and quantify the formation of phenoxy radicals on the fibre surfaces. These data were compared with the analysis of lignin chemical structure present in different fibres, as assessed by heteronuclear single quantum coherence - nuclear magnetic resonance (2D-HSQC-NMR) spectroscopy, nuclear magnetic resonance spectroscopy of carbon (^{13}C-NMR) and phosphorous (^{31}P-NMR), and gel permeation chromatography (GPC) and with the oxidative potential of enzymes. The changes in chemical structure, achieved by lignin units under oxidative treatments, were assessed by hetero-nuclear single quantum coherence (HSQC), by ^{13}C and ^{31}P-NMR spectroscopy and by GPC.

Optimisation of gene expression in recombinant xylose and arabinose fermenting yeast by kinetic modelling

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Production of fuel-grade ethanol from lignocellulosic materials requires that all available sugars are fermented to ethanol. The most abundant sugars in a hydrolysate of agriculturally derived lignocellulosic materials are glucose, xylose and arabinose. Since *Saccharomyces cerevisiae* cannot naturally ferment pentose sugars to ethanol it needs to be engineered to have this feature. A kinetic model of the xylose pathway introduced in yeast has been developed and the optimum expression ratios between the three enzymes involved in the pathway were determined under steady state conditions [1]. An industrial strain of *S. cerevisiae* capable of co-fermenting glucose and xylose has been constructed and evaluated using microarray analysis [2,3]. More recently a strain capable of co-fermenting the three sugars glucose, xylose and arabinose has been engineered [4]. However, relatively little is still known about the kinetics of the involved enzymes and how they affect each other. The introduction of an arabinose pathway leads to new reactions and formation of new compounds that were not included in the previous model. The old model therefore has to be revised and expanded to include the new reactions so that the optimum ratios between all six enzymes can be determined. The emphasis of the project will be to determine kinetic properties of the six enzymes at intracellular conditions. The enzymes will also be characterised with selected alternative substrates, with preference for compounds from the other pathway. Key compounds from the glycolysis and the pentose phosphate pathway (PPP) will also be investigated. The data obtained will be used to create a computer model in which optimum expression levels can be determined. It would also be desirable to integrate the pathways of glycolysis and PPP into the model together with thermodynamic constrictions to get a full view of the process and to identify potential limitations in the metabolism

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1. Eliasson et al. (2001) Enzyme Microb Technol, vol 29, p. 288
2. Wahlbom et al. (2003) FEMS Yeast Res, vol 3, p. 319
3. Wahlbom et al. (2003), Appl. Environ. Microbiol., vol 69, p. 740
4. Karhumaa et al. (2006), Microb Cell Fact, vol 5:18

Ethanolic fermentation as a tool for high added value products purification from biomass

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The biorefinery concept implies the development of processes in order to valorize the whole part of a crop. In this concept, white biotechnologies are usually considered for the production of new compounds or compounds actually derived from the oil industry.

For example, interest in ethanolic fermentation is growing nowadays, mainly for its energetic applications, starting from saccharose or starch. Biorefineries integrating lignocellulosic materials are under development. Whatever the initial substrate may be, wet technologies would produce sugars and other soluble materials from the biomass.

Different results could be expected:
Most of the hydrolysed molecules are used by the micro organisms.
Some molecules could be inhibitors for the fermentation.
Some molecules are "indifferent ones" and could be concentrated by the fermentation process followed by distillation

In the latest case, these molecules are concentrated in the residual medium and could be more easily recuperated. An example of this exists in the production of ethanol from beet. The different juices (raw, thin or thick juice) contain molecules such as raffinose, glutamine, betaine, saponins or pectic materials. Some products are used by the yeasts and others are concentrated. Our purpose is to summarize the results obtained, allowing the development of the concept in others biorefinery applications.

Evaluation of Streptomyces for biomechanical pulping. Pyrolysis-GC/MS, confocal (CLSM) and environmental scanning electron microscopy (ESEM) assessment

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The biomechanical pulping offers the potential for saving energy and to strengthen the fibres quality. These advantages will lead to economic benefits and the enhancement in the environmental sustainability of the pulping process. Previous studies have demonstrated the suitability of streptomycetes for biopulping of agricultural residues and Picea wood, provided they produce a range of enzymes related with lignocellulose degradation from which laccases are remarkable.

In this study, the evaluation of different *Streptomyces* strains to modify pine wood structure in solid-state fermentation (SSF) to be applied for biomechanical pulping was examined through Pyrolysis-GC-MS. In addition, biomechanical pulp obtained after fermentation of *S. ipomoea* was analysed using confocal laser scanning microscopy (CLSM) and environmental scanning electron microscopy (ESEM) techniques.

A stepwise pyrolysis (500 ºC) was applied to the samples in order to establish differences between the types and/or abundance of the compounds derived from the carbohydrate and lignin moieties. The pyrograms of sound pine wood confirm the presence of only G units as representative of gymnosperms. Some differences were observed in the relative abundances of the released lignin and carbohydrate-derived compounds in woods treated with different *Streptomyces* strains. Some products arising from pyrolysis of carbohydrates could be recognized, such as furfural, 2-acetylfuran, 2,3-dihydro-5-methylfuran-2-one and 3-hydroxy-2-methyl-(4H)-pyran-4-one. The relative abundance of these compounds is generally higher in treated woods compared with the control. When performing the lignin pyrolysis, new oxidized products derived from G units were identified in treated woods such as homovanillin and propiovanillone. The abundance of other oxidized compounds such as vanillin, acetoguaiacone and guaiacylcetone was clearly increased after bacterial treatment of wood. Trough this technique either oxidation or oxidation followed by cleavage of C3 alkyl chain from guaiacyl units of the lignin can be inferred.

In order to know the efficiency of the bacterial pre-treatment on the mechanical pulp, hand sheets were analysed for physico-chemical and optical properties. Some of them such as braking length and Gurley index were improved compared with untreated pulp.

Moreover, the analysis of these pulps by CLSM showed changes in fluorescence intensity of pulp fibres according with lignin degradation. The application of ESE microscopy allowed observing structural changes in the wood fibres corresponding to treated woods.

Importance of morphology of Trichoderma reesei for production of cellulases

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A major bottleneck in developing an economically feasible process for enzymatic hydrolysis of cellulose is the high cost of the enzyme production. *Trichoderma reesei* has long been considered to be the most efficient producer of cellulases and it is currently used for production of commercial cellulolytic enzymes (e.g. Celluclast). However, further improvements in the enzyme production process are necessary if the cost of the enzymes is to be lowered enough to make second generation bioethanol production economically feasible. *T. reesei* has been well characterised on a molecular level and the genome has been sequenced, but little has been done to transfer this knowledge to process relevant conditions. Therefore, a physiological characterisation of T. reesei focusing on the enzyme profile and levels produced in relation to conditions with relevance to those found during full scale production is needed.

Enzyme productivity is strongly connected to fungal morphology in *T. reesei* and investigating this relationship is a major step towards designing improved fermentation processes for cellulase production. In this presentation the effect of pH and agitation on morphology and enzyme production of *T. reesei* Rut-C30 in batch fermentations is described. Enzyme activity has been investigated by measuring the total cellulolytic activity as well as investigating the detailed composition of the enzyme mixture. In contrast to the traditionally used assays for measuring enzyme activity, methodology for investigating the detailed enzyme profile is not well established. For this purpose, we will develop methodology using capillary electrophoresis or protein chips that will allow determination of the amount of single enzymes.

Microarray data reveals change in expression level, what next?

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Recombinant *Saccharomyces cerevisiae* strains overexpressing the *Pichia stipitis XYL1* and *XYL2* genes, as well as the endogeneous *XKS1* gene, grow slowly on xylose. By chemical mutagenesis, adaptation or breeding, strains with enhanced aerobic xylose growth have been generated. However, the genetic modifications which are responsible for the xylose growing phenotype are unknown. Four strains with enhanced xylose growth (TMB3400, C1, C5 and BH42) were compared with two control strains (TMB3399, TMB3001) through genome-wide transcription analysis in order to identify novel targets for metabolic engineering. A subset of 13 genes with changed expression levels in all improved strains was selected for further analysis. Thirteen validation strains and two reference strains were constructed in order to investigate the effect of overexpressing or deleting these genes in xylose-utilizing *S. cerevisiae*. Improved growth rates were observed in five out of thirteen cases when growing these strains aerobically with xylose as carbon source.

Ethanol production from wheat straw hydrolysate by Ca-alginate immobilised Saccharomyces cerevisiae and Mucor indicus

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Abstract

Lignocellulosic biomass has great potential to become feedstock for production of bioethanol. To obtain fermentable sugars lignocellulose needs to be depolymerised e.g. by a pretreatment process such as wet oxidation, hydrothermal treatment, or steam explosion followed by enzymatic hydrolysis. The drawback to the lignocellulosic process is the content of pentose sugars in the raw materials and the formation of fermentation inhibitors during processing of the material. To be able to efficiently convert this feedstock into ethanol a pentose fermenting and inhibitor tolerant microorgansm is needed.

Strains of Mucor have been shown to exhibit favourable characteristics in lignocellulosic hydrolyzates such as: Ethanol yield on glucose comparable to that of *S. cerevisiae*, ability to take up xylose as well as hexoses, high productivity on glucose and xylose, no lactate formation, and high inhibitors tolerance (Millati et al., 2004). In this study continuous fermentations were carried out by immobilized *M. indicus* on both synthetic substrate (with glucose, arabinose and xylose as carbon source) and wheat straw hydrolysate (with added glucose) pretreated at the Danish IBUS-pilot plant. The fermentation using wheat hydrolysate gave an ethanol productivity of 0.38g/h. Cascade process was carried out using two different reactors to consume glucose and xylose by immobilized *S. cerevisiae* and immobilized *M. indicus*, respectively. The ethanol concentration reached 7.294g/l in the first reactor and 7.871g/l in the second reactor. The specific ethanol productivity reached 1.4g/h and 0.752g/h, by *S. cerevisia* and *M. indicus* respectively.

References

Millati R, Edebo L, Taherzadeh M J (2004) Performance of Rhizopus, Rhizomucor, and Mucor in ethanol production from glucose, xylose, and wood hydrolysates, Enzyme and Microbial Techonolgy 36, 294-300.

Ethanol production and co-subtrate consumption in xylose-utilizing *Saccharomyces cerevisiae*

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In this study, the effect of glucose signaling on the fermentative behavior of xylose-utilizing Saccharomyces cerevisiae TMB 3400 was investigated. The yeast metabolic response to situations of excess and limitation of glucose and xylose was evaluated in chemostat culture. Co-substrate pulsing using glucose, 2-deoxyglucose and acetate, was investigated in xylose-limited chemostat cultures. For carbon-limited cultures, biomass yield and substrate uptake rate were similar during glucose and xylose limited conditions. On the other hand, for excess-carbon cultures, the strain behaved differently in response to the presence of glucose and xylose, respectively. While in both cases a higher production of glycerol occurred, ethanol production was only observed for excess-glucose cultures. Pulsing acetate to xylose limited cultures stimulated transient overflow metabolism. Glucose pulses above approximately 2 g/L in xylose steady-state cultures inhibited xylose uptake, whereas lower glucose concentrations enhanced the xylose uptake rate. Pulse addition of 2-deoxyglucose, which is taken up but not metabolized by the cells, was also effective in redirecting the metabolism to respiro-fermentative. Results are discussed in relation to regulation of sugar metabolism in Crabtree-positive and –negative yeast.

Design of new bio-processes for conversion of lignocellulose wastes into energy and high added value products

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The crucial aspect of our research is the development of new systems of ethanol production from raw materials so far never used, such as environmentally challenging lignocellulose wastes. In fact, one of the main steps of the project is finding an alternative to conventional biomasses used for ethanol production. For this purpose, we are investigating the feasibility of microbial processes to bio-refine different fractions of agro-industrial, forestry and urban wastes. Transforming these wastes into sugars to be used as substrates of alcohol fermentation will ensure a cleaner environment and will allow for the production of lignocellulose-based bio-fuels, giving both economic and environmental advantages.

Data were collected on discharged amounts, legislations on dischargeable amounts, production times and, when available, on composition. We chose to use a panel of agro-industrial wastes such as those from peach, apricot, apple, pear and tomato processing, because of the presence of local factories with disposal problems. In spite of a short time of production of each of these wastes, employment of a panel of different wastes can guarantee a more continuous supply of wastes to the ethanol production plant. We also selected vegetable residues, having the advantage of containing high cellulose levels. The other classes of selected wastes consist of greengrocer's wastes and organic fraction of urban solid wastes that are continuously produced during the whole year, giving disposal problems for the huge discharged amounts. In Europe 1,3 billion tons of solid wastes and 700 millions of tons of agricultural wastes were produced in 2003 (Eurostat).

Analyses of macro-molecular composition of the selected wastes were performed, verifying that wastes from peach, apricot, and tomato processing and vegetable residues contain low level of lignin and high content of cellulose, thus these are expected to be appropriate for conversion into fermentable sugars. Exploitation of the ability of fungi to transform cellulose, lignin and other macro-molecular components of wastes in sugars through "solid-state" fermentation (SSF) of fungi on wastes represent the second phase of the project. Such a process will allow attaining degradation of lignin in wastes by in situ-produced fungal lignolytic activities avoiding the conventional acid hydrolysis; the more accessible cellulose will be then hydrolyzed by in situ-generated fungal cellulolytic activities, substituting hydrolysis by purified cellulases, and thus reducing the cost of the process. Avoiding the use of chemical additives and of the harsh conditions of chemical treatments, increasing the efficiency and reducing the cost of enzymatic treatment will be of high added value in bio-fuel production.

So far, a commercial microbial consortium suitable for waste conversion has been selected. At the same time, rationally formulated microbial consortia usable for conversion of selected wastes will be developed on the basis of the ability of fungi to convert different macro-molecular components of wastes producing cellulolytic activities, lignolytic activities, xilanolytic activities, etc. and taking into account macro-molecular composition of the wastes to be converted. These fungal consortia will be used to optimize waste conversion by selecting the most appropriate conditions of SSF for each waste-consortium system.

On the basis of the composition of the mixtures produced by fungal treatment of wastes, different tailor-made microbial systems for conversion of these products into bio-fuels, fine chemicals and biopolymers will be developed. In fact, the design of integrated activities and technologies is crucial to make the whole process feasible and economically viable. Sugar mixtures will then be used as substrates of natural or engineered yeasts settled up to convert with high efficiency all the sugars present and to produce bio-ethanol and added-value chemicals.

Environmental and economical advantages of the local scale: a pilot biodiesel production line in the Province of Siena, Italy

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The S.I.En.A. project started in 2007 and is aimed at the construction of a pilot production chain of biodiesel from sunflower. All the steps of the production row (agricultural phase, extraction, esterification and final use) are entirely closed in a small area (the Province of Siena, Tuscany, Italy). The construction of such a short production line where the "cradle-to-grave" cycle is entirely closed in a small area has been possible by means of a planning operation that put together the farmers, the transformation industries and the final users. In comparison to traditional large-scale industrial processes where the raw materials generally are produced far from the transformation plant (typically imported from the South East Asia), a local province-scale process shows several advantages:
fuel and energy transport demand are strongly reduced, and the environmental benefits maximized;
all the environmental benefits arising from substitution of mineral diesel with biofuel remain in the area;
all the actors (farmers, manufacturers and final costumers) are directly involved in the chain.

The aims of this paper are a) to make a comparative evaluation between the biodiesel production row in a provincial area (Siena province) and the traditional large scale production b) to account for the benefits of the substitution of mineral diesel in the local public bus system with the biodiesel fuel.

The project involves 5 farmers for a total 150 ha crop extension while the maximum theoretical extension of sun flower crop in the province of Siena should reach 10.000 ha (land actually not used). Once harvested, sunflower seeds are transformed into biodiesel by means of oil extraction (hot crushing + hexane) and transesterification (KOH+MeOH). A better alternative based on the use of an enzymatic process will be attempted and evaluated. This approach, very promising because of his selectivity and mild operative conditions, might be tried by use of enzymes able to carry out esterification and transesterification reactions.

Preliminary results show that the production of biodiesel on local scale reduces the amount of CO_2 emission and improve the efficiency of the whole process. Moreover the local scale production row makes the "local" biofuel more sustainable and reduces also the economic cost of the entire process, increasing the opportunity for a virtuous agricultural economy.

Implementing novel glycan arrays in analyzing changes in polysaccharide composition due to hydrothermal pretreatments

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Ethanol obtained by the fermentation of sugars from plants is one strategy for reducing the world's dependence on petroleum-derived fuels. In particular, lignocellulosic biomass is a desirable feedstock for the future supply of ethanol since it is the largest known renewable carbohydrate source.

However, hydrolysis of lignocellulose requires considerable energy input through pretreatment, involving temperatures up to 200 oC and the use of hydrolytic enzymes. It is likely that the pretreatment can be optimized, but the precise changes that occur in cell wall architecture during processing are largely unknown.

We used a recently described microarray-based technique (Comprehensive Microarray Polymer Profiling, CoMPP, Møller et al., 2007) to track the detailed changes in cell wall polymers in wheat straw subjected to pretreatment at the DONG Energy IBUS pilot plant at four different temperatures. This technique combines the use of monoclonal antibodies specific for different cell wall polysaccharides and the high-throughput capacity of the microarrays allowing rapid detection of several carbohydrate structures.

Influence of different enzymatic bleaching stages on hexenuronic acids

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Environmental pressure has led the pulp and paper industry to develop new technologies with the aim of reducing or suppressing the presence of various pollutants in effluents from bleaching plants. One of the choices for this purpose is enzyme-based biotechnology like the use of a xylanase treatment (X) or a laccase-mediator treatment (L).

On the other hand, some 4-O-methylglucuronic acid groups present in xylans are known to be converted into the corresponding unsaturated hexenuronic acids by release of methanol during the alkaline cooking of wood. For this reason, HexA are highly likely to occur in kraft pulp. The significance of the HexA presence lies in their role in the bleaching process and in their influence on the properties of the final pulp. A xylanase treatment could theoretically decrease the hexenuronic acid content of pulp because of its action on pulp xylan, but in this work special interest was given to the laccase-mediator system effect on these acids.

Firstly, novel bacterial xylanases from different glycosil hydrolase families (5, 10 and 11) were applied as a pretreatment stage (X) in ECF sequences giving special importance on its effect on the hexenuronic acid (HexA) content. A xylanase from the family 11 was the most efficient one. Secondly, a laccase-mediator stage (L) was optimized using a three-variable sequential statistical plan over the following ranges: 1-20 U/g o.d.p. laccase dose, 0.5-2.5% o.d.p. mediator (HBT) dose and 1-7 h reaction time. L stage was followed by an alkaline extraction stage (E). The influence of variables on pulp delignification and brightness increase was examined after L and E stages. The HexA content of pulps was measured in some experiences after L and E stages (LE sequence) and the effect of a xylanase pretreatment on these acids was also evaluated (XLE sequence). Finally, LP and XLP sequences were also studied.

It is possible to reduce the hexenuronic acid content of pulps by a xylanase pretreatment stage (X) and by a laccase-mediator stage (L). Moreover, an X stage before an L stage boosts its effect on the release of these acids more efficiently than boosts the effect of a chlorine dioxide stage (D).

Optimization of process parameters in a laccase mediator stage in TCF bleaching of flax pulp

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In the present work, the influence of variables in a laccase-mediator system (laccase and mediator dosages, treatment time and oxygen pressure) on pulp properties (kappa number, brightness and viscosity) was evaluated. Pulp properties were analyzed after TCF sequences (L, LE, LRE, LP and LRP), where L is an enzymatic treatment with laccase and HBT, E is an alkaline extraction stage, R is a reductive stage and P is a hydrogen peroxide stage. The L stage was optimized by a sequential statistic plan of four variables. Variation margins were: laccase dosage (1 to 20 U ·g-1), HBT dosage (0.1 to 2 %), treatment time (0.5 to 6.5 h) and oxygen pressure in reactor (2 to 6 bar). Effluent properties (toxicity, residual enzymatic activity, COD and colour) after each bleaching stage are also evaluated. An additional treatment was performed in a 20 L reactor in a pilot plant in CELESA mill.

Results and discussion

Mathematical models obtained predict kappa number, pulp brightness and enzymatic residual activity, COD and color. Oxygen pressure variation between 2 and 6 bar does not affect pulp and effluent properties. In general, an increase in reagent doses and reaction time produces a kappa number decrease and a pulp brightness increase. Models predict limit reagents doses so an increase in those doses does not produce an additional decrease of kappa number or an increase in brightness. Initial toxicity and color in effluent increase during enzymatic treatment. COD is due only to the presence of commercial laccase. Decrease in enzymatic activity is related with mediator dose. The obtained surface graphs for kappa number, brightness in LP sequence and residual enzymatic activity in L stage are showed in figure 1.

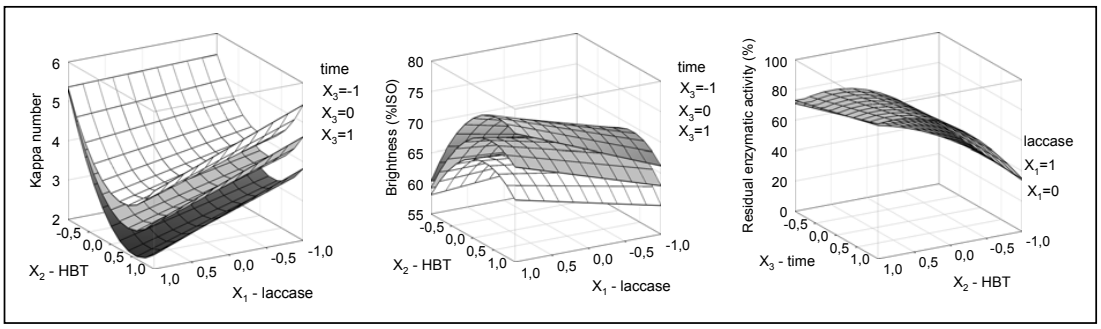

Fig. 1. Kappa number and brightness in LP sequence and residual ezymatic activity in L stage.

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The Effects of Biotreatment on Kraft, Kraft-AQ and Kraft-NaBH4 Pulp and Paper Properties

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Several studies have examined the effect of additives (AQ and $NaBH_4$) and biodelignification prior to kraft pulping. However, the effects of biodelignification prior to AQ and $NaBH_4$ additive-modified kraft methods (kraft-AQ and kraft-$NaBH_4$) are not investigated yet. The aim of this study was to evaluate untreated and biotreated pulps for each of these methods. Untreated and biotreated pulps were produced under the same cooking conditions conducting kraft, kraft-AQ and kraft-$NaBH_4$ methods. Kraft method was modified by adding 0.1 % AQ and 2 % and 4 % $NaBH_4$ in this study. The obtained pulps were refined in a PFI mill and handsheets properties were also studied.

Modifying kraft method by AQ and $NaBH_4$ resulted in an increase in pulp yield and reduction in both kappa number and screening rejects. The benefits of $NaBH_4$ addition into kraft pulping were a significant reduction in kappa number and screening rejects and a significant increase in pulp yield. The most notable outcome of $NaBH_4$ was 66.6% increase in pulp brightness when 4% $NaBH_4$ was added into kraft pulping. Of unrefined pulps, unrefined kraft pulp displayed the highest strength of pulp, which is described as tear index at a constant tensile index. Of refined pulps, kraft-AQ showed the highest pulp strength when refined to 6000 and 12000 revs in PFI mill.

Biotreatment resulted in an increase on pulp yield and reduction on kappa number compared to the control kraft method. The results also showed that pulp rejects were lower for biokraft pulp and a significant reduction was observed when biokraft process was modified by $NaBH_4$ compared to the control kraft method. Also, adding AQ and $NaBH_4$ into biopulping led to positive results with regards to pulp yield and kappa number compared to biokraft pulp. The results indicated a major increase in pulp brightness when biokraft pulping was modified with 2 % $NaBH_4$. On the other hand, biopulps gave lower tear but higher burst index compared to the control kraft pulp. Tensile index of the biopulps were slightly lower; however, untreated kraft pulp was found to be easier to refine and when pulps were refined to 12000 revs. in PFI mill, biokraft-AQ pulp showed a significant improvement on tensile index.

In conclusion, the obtained results indicated that biodelignification prior to pulping resulted in an increase on pulp yield and a decrease on kappa number for each method. However, the strength properties of the biodelignified pulps were found to be lower compared to the untreated pulps.

Key Words: Biopulping, kraft, AQ, $NaBH_4$, physical and optical properties.

Effect of pretreatment temperature in combination with hemicellulases on cellulose conversion

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Pretreatment is an essential step in the conversion of lignocellulosic materials into fermentable sugars. A hydrothermal pretreatment process developed and operated at pilot scale (The DONG Energy IBUS-process) has been shown to be effective in preparing wheat straw for enzymatic hydrolysis without the application of additional chemicals (Thomsen et al., 2006).

The optimal pretreatment temperature is around 180-200 °C, but the applied temperature has a significant effect on hemicellulose removal and formation of degradation products and inhibitors. Higher temperature results in removal of more hemicelluloses but also increased inhibitor formation. Removal of hemicelluloses is believed to play a role in convertibility of cellulose. It has been proven that addition of hemicellulases in combination with cellulases results in increased cellulose conversion, even in materials with low amounts of residual hemicelluloses (Berlin et al., 2007; Öhgren et al., 2007).

In this study, the ability to compensate for lower pretreatment temperature by including hemicellulases in the enzymatic hydrolysis has been investigated. Wheat straw was pretreated at four temperatures from 160 to 195 °C and the cellulose conversion obtained using a commercial cellulase alone or in combination with various hemicellulases was tested. In addition, the ability to improve cellulose conversion by optimising the hemicellulase addition was tested.

Reference List

Berlin A, Maximenko V, Gilkes N, Saddler J. 2007. Optimization of enzyme complexes for lignocellulose hydrolysis. Biotechnol Bioeng 97:287-296.

Öhgren K, Bura R, Saddler J, Zacchi G. 2007. Effect of hemicellulose and lignin removal on enzymatic hydrolysis of steam pretreated corn stover. Bioresour Technol 98:2503-2510.

Thomsen MH, Thygesen A, Jørgensen H, Larsen J, Christensen BH, Thomsen AB. 2006. Preliminary results on optimisation of pilot scale pretreatment of wheat straw used in coproduction of bioethanol and electricity. Appl Biochem Biotechnol 129-132:448-460.

Functional fibres pave the future for pulp and paper industry

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Upgrading of lignocellulosic fibre materials to enhance the value or properties of traditional fibre products and to create new applications for fibres is a constant challenge for the fibre-producing industry. The presence of surface lignin in pulp fibres offers possibilities to enhance or even to create completely new and innovative paper and board products by chemo-enzymatic means.

Chemo-enzymatic functionalisation is based on the use of lignin as a bonding matrix for designed attachment of novel functional groups to pulp. Functionalisation includes enzymatic activation and enzymatic bonding steps resulting in enhanced fibre properties. Thus, this method enables manufacturers and end users to customize fibres according to specific needs and requirements. Native surface properties of lignin rich fibres can be enhanced and completely unique properties can be brought to fibre materials.

Short rotation forestry: Production of biomass from plantations of fast-growing forest tree species - possibilities of utilization for energy and chemical use

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Short rotation forestry has been researched since the '50s and mainly refers to plantations of fast growing broadleaved species, aiming at production of woody products and biomass for energy or other uses. In forest biomass plantations (in Europe), fast growing tree species are used, mainly of the following genera: Populus, Salix, Platanus, Eucalyptus, Robinia (R. pseudoacacia), Acer, Alnus, Ulmus, Fraxinus, Tamarix, Castanea, Morus, Betula and to less extent the conifers Pinus maritima, P. radiata, P. halepensis, P. brutia and Cupressuss sempervirens.

In short rotation forestry, narrow planting spacing and intensive culture technologies are applied, similar to those used in agricultural crops. Tree species selected for use in biomass/energy plantations should be characterized by fast growth, easy reproduction, high coppicing ability and production of biomass for multiple uses. Energy production from biomass is feasible by the application of various technologies such as pyrolysis and gasification, liquefaction, biological conversion in hydrolysis (mainly wood decay fungi - and some bacteria) and chemical conversion.

Biomass from fast growing tree species represents a renewable energy source and produces lower emissions in comparison to the emissions produced by the use of the most conventional fuels (e.g. coal or petrol). Biomass plantations can also be an alternative and attractive use of marginal lands and abandoned or out-of-use agricultural lands. The main objective of this paper is to present a general review and analysis at different levels (economical/political, scientific, technological, management and improvement) of plantations of intensive forestry and the possibilities of utilization of biomass for energy and chemical use.

The IBUS process – scale-up of enzymatic hydrolysis and SSF in a biorefinery working at very high dry matter content

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In the IBUS process, all process steps are carried out at high dry matter content thus minimising water and energy consumption. This gives rise to special challenges in pre-treatment and especially in enzymatic hydrolysis and SSF when working with dry matter contents above 25 % (water insoluble solids).

A very central part of a lignocelluloses-to-ethanol plant is pre-treatment. This very energy demanding process step is needed to make the cellulose accessible for enzymatic degradation. Pretreatment is carried out without addition of chemicals, thus yielding two fractions: a fibre fraction containing most of the cellulose and lignin and some of the hemicelluloses, and a liquid fraction containing most of the hemicellulose, some cellulose and also degradation products from pretreatment.

After pre-treatment, the fibre fraction is hydrolysed and fermented in an SSF process at very high dry matter content. This process is carried out in specially designed reactors in a patented process. Here the results of scale-up of enzymatic hydrolysis and SSF from 60 L to 11 m^3 are presented.

Enzymatic conversion of softwood fiber

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There is an increasing need to use renewable materials as industrial raw materials and energy sources, since the reserve of mineral oil will be exhausted. Lignocellulosic biomass is one of the most promising raw materials. Forestry products and by-products may be attractive feedstock for biorefineries. With the separation of the major components there is a possibility to use cellulose, hemicellulose and lignin separately. With an appropriate method, hemicellulose fraction can be separated and utilised. If cellulose is hydrolysed by cellulolytic enzymes, the remaining solid lignin is a potential raw material for valuable chemicals.

Lignocellulosic biomass needs to be pretreated in order to break up the structure and enhance the enzymatic digestibility of the components. Steam pretreatment, which can be combined with acidic or alkaline catalysis, is one of the promising techniques. During the process, raw material is treated with high-pressure saturated steam and then decompressed into atmospheric pressure, which causes an explosion in the fibres. During steam pretreatment, hemicellulose fraction of the lignocellulose complex is mainly solubilised, while cellulose and lignin are present in the fiber fraction. The liquid fraction contains also the degradation products of lignin and sugar, such as furfurol, HMF or levulinic acid. In case of spruce, hemicellulose contains mainly hexoses, while in case of herbs it contains significant amount of pentoses. Spruce hemicellulose hydrolysate can be fermented into ethanol by yeast or can be used as carbon source for cellulase production.

In our experiments, spruce (Picea abies) was used as raw material. Steam pretreatment of spruce was done at Lund University, Department of Chemical Engineering. Spruce chips were initially impregnated with 2.5 % SO2 and then pretreated at 210 °C for 5 minutes. In the focus of our interest was the examination of enzyme adsorption-desorption during enzymatic hydrolysis of cellulose. Previous results showed that addition of polyethylene glycol (PEG) can enhance cellulose hydrolysis [1]. In our experiments, washed fibre fraction of steam pretreated spruce (SPS) was used as substrate. Enzymatic hydrolysis was performed with and without addition of PEG 4000, using Celluclast 1.5 L (Novozymes) cellulase and Novozym 188 (Novozymes) ß-glucosidase. Besides the determination of produced sugar concentration, enzyme activities in the supernatants were also measured during the hydrolysis (FPA, CMCase, ß-glucosidase). Effect of PEG addition on conversion of enzymatic hydrolysis and enzyme adsorption was evaluated.

[1] Börjesson J., Engqvist M., Sipos B., Tjerneld F. (2007) Effect of poly(ethylene glycol) on enzymatic hydrolysis and adsorption of cellulase enzymes to pretreated lignocellulose; Enz. Microb. Technol. 41:186-195

Fuel ethanol from sweet sorghum juice, kernel and bagasse

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For the growing fuel ethanol industry, a very important question is to find the suitable feedstock in a long term view. With the utilization of starch-containing substrates, like corn seed, a competition for food and feed industry has emerged, which has created repugnance in the society. The use of lignocellulosic materials as feedstock could be the solution, since agricultural by-products and energy plants mostly consist of lignocellulose. In our work, we demonstrate that ethanol potential of by-products is especially considerable. For this purpose, a lesser-known plant, sweet sorghum has been chosen.

The useful parts of the plant for this purpose are the stalk with high sucrose and cellulose content and the seed with high starch content. In general, the stalk makes up to 75% of the whole plant mass, while the kernel up to 7%. The optimal harvest period is in September – October because the sugar content of the stalk is the highest in this period. After harvesting, the stalks get pressed to extract the juice. The left-over of the stalks, called bagasse, consists mainly of lignocellulose and is regularly burned. Ethanol production is possible from all these separated parts, namely from the kernel, the juice and also from the bagasse.

In our study, the ethanol potential of the whole plant was estimated. First the bagasse was ground and pretreated. The conditions of pretreatment at elevated and at room temperature were studied. Therefore the influence of different diluted acids and bases on subsequent enzymatic hydrolysis was investigated. Efficiency of hydrolysis was characterized by reducing sugar concentration. Samples showing good hydrolysis results were fermented to ethanol using baker's yeast. Kernel was also ground and fermented after amylolytic hydrolysis. Juice was directly fermentable, since it contains sucrose. According to these experiments, ethanol yield per hectare was calculated.

Mechanical pre-treatments effects on cellulose enzymatical hydrolysis.

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Lignocellulosic materials are very interesting for the production of bioethanol after chemical or enzymatical hydrolysis of cellulose.

To optimise production rate and increase the output of these processes, different kinds of pre-treatments (grinding, steam explosion etc.) are often needed for opening the fibrous structure of the cellulose and increase the specific surface of the materials.

The present study is focused on two models of "pure" cellulose (cellulose C200 microtechnik, cellulose FD100 FMC biopolymer) in order to analyse the effects of mechanical pre-treatments on the kinetics and yields of hydrolysis. The crystallinity index differentiates the two celluloses.
Hydrolysis is realized by a cellulase mix (cellulase Trichoderma reesei C2730, Novozyme; cellobiase Aspergillus niger C6105, Novozyme).

Results compare the influence of two pre-treatments (homogenization and microfluidization) and analyse differents parameters of those technologies (e.g. pressure, number of pass, concentration and viscosity).

Influence of xylanase application in pulp bleaching in Jacarei mill (Votorantim Celulose e Papel)

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Enzymes are catalysts produced from live organisms. Xylanases are enzymes that catalyse hydrolysis of xylans that are part of hemicelluloses present in cellulose fibers. Xylanase treatment allows an increase of pulp delignification and brightness, bleaching reagents savings and a reduction of halogenated compounds in mill effluents.

Factors that concern the efficiency of xylanase treatment are associated with enzyme properties (type, activity, optimum pH and temperature) and pulp properties (lignin content, bleaching sequence, substrate composition and accessibility) and others like reaction time. In the beginning, enzyme applications were performed at acid pH. Kraft process and oxygen delignification are performed at alkaline pH and high temperature. These conditions led enzyme producers to develop formulations that were stable to these pH and temperatures so they could be applied under industrial conditions. Though the pulp wash is carried out after the bleaching stage, this wash is not exhaustive, since in this operation a great quantity of water is consumed. So pulp goes to the following bleaching stage with a certain content in organic matter and chemical residual compounds used in the previous processes. When the circuit of water process is closed, the DQO content in the filtrates associated with pulp increases. In those mills with a high degree of circuit closure as is the case of Jacarei mill, pulp contains a high level of DQO. The study of DQO and pH influence in xylanase bleaching allows a more realistic evaluation of xylanase efficiency in industrial processes.

This study evaluates xylanase application at high temperature and pH and also pH and DQO influence in treatment efficiency in post-O eucalyptus pulp. Influence of enzymatic treatment in pulp properties is studied with nine commercial xylanases. The objective of this work is to assess which is the most effective enzyme in the conditions similar to the storage tower situated in fiber line B. This point was identified as the most favorable point of enzyme application in the bleaching sequence in Jacarei mill (Votorantim Celulose e Papel). Pulp properties (kappa number, brightness and viscosity) were analyzed after the enzymatic treatment. Pulp properties after enzymatic treatment were also analyzed at different initial pH and DQO with two commercial xylanases.

The obtained results indicate that: I) Two out of the nine enzymes present the best influence in pulp properties, II) Xylanase treatment allows a decrease of 1,5 points of kappa number and an increase of 2,5 %ISO in brightness, III) No differences in viscosity are observed between pulps treated enzymatically and those without enzymatic treatment and IV) Increase of initial DQO and / or pH over 16 kgO2 t-1 and 9,5, has a negative effect on enzyme efficiency.

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Influence of laccase-mediated enzymatic system on refining bleached flax pulp

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One of the goals of the application of biotechnology on pulp and paper industry is to improve the properties of the current paper products and, at the same time, to develop new ones. In this way, the aim of this work is to study the effects of a laccase-mediated system (LMS) on bleached flax pulp.

A laccase-mediated system has been applied on ECF and TCF bleached flax pulp in order to study its effects on the physical properties of papers. The raw pulp has been beated with a Valley beater previously to all the treatments to ease the refining on the PFI mill. A commercial laccase supplied by Novozymes has been used together with HBT mediator. The treatments have been carried out at 50ºC, 6 bar and pH 4 during four hours. Three series are compared; no treated pulp (blank), control treatment (same conditions as enzymatic system but without enzyme), and the laccase-mediated system. After each treatment the pulp has been refined at different points (0, 1000, 3000, 5000 and 7000) and afterwards paper sheets have been made with a Rapid-Köthen former.

Physical properties, Schopper-Riegler drainability, water retention value and air permeability have been studied. No changes in any mechanical property (tensile strength, burst, folding endurance and tearing resistance) are appreciated for both ECF and TCF pulps. However, the air permeability has experienced some variation. In the case of TCF flax pulp, the enzymatic system diminishes slightly the air permeability of papers compared to the non-treated pulp samples during the first stages of refining. There is no degradation of carbohydrates due to the enzymatic action as there is no loss of viscosity. Besides, no carbonyl groups are formed on cellulose chains because there is no gain of viscosity after a reduction treatment ($NaBH_4$).

The use of natural mediators on bleaching of kenaf pulp

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Laccases have been widely studied as lignin oxidation promoters in presence of non-phenolic mediators, and the so-called laccase-mediator systems (L) are the most promising enzymatic systems for pulp bleaching. The use of natural mediators easily obtained from natural substrates could provide environmental and economical advantages. *Diferent* laccases and five plant phenols, namely acetovanillone (AV), vanillin (V), acetosyringone (AS), syringaldehyde (SA) and p-cumaric acid (PC), were selected as laccase redox mediators. The aim of this work is to study the influence of L stage on bleaching of kenaf pulp (LP sequence). The effects of these natural mediators were compared with those obtained using the synthetic mediator 1-hydroxybenzotriazole (HBT).

The present study provides one first screening for natural mediators in unbleached kenaf pulp. The characteristics of the pulp after the bleaching sequence LP were evaluated in terms of kappa number, brightness and ADR (% removal of colored compounds in the pulp). The treatment with laccase in the presence of acetosyringone (AS) and syringaldehyde (SA), increased brightness and the removal of colored compounds (ADR), and decreased kappa number, with respect to a control (2_4LP). The treatment with laccase in the presence of syringaldehyde (SA) gave the best results. However, the obtained properties using the natural mediator SA in $2_4L_{SA1.5}P$ (employing the less quantity of mediator) were lower than the obtained using HBT (Table 1).

In stage L, all natural mediators increased the kappa number and decreased brightness. It has to be pointed out that an increase in ADR corresponded to an increase in colored compounds. It was observed the different behavior of both natural mediators, compared with HBT. These results prove that the natural mediators are fixed into the fibers during the stage L.

The good values obtained with $2_4L_{SA1.5}P$ sequence (7.6 kappa number, 71.0 % brightness and 90.97 % ADR) compared to $2_4L_{HBT3}P$ sequence (6.0 kappa number, 76.5 % brightness and 94.15 % ADR) provide the evidence that syringaldehyde could be an alternative to HBT for kenaf pulp biobleaching; however, the operational conditions of the process have to be optimized in future research work.

Synergistic effects of endoglucanase Cel9B and cellobiohydrolase Cel48C on the strength properties of paper

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The refining process is an essential part of papermaking because the strength properties of paper sheets depend on this mechanical treatment. The refining requires a substantial amount of energy to modify the fibres, thus any treatment of pulp that significantly decreases the energy requirement will have a significant beneficial effect. In this context, enzymatic treatments with cellulases have been proposed.

In this study, we have compared the enzyme effects on pulp before and after PFI mill refining at different revolutions. Two cellulases: *endoglucanase* Cel9B and *cellobiohydrolase* Cel48C of *Paenibacillus sp. BP-23*, have been applied separately and also together to ECF-bleached kraft pulp (*Eucalyptus globulus*) in order to estimate their synergy. The enzymatic effects have been evaluated on paper strength properties and on the surface properties of fibres using Scanning Electron Microscopy (SEM). Cel9B treatments had a beneficial effect on paper sheets properties such as tensile, permeability and burst indexes. The effect produced by Cel9B can be considered a biorefining process. The Cel48C treatment had a smaller effect on physical properties than the Cel9B. The combined action of both cellulases Cel9B:Cel48C did not imply an enhancement of the mechanical properties of paper in order to be higher than the sum of the individual effects of Cel9B and Cel48C.

Potential of natural mediators for flax pulp bleaching by laccase mediator system

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Synthetic laccase-mediators have a high cost and a growing concern exists about their possible toxicity. The use of natural mediators easily obtained from natural substrates could provide environmental and economical advantages.

Three plant phenols, namely acetosyringone (AS), syringaldehyde (SA) and p-coumaric acid (PCA), were selected as laccase redox mediators to study the stability of *Pycnoporus cinnabarinus* laccase for total chlorine free (TCF) bleaching of flax pulp. The effects of these natural mediators were compared with those obtained using the synthetic mediator 1-hydroxybenzotriazole (HBT). Temperature, time and amount of mediator influence on laccase activity were measured both in presence and in absence of flax pulp. Laccase inactivation by HBT (97 % in 5 h at 50 ºC) decreased 35 % in flax pulp presence, PCA also produced laccase inactivation (100 % in 3 h at 50 ºC) but in flax pulp presence the activity lost was only 25% in 5 h. AS and SA did not cause laccase inactivation. The same plant phenols were selected as laccase redox mediators to investigate the enzymatic delignification of flax pulp in combination with peroxide bleaching and the results were compared with those obtained by HBT. Laccase-syringaldehyde treatment and subsequent peroxide bleaching caused an important decrease of kappa number (67 % with respect to the control). The use of acetosyringone and syringaldehyde enabled to increase final brightness over 20 %.

Natural mediators represent a powerful alternative for pulp biobleaching, moreover their wide availability from plant materials and pulping liquors makes them a new way to study an environmentally friendly delignification of paper pulp.

Recovery and enzymatic modification of galactoglucomannan, the major soft-wood hemicellulose

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The interest in using non-cellulosic polysaccharides of the plant cell-wall as a renewable resource for novel oligomeric and polymeric products is currently increasing. Potential applications are e.g. new films, gels and food/feed additives with health promoting properties. These applications depend on the saccharide/glycan structure and knowledge of structure and properties is an important contribution to this development as well as the development of enzymatic tools for saccharide/glycan modification. With this focus we combine protein crystallography, enzyme kinetics and carbohydrate analysis in our studies of the major softwood hemicellulose: *O*-acetyl galactoglucomannan (GGM). It is a complex branched heteropolysaccharide containing an *O*-acetylated β-(1→4) linked glucomannan backbone with α-(1→6)-D-galactosyl side groups attached to some of the mannosyl units. Previously, we developed a method for the water extraction of GGM from spruce chips by microwave heat treatment and further fractionation using size exclusion chromatography, and we showed that the time, temperature and pH conditions during the extraction of GGM influence the molecular weight, substitution and yield of the extracted material (*1*). Recently, we compared the recovery of GGM from side-streams of technical–mechanical pulping (TMP) by size-exclusion chromatography with the recovery using membrane filtration (*2*).

α-Galactosidase from *Aspergillus niger* (*3*) was used for partial side group hydrolysis of water-soluble and aggregated GGM. The effect on polymer properties (association, rheology, and solubility) will be discussed. We are also studying glycoside hydrolases which catalyse brealdown of the GGM backbone (β-mannanase, β-mannosidase). Two recently solved 3D structures of family 5 (*Me*Man5A) and family 26 β-mannanases (*Cf*Man26A) will be discussed with a focus on implications on enzyme-substrate interactions, important for specificity and recognition of the different substrate monomers (*4*,*5*). These enzymes can perform transglycosylation reactions which may be useful for the biosynthesis of new hemicellulose-based glyco-conjugates. However, only *Me*Man5A was shown in practise transglycosylate and CfMan26A did not. Fine structural differences in the subsite organisation may be the cause of this observation. The studied enzymes are useful tools in the characterisation of GGM and promising tools in the structural-functional modification of GGM-derivide saccharides for novel applications. Results describing the discovery and characterisation of novel *Aspergillus* sps mannanases and α-galactosidases will be presented.

1. Lundqvist J., Jacobs A., Palm M., Zacchi G., Dahlman O., & Stålbrand H. *Carbohydrate Polymers*, **2003**, 53, 203-211.
2. Andersson A, Persson T, Zacchi G, Stålbrand H and Jönsson A-S. Applied Biochemistry and Biotechnology, **2007**, 137, 971-983
3. Ademark P. de Vries R. P. Hägglund P. Stålbrand H. & Visser J. *European Journal of Biochemistry*, **2001,** 268, 2982-2990
4. Le Nours J., Anderson L., Stoll D., Stålbrand H. & Lo Leggio L. *Biochemistry*, **2005**, 38, 12700-12708
5. Larsson, A.M., Anderson, L, Xu, B., Munoz, I.G., Uson, I., Janson J.-C., Stålbrand, H., and Ståhlberg, J. *Journal of Molecular Biology*, **2006**, 357, 1500-1510

Novel device for aerobic biodegradability testing

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We have developed a novel device for testing the biodegradability of biopolymers based on ISO standard 14855-1 "Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions – Method by analysis of evolved carbon dioxide". The device can contain up to 18 glass vessels of 3 L, which are pressure resistant and have airtight connections. Each vessel is a separate experimental unit, made independent from the other vessels by its own pressure and flow regulation system. Temperature of the compost inside the vessels is maintained by placing the entire system inside an incubator at 58 °C. A LabView-based program regulates the gas switching system, which selects the vessel to be measured and connects it to the sensor array. The system measures and logs the flow rate, CO_2 concentration, O_2 concentration, temperature and humidity, and calculates the total amount of CO_2 produced in real time. In between measurements, which usually occur in sequence, the sensor array is purged with dry normal air.

The main advantage of the new device is the presence of the pressure and flow regulation system, the gas switching system and the sensor array inside the incubator with the vessels, avoiding problems with condensation inside the sensors and thus making condensers prior to the sensors redundant. This also allows for short tubing and low dead volumes between the vessels and the sensors, resulting in a response time of the sensors after switching to a different vessel of mere seconds. The system measures all relevant parameters and is purged and ready for a new measurement in one minute. Correct humidity of the compost can be maintained with individual humidifiers per vessel inside the incubator or by a large humidifier tank outside of the system prior to the pressure regulators. Canisters of certified CO_2 containing air (0 ppm and 5000 ppm) are also connected to the gas switching system allowing for each measurement series to have measures for validated values of CO_2 allowing for a continuous check of the system's performance.

The system has been used successfully for the 12-week biodegradability testing of PLA by compost of a municipal composting facility.

References

Text of reference

ISO standard 14855-1 "Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions – Method by analysis of evolved carbon dioxide"