The Final Workshop of COST FP0602

Biotechnology for lignocellulose biorefineries

September 7-8, 2011

Tuscia University, Viterbo Italy

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Scientific and Organizing commettee

Claudia Crestini, Tor Vergata University, Rome, Italy

Federica Melone, Tor Vergata University, Rome, Italy

Silvia Decina, Tor Vergata University, Rome, Italy

Liisa Viikari, University of Helsinki, Finland

The abstract book was edited by

Claudia Crestini and Liisa Viikari

Preface

On behalf of the Organizing Committee we would like to express our gratitude to all persons who have contributed to organizing this meeting. We also wish to thank our hosts at Tuscia University the for offering excellent meeting facilities in Viterbo.

We wish you all a fruitful meeting of scientific excellence and an enjoyable stay in Italy.

07-09-2011

Programme

September 7th, 2011

10.00 MC Meeting

14.00 Poster session

20:00 Social dinner

September 8th, 2011

9:00 *Opening the workshop*

Chair of the Action (Liisa Viikari)

Hosting Organization (Claudia Crestini)

9::15 Session 1. BIOTECH FOR BIOREFINERIES

Jeff Tolan, IOGEN. Iogen's experiences operating a wheat straw-to-ethanol demonstration plant

Terhi K. Hakala. Application of enzymes in wood biorefinery for high-value products

10:15 Coffee

10:45 Session 2. NEW GLYCOSYL HYDROLASES

Bjorge Westereng. Efficient separation of oxidized oligosaccharides from recalcitrant polysacchatrides generated by a new class of enzymes

Paul Christakopoulos. Glycosyl hydrolases from Sporotrichum thermophile with biotechnological potential, Greece

Petr Baldrian. Targeted search for fungal cellulase genes in soil metagenomes using next-generation sequencing

George Anasontzis, CTH, Sweden: Screening the natural resources for enzymes with wood degrading and modifying properties

12:30 Lunch

14:00 Session 3. ENZYMES FOR NEW PRODUCTS

Elisabetta Aracri. Laccase-tempo oxidation of sisal pulp for improving paper strength properties: influence of the operating conditions

Janez Štrancar. Inspecting laccase action for tailoring lignin functionalization

Raffaele Saladino. LbL immobilized oxidative enzymes in natural phenolic and polyphenolic compounds modification

15:30 Coffee

16:00 Session 4. HYDROLYSIS AND FERMENTATION

Claus Felby. Recycling of cellulases from wheat straw

Aniko Varnai. Simultaneous hydrolysis of both xylan and glucomannan improve the total hydrolysis of lignocelluloses

17:00 Closing remarks of the workshop, final conclusion of the MC

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HYDROLYSIS AND FERMENTATION

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Oral presentations

Iogen's experiences operating a wheat straw-to-ethanol demonstration plant

Jeff Tolan

Iogen Corporation has been operating a demonstration plant in Ottawa, Canada for the conversion of wheat straw to ethanol since 2004. The process includes a pretreatment with steam and dilute sulfuric acid, followed by enzymatic hydrolysis of the cellulose, and then fermentation of the glucose and xylose to ethanol by using recombinant Sacchaomyces. The ethanol is finished as E85 fuel for commercial and fleet use. The demonstration plant is the final step in scale-up of the process prior to commercial operation. This presentation describes the operation of the demonstration plant and our experiences in scale-up of the process, including both the successes and the difficulties encountered.

Application of enzymes in wood biorefinery for high-value products

Terhi K. Hakala*, Tiina Liitiä, Anna Suurnäkki

Address: VTT Technical research centre of Finland, PO Box 1000, FI-02044 VTT, Finland

Email: Terhi.Hakala@VTT.Fi

The aim of this the study was to enhance alkaline extraction of xylan from bleached kraft pulps, tailor molecular weight distribution of extracted polymers and to obtain hemicellulose-poor pulps by enzymatic pulp treatment prior to alkaline extraction. Enzyme treatments of HW kraft pulp were carried out prior to one or in between two subsequent alkaline extraction steps with purified Trichoderma reesei xylanase (pI 9) and endoglucanase II (Cel5A) and with a commercial monocomponent endoglucanase product (Novozym 476). After the first extraction pulps pre-treated with xylanase or with Novozym 476 had the lowest xylan content, whereas enzyme treatments had no clear effect on pulp composition after the second extraction. Xylanase treatment before one alkaline extraction step hydrolysed up to 12% of pulp xylan to xylo-oligosaccharides (XOS) with DP 2-6. Xylan yield in the first extraction varied between 50 to 60% of original pulp xylan. Xylan extracted in the second stage had higher molar mass than in the first stage, but the yield was only 7 to 9% of original xylan. Xylan yield after xylanase treatment was reduced by up to 10%, which corresponds to the amount of XOS released to the pulp filtrate. EG product Novozym 476 slightly enhanced xylan extraction without affecting molar mass of extracted xylan. The most beneficial process combinations were either EG treatment before extraction to enhance extraction of xylan or xylanase treatment after extraction to produce XOS in addition to xylan.

Efficient separation of oxidized oligosaccharides from recalcitrant polysaccharides generated by a new class of enzymes

Bjørge Westereng, Jane Agger, Yngve Stenstrøm, Gustav Vaaje-Kolstad, Svein J. Horn and Vincent G.H. Eijsink

Enzymatic conversion of crystalline carbohydrate rich material is a key technology in the production of biofuels and high value products from biomass. The degradation of this biomass is challenging and there is a big effort going on to find and develop more efficient enzymatic systems. Recently, a growing interest in proteins categorized in CAZy as CBM33 and GH61 has emerged. Hitherto, these proteins were known as helper proteins in polysaccharide degradation [1, 2]. Using CBP21 [1] and other CBM33 members, we have shown that these proteins degrade polysaccharides by a exerting an unprecedented enzymatic activity and may have a great potential to make conversion of recalcitrant carbohydrate rich biomass more efficient. In 2010 we showed that CBM33 proteins cut polysaccharides via an oxidohydrolytic mechanism [3] and have found additional examples since. The discovery of this novel activity has been critically dependent on development of methods required for analysis and detection of the oxidized products. Oxidation of the reducing end yields aldonic acids and drastically changes the physicochemical properties of the oligosaccharide products, making analysis challenging. Our studies thus have a major focus on the analytical tools needed for product identification and this will be included in the presentation.

1. Vaaje-Kolstad, G., et al., *The non-catalytic chitin-binding protein CBP21 from Serratia marcescens is essential for chitin degradation.* Journal of Biological Chemistry, 2005. **280**(31): p. 28492-28497.

2. Harris, P.V., et al., *Stimulation of Lignocellulosic Biomass Hydrolysis by Proteins of Glycoside Hydrolase Family 61: Structure and Function of a Large, Enigmatic Family.* Biochemistry, 2010. **49**(15): p. 3305-3316.

3. Vaaje-Kolstad, G., et al., *An Oxidative Enzyme Boosting the Enzymatic Conversion of Recalcitrant Polysaccharides.* Science, 2010. **330**(6001): p. 219-222.

Glycosyl hydrolases from Sporotrichum thermophile with biotechnological potential

Paul Christakopoulos, Evangelos Topakas, Maria Dimarogona, Maria Moukouli, Anthi Karnaouri

BIOtechMASS Unit, Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, 9 Iroon Polytechniou Street, Zografou Campus, 15700 Athens, Greece

Considering the current dependence on acid and heat-pretreatment in the deconstruction of lignocellulose, it is clear that enzymes that are stable and active at low pH values and at high temperatures are of particular value. Filamentous fungi growing in the plant litter and soil are known to be good sources of polysaccharide degrading and modifying enzymes. Frequently isolated from soil and self-heating compost, the thermophilic, filamentous fungus Sporotrichum thermophile grows optimally between 45^{o}C and 50^{o}C. Since high temperatures help to solubilise some components of lignocellulosic feedstock and decrease the viscosity of slurries of biomass, thermophilic enzymes would have strong process advantages. Thermophilic enzymes would also have advantages in stability during the course of harsh process conditions, and increased catalytic rates at higher temperatures. The genome sequence of S. thermophile has also come available recently. Overall, these facts make S. thermophile as a very promising source for novel thermophilic enzymes. The enzymes and enzyme cocktails derived from this species would promote the development of advanced technologies for the biomass derived fuels and chemical sector and many other industries.

Novel enzyme activities such as feruloyl esterases and glucuronoyl esterases, responsible for the cleavage of lignin-carbohydrate linkages for the tailored made modification of lignin-carbohydrate complexes in order to enhance their biological activity, xylanases, endoglucanases, β-glucosidases and glycoside hydrolases family 61 (GH61) which are capable of enhancing the biomass degrading activity of common cellulolytic preparations via an unknown mechanism, were functionally expressed in the methylotrophic yeast Pichia pastoris. The role of these enzymes in plant biomass degradation but also their use as biosynthetic tools has been studied.

Targeted search for fungal cellulase genes in soil metagenomes using next-generation sequencing

P. Baldrian, J. Voříšková and M. Štursová

The speaker's name in bold type followed by other co-authors

Laboratory of Environmental Microbiology, Institute of Microbiology ASCR, Praha, Czech Republic

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Abstract

Cellulose represents the most abundant biopolymer on the Earth and consequently an important renewable resource. Its decomposition is a key step in several biotechnological processes. Here we report on the characterization of diversity and sequence variability of fungal GH7 cellobiohydrolase cbhI genes derived from metagenomes of hardwood and coniferous forest litters. Tens of different cbhI gene sequences were obtained from both environments and the total diversity was estimated to range from 80 in the Quercus forest to more than 200 in more diverse the Picea forest. The analysis of the 50 most abundant genes in both ecosystems showed that the genes are largely ecosystem-specific and belong to both the Ascomycota and the Basidiomycota. The translation of genes and the subsequent analysis of internal peptides of the CBHI proteins demonstrated that the analysed 96-amino acid region harbours 0-3 introns and includes both highly conserved and diverse regions. We show that the combination of targeted metagenomics with next-generation sequencing can be useful for the analysis of both the diversity and structure of microbial enzymes in the environmental samples. In a similar way, other genes with importance for biotechnology produced by prokaryotic or eukaryotic microorganisms may be specifically recovered from metagenomic DNA.

Screening the natural resources for enzymes with wood degrading and modifying properties

George E. Anasontzis and Lisbeth Olsson

Department of Chemical and Biological Engineering Chalmers University of Technology SE-412 96 Gothenburg, Sweden

e-mail: george.anasontzis@chalmers.se

The production of high added value compounds from forest and agricultural biomass has become one of the main targets of contemporary carbohydrates research. The renewability of the biomass, the potential use of waste residues and the complete or partial biodegradability of the products have made the whole approach an attractive perspective towards the sustainable and green ideal. However, most of the already developed biomass separation and modification processes are based on aggressive chemical reactions in extreme conditions that are costly and harmful for the environment. Enzymatic and microbial catalyzed processes present an interesting alternative. The development and discovery of novel biological approaches in the modification, degradation and separation of wood biomass is one of the main activities of the Industrial Biotechnology Group in Chalmers University of Technology, also as part of the Wallenberg Wood Science Center.

Presently, we pursue this aim through a triple approach:

Multiple enzymatic screening of phytopathogenic and wood degrading filamentous fungi, such as Trametes hirsuta and Penicillium pinophilium, as well as screening newly isolated microorganisms. We seek enzymes with industrially interesting activities and unique properties, such as reactivity under extreme conditions.

Microorganisms efficient in degrading lignocellulose produce enzyme in response to the environmental conditions. In collaboration with Associate Professor Gianni Panagiotou, Center for Biological Sequence Analysis, DTU, we are looking for sequenced, but still unclassified proteins that are related to the degradation of plant biomass using information from transcriptomics analysis of Aspergillus oryzae grown on different carbon sources.

Novel enzymes can only be identified by new methods. We investigate the properties of synthetic model compounds that can simulate the natural substrates and the implementation of different analytical methods for the identification of the sometimes complex and singular enzymatic activities. In collaboration with Associate Professor Paul Christakopoulos, BIOtechMASS Unit, School of Chemical Engineering, National Technical University of Athens, we also attempt to isolate model compounds from plant cell wall material.

Laccase-tempo oxidation of sisal pulp for improving paper strength properties: influence of the operating conditions

Elisabetta Aracri*, Teresa Vidal

Universitat Politècnica de Catalunya, Textile and Paper Engineering Department, Colom 11, E-08222 Terrassa, Spain. elisabetta.aracri@etp.eupc.edu

This study was conceived to investigate the oxidation of sisal pulp by laccase-TEMPO system which has not been previously examined. TEMPO-mediated oxidation is a well-known approach to introduce carboxylate and aldehyde functional groups into cellulose under aqueous and room temperature conditions. Although the ability to use laccase to catalyze the regenerative oxidation of TEMPO has been demonstrated, the reaction is commonly carried out in the presence of NaOCl/NaBr as a co-oxidizer system. TEMPO-mediated oxidation has been successfully exploited to improve several physical properties of pulp fibers including the inter-fiber bonding capacity and thus the strength properties of the resulting papers. The improvement of wet strength in papers obtained from TEMPO-oxidized fibers was recently reported in various studies and ascribed to the formation of large amounts of surface aldehyde groups as intermediate structures during TEMPO-mediated oxidation which are able to form inter-fiber covalent bonds through hemiacetal linkages with hydroxyl groups of adjacent fiber surfaces.

In this work a low-lignin content sisal pulp was treated with laccase from Trametes villosa and TEMPO in order to evaluate the potential of this system to improve the paper strength properties. TEMPO-mediated oxidation was found to cause the formation of aldehyde and carboxyl groups in proportions dependent on the particular reaction conditions. The increase in aldehyde content was found to be closely aligned with enhanced wet strength. The influence of process variables in the laccase-TEMPO treatment (viz. laccase dose, TEMPO dose and reaction time) on the properties of the pulp and the resulting handsheets was assessed by using a three-variable sequential statistical plan.

Acknowledgments

The authors are especially grateful to the projects FUNCICEL (CTQ2009-12904) and BIOFIBRECELL (CTQ2010-20238-CO3-01) within the framework of Spain's MICINN.

Inspecting laccase action for tailoring lignin functionalization

J. Štrancar[1], M. Božič[2], S. Gorgieva[2], A. Mehle[1], V. Kokol[2]

[1]Jožef Štefan Institute, Laboratory of Biophysics – EPR center, Ljubljana, Slovenia, janez.strancar@ijs.si
[2]University of Maribor, Institute for Engineering Materials and Design, Slovenia

vanja.kokol@uni-mb.si

Abstract
Lignocellulose-based composites made from plant fibers are becoming extremely important and highly perspective sustainable and renewable natural materials. Fibre modification enhancing their existing properties or even creating completely new ones can be obtained to broaden the application areas. In response to shortcomings of traditional chemical and physical methods, enzymes and chemo-enzymatic methods have emerged as important eco-friendly catalysts that work under mild conditions and enable tailoring of the material surface properties by substrate specificity and regional selectivity. Recently, binding of different functional molecules to lignin-rich fibres by using an oxidative enzyme (e.g. laccase) has been reported leading to their functionalisation through free radical reactions.

By the application of electron paramagnetic resonance spectroscopy (EPR) and cyclic voltammetry laccase action was inspected. Firstly, consumption of phenol-type substrates, like caffeic and gallic acids as monomers and commercial lignins as complex polymers, was investigated and their polymerization was traced via HPLC-SEC and FTIR spectroscopy. In this case some stable radical intermediates were detected with EPR spectroscopy when substrate molecules were in contact with active enzymes. Secondly, oxidation of mediators like nitroxides was determined via EPR spectroscopy of stable water-soluble nitroxide radicals. Finally, the generation of short-lived radicals as well as their reduction kinetics was measured via EPR spin trapping using DMPO as sensitive water soluble spin trap converting short-lived radicals into long-lived radical DPMO-adducts.
Acknowledgement: This research was supported by Slovenian Research Agency (grant no. L4-3641).

LAYER BY LAYER IMMOBILIZED OXIDATIVE ENZYMES IN NATURAL PHENOLIC AND POLYPHENOLIC COMPOUNDS MODIFICATION

R. Saladino, M. Guazzaroni, F. Melone, C. Crestini

Selective procedure for the oxidative functionalization of phenol and polyphenol derivatives are of great interest in the field of medicinal chemistry and in the design of novel materials derived from biopolymers. Here we describe the preparation of different biocatalysts based on the immobilization of oxidative enzymes, such as tyrosinase and laccase, and their use for the oxidation of phenol and polyphenol derivatives. Procedures working in buffer and in organic solvents are reported, as well as the stability of biocatalysts for more runs.

Recycling of cellulases from wheat straw processing

Claus Felby[1], Piotr Chylenski[1], Jane Lindedam[1], Henning Jørgensen[1], Miguel Gama[2], Mai Østergård Haven[1,3]

1 University of Copenhagen, Faculty of Life Sciences
2 Departamento Engenharia Biológica, Universidade do Minho, Campus de Gualtar 4710-057 Braga, Portugal
3 Inbicon, Kraftværksvej 53, Fredericia, Denmark

The development of a sugar platform based on lignocellulose has improved significantly during the past decade. Both enzyme and process technology has progressed to a level where commercial processing is now coming on line. But even though enzyme technology has improved, due to the very nature of the lignocellulose substrate the amount of enzyme protein required for cellulose hydrolysis will be orders of magnitude higher than for e.g. starch processing. Therefore the possibility of recycling cellulases could significantly reduce the cost of processing lignocellulose to sugars.
In this presentation we present our results on the basic issues and challenges in relation to cellulase recycling from wheat straw processing. This includes studies of enzyme location on fermentation and distillation residue, protein stability, protein adsorption and desorption to lignin and cellulose as well as recovery of active enzyme protein. The presentation will address basic issues in relation to enzymology as well as practical use of cellulase recycling.

Simultaneous hydrolysis of both xylan and glucomannan improve the total hydrolysis of lignocellulose

Anikó Várnai a, , Laura Huikko a, Jaakko Pere b, Matti Siika-aho b, Liisa Viikari a

a Dept. of Food and Environmental Sciences, University of Helsinki, Helsinki, Finland

b VTT Technical Research Centre of Finland, Espoo, Finland

Lignocellulosic biofuels have an increasingly important role in partially replacing crude-oil based transportation fuels. Already several pilot units and demonstration plants have been established world-wide for bioethanol production. However, the hydrolysis efficiency of lignocellulosic substrates still needs improvement in order to increase the feasibility of the conversion process.

The hydrolysis is hampered by the recalcitrant structure of the raw material. Lignin and hemicellulose have been recognised to decrease the hydrolysability by forming a complex network, a physical barrier, around the cellulose. To avoid limitations of hydrolysis by hemicellulose and to reduce the overall enzyme loading, accessory enzymes may be supplemented to cellulase preparations. Understanding which enzyme components are required for the enhanced total hydrolysis is crucial to design the best suitable commercial enzyme preparations.

This work focuses on the role of the hemicellulolytic enzymes in the total hydrolysis of lignocellulosic substrates. The hydrolysis of various lignocellulosic materials using enzyme mixes with various combinations of purified cellulases and hemicellulases was studied. Two xylanolytic enzymes, TrCel7B endoglucanase I and TrXyn11 endo-β-xylanase, as well as one mannanolytic enzyme, TrMan5A endo-β-mannanase were used. In the study, nanofibrillated cellulose was included as model substrate. The results reveal novel information on the lignocellulosic structure, i.e. a linear correlation between the solubilisation of lignocellulosic polysaccarides.

Reference:

Várnai A, Huikko L, Pere J, Siika-aho M, Viikari L (2011). Synergistic action of xylanase and mannanase improves the total hydrolysis of softwood. Manuscript submitted to Bioresour Technol.

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Poster presentations

Chemo-enzymatically modified CTMP in biocomposites

Suurnäkki Anna*, Mikkonen Hannu, Immonen Kirsi

Address: VTT Technical research centre of Finland, PO Box 1000, FI-02044 VTT, Finland

E-mail: anna.suurnakki@vtt.fi

Use of wood fibres together with biopolymers offers an interesting opportunity for new, sustainable composite materials. The poor compatibility of hydrophilic lignocellulosic fibres with hydrophobic biopolymers is, however, limiting the wider application of these short fibres in biocomposites. In this work, the effect of laccase-catalysed chemo-enzymatic hydrophobisation of spruce chemi thermo mechanical pulp, CTMP, on the properties of biocomposites was studied. CTMP surface was modified with commercial and tailor-made phenolic compounds using either experimental or commercial laccase preparation. The modified fibre materials were compounded with polylactic acid (PLA) or polyhydroxybutyrate (PHB) and the properties of composites were studied. Laccase-catalysed modification with dodecyl gallate (DOGA) resulted in improved dispersion of fibres both with PLA and PHB. In addition, mechanical properties of composite specimens with modified fibres were found to be similar or better than those with reference fibres. The effect on mechanical properties was different with different biopolymers and the combination of fibres modified with DOGA and PLA as a matrix polymer was found to be most advantageous in biocomposites.

Isolation and characterisation of lignin from brewer's spent grain

Piritta Niemi[1], Luca Zoia[2], Tarja Tamminen[1], Johanna Buchert[1], Marco Orlandi[2]
[1] VTT Technical Research Centre of Finland, P.O. Box 1000, FI-02044 VTT, Finland
[2] University of Milano Bicocca, Piazza della Scienza 1, 20126 Milano, Italy

Brewer's spent grain (BSG) is a cereal side stream produced by the brewing industry. It is rich in carbohydrates, protein and lignin which could be valuable raw materials for various applications if they can be efficiently separated. The objective of this work was to isolate lignin from BSG and to characterize it. The methods used for lignin isolation were acidolysis and EMAL (enzymatic mild acidolysis). The obtained lignin fractions were analyzed by Klason lignin, elemental analysis, GPC, ^{31}P NMR and 2D-HSQC-NMR. The purities of the lignin fractions obtained by the two methods were compared, as well as their average molecular weights. The ^{31}P NMR spectra showed that lignin from BSG contains p-hydroxyphenyl and guaiacyl units. In addition significant amounts of aliphatic hydroxyl groups and carboxylic acid groups were present in the samples. The intermolecular bonds of lignin were analyzed by 2D-HSQC-NMR.

This study was part of the research project Lignin Fibre financed by the Academy of Finland (133091).

PARTICLE DYNAMICS AND STRUCTURE OF A STABILIZED LIGNIN SUSPENSION AFTER TREATMENT WITH PENICILLIUM CHRYSOGENUM VAR. HALOPHENOLICUM

Marlene Remédios[1], Filomena Carvalho[2], Nuno Santos[2], Ana Lúcia Leitão[1]*

[1]Departamento de Ciências e Tecnologia da Biomassa, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Campus de Caparica, 2829- 516 Caparica, Portugal.

[2]Unidade de Biomembranas, Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Av. Prof. Egas Moniz, 1649-028 Lisboa, Portugal

*E- mail corresponding author: aldl@fct.unl.pt

Abstract

Lignins are complex phenolic polymers that can be found in trees and agricultural crops as an abundant and renewable vegetable resource. Due to their very complex structure, lignins are classified as three-dimensional amorphous polymers with several important chemical functional groups such as hydroxyl among others. The potential of use of lignins is gaining attention as a product that can bring health benefits and new industrial applications.

Our group have been isolated a Penicillium strain from soil samples of a salt mine in Portugal. This halotolerant strain was characterized and identified as a new strain of Penicillium chrysogenum var. halophenolicum showing a high capacity to degrade aromatic compounds under osmotic pressure (Leitão et al., 2007 and 2011).

In the present study, degradation and stabilization of the alkali lignin by Penicillium chrysogenum was investigated in batch system under aerobic conditions. Atomic force microscopy and dynamic light scattering were used to follow the changes of lignin structure during bioprocessing. Further undergoing studies will explore the potential of this halotolerant Penicillium strain on food field.

References

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Inverting character of family GH115 α-glucuronidases

Mária Vršanská, Katarína Kolenová, Olena Ryabova, Peter Biely

Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia

α-Glucuronidases of glycoside hydrolase family 115 of the xylose-fermenting yeast *Pichia stipitis* and wood destroying fungus *Schizophyllum commune* liberate 4-*O*-methyl-D-glucuronic acid residues from aldouronic acids and glucuronoxylan. The specific activities of both enzymes depended on polymerization degree of the acidic xylooligosaccharides and were inhibited by linear β-1,4-xylooligosaccharides. These results suggest interaction of the enzyme with several xylopyranosyl residues of the xylan main chain. Using ^{1}H NMR spectroscopy and reduced aldopentaouronic acid (MeGlcA^{3}Xyl$_{4}$-ol) as a substrate, it was found that both enzymes are inverting glycoside hydrolases releasing 4-*O*-methyl-D-glucuronic acid (MeGlcA) as its β-anomer.

Adsorption of fungal cellulases on lignin-rich residues

Jenni Rahikainen, Kaisa Marjamaa, Saara Mikander, Sami Alakurtti, Tarja Tamminen, Liisa Viikari* and Kristiina Kruus

VTT Technical Research Centre of Finland P.O.BOX 1500, FIN-02044VTT, Finland

*University of Helsinki, Department of Applied Chemistry and Microbiology P.O. Box 27, 00014 Helsinki, Finland

Lignin is one of the major components in all lignocellulosic materials. It is also an important factor in limiting the enzymatic hydrolysis of biomass. Numerous studies have shown an inverse correlation between the lignin content and the rate of biomass hydrolysis. The mechanisms of lignin interference in enzymatic hydrolysis are related both to the structure of the lignocelluloses and to the properties of the enzymes. Lignin appears to limit the hydrolysis by creating a physical barrier restricting the access of the enzymes to carbohydrate polymers. On the other hand adsorption of the enzymes on lignin surfaces has been shown to be a major contributor in the inhibition of the hydrolysis. Binding of enzymes onto lignin is considered to take place mainly via hydrophobic interactions. However, due to the complex structure of lignin-rich hydrolysis residues, adsorption is likely to occur by several simultaneous and competitive mechanisms. Relatively little is still understood about the binding mechanisms and factors affecting on the binding of the enzymes on lignin.

The paper will describe adsorption of Trichodema reesei cellulase mixtures and monocomponents onto different lignins isolated from relevant technical feedstocks. The origin of the lignin as well as the isolation method was shown to have a crucial impact in the binding.

Using fluorescence microscopy to probe cell wall ultra structure and susceptibility to enzyme binding.

Budi Juliman Hidayat1, Lisbeth G. Thygesen1, Katja Salomon Johansen2, Claus Felby1.
1 Forest & Landscape, Faculty of Life Sciences, University of Copenhagen, Rolighedsvej 23, DK- 1958 Frederiksberg C, Denmark
2 Novozymes A/S, Krogshøjvej 36, DK-2880 Bagsværd, Denmark

Dislocations are sites in fibers where cellulose microfibrils are misaligned (Nyholm et al, 2001).

Dislocations have been investigated using light microscopy, revealing its structure at the micrometer scale, but leaving little information on both the cellulose supramolecular structure and the environment within dislocations. Fluorescence microscopy technique has not been used for structural studies of fibers. Here we present two approaches by which this technique is exploited to study aspects of dislocations.

In the first approach enzymes are labeled with fluorophores and their preferences for dislocations are investigated. Competition for binding to dislocations is assessed by using different labels and by simultaneously/sequentially mixing the enzymes with fibers. As the modes of action of the enzymes are known, the supramolecular structure of dislocations may be inferred by assessing which enzymes bind to dislocations.

The second approach involves the use of a pH sensitive fluorophore to probe the environment within dislocations. Voids have been observed within artificially made dislocations (Terziev et al, 2005) and may also be present in natural dislocations. pH is an important factor in enzymatic degradation of biomass and pH in a small confined environment, such as voids, could be different from that of the bulk solution (Fält, 2003). Our results show that pH within dislocations does not differ significantly from that of voids within the surrounding cell wall. However, in a low conductivity environment a pH difference could be observed between voids/dislocations and the bulk solution.

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Cellulase recycling during the enzymatic hydrolysis of lignocelluloses by alkaline elution

A.C. Rodrigues[1], F. M. Gama*[1]
[1] *Centre of Biological Engeneering, University of Minho, Braga, Portugal*

There is a growing demand for viable alternatives to fossil fuels because of increasing oil price spikes, the need for increased energy security and -climate change. In response to the growing global demand the production of biofuels, such as bioethanol, has gained significant notoriety. Countries like Brazil and the USA lead world production of bioethanol; they produced between them, in 2010, 88% of the world's bioethanol through first generation production (www.ethanolrfa.org/pages/statistics#E: May 2011).
The aim of this work is to develop an enzymatic recycling method post-bioethanol production, from lignocellulosic material and more to evaluate the recycling of the enzyme activity using low molecular mass fluorogenic substrates specific substrates (4-methylumbellyferyl-cellobioside) for determining the activities of adsorbed cellulases (in this case, exoglucanase Cel7A), instead of non-specific methods, which measure the total protein concentration (for example Bradford and ninydrin assay). This method may be relevant for industrial purposes. In this work, we studied how the presence of cellulose and lignin interfere in the recycling of enzymes. We also analyzed the effect of temperature on the enzymatic hydrolysis of straw and subsequent enzymatic recovery. We suggested that with a simple change in pH, from the optimum pH of the enzymes to an alkaline pH (pH 9 or 10), it is possible to obtain a high yield of desorption. These experiments show that over 60% of the MUC activity adsorbed are recoverable for each of the different substrates studied (lignin, straw and cellulose CF 11).
We observed that these enzymes, after the alkaline wash, did not lose their catalytic activity or suffer any irreversible conformational changes. This finding is important since it allows for recovered enzymes to be recycled and reused in the production of bioethanol, consequently leading to a decrease in production costs. Another important finding was related to the effect of temperature on the rate of hydrolysis and enzyme desorption, we found that high temperatures cause denaturation of the enzymes which translates as a decrease in the rate of hydrolysis of the lignocellulosic biomass, and also impairs enzyme desorption. These experiments lead to the conclusion that the temperature of hydrolysis and subsequent denaturation interfere with the reversible adsorption of cellulolytic enzymes to the substrate. Another finding that makes this work even more interesting is that, the presence of lignin does not pose a problem for enzyme recycling, contrary to what many others authors have reported, but that the presence of unhydrolyzed cellulose residues from the bioethanol production process are contributing to the incomplete desorption of the enzymes.

Cellulase expression specific gene regulation in Trichoderma reesei

Doris Tisch, Andre Schuster and Monika Schmoll*

Vienna University of Technology, Research Area Gene Technology and Applied Biochemistry, Gumpendorfer Straße 1a, 1060 Wien, AUSTRIA

*presenting author, oral or poster presentation

email: monika.schmoll@tuwien.ac.at

Trichoderma reesei (anamorph Hypocrea jecorina) is one of the most prominent fungi in biotechnology. Enzyme cocktails of Trichoderma reesei are used in the pulp and paper industry, food industry or for production of the second generation of biofuels. Recently it was shown that light has a positive effect on most glycoside hydrolases, like the major cellulase gene of T. reesei cbh1/cel7a (cellobiohydrolase I, GH7 family). Furthermore, the environmental signal light has an influence on multiple important components of the signal transduction cascade in T. reesei, such as G protein subunits, pheromone precursors, kinases or dehydrogenases. We were now interested in how another environmental signal – the kind of the carbon source – is integrated in this signal transduction cascade and influences cellulase gene expression. Additionally, this analysis can reveal cellulose expression specific genes and hence provide novel targets for strain improvement. While upon growth on the cellulase inducing carbon sources lactose and sophorose more than 300 genes are responsive to light (>2fold differential expression, $p<0.001$), on the cellulase repressing carbon source glucose just 148 genes are responsive to light. Among these light regulated genes, only about 20 of them are identical between these different carbon sources, indicating a sophisticated fine-tuning mechanism of the light signaling cascade in dependence on the carbon source availability. A comparison with two more carbon sources – cellulose, which mimics the natural substrate of T. reesei, and glycerol as a non-repressing and non-inducing carbon source, reveals that 6 genes are responsive to light ubiquitously. The atypical pheromone precursor HPP1 and the light regulatory protein ENVOY are among the targets of light not affected by the carbon source, suggesting that these two proteins have a unique and the most important position in regulation of the light response and sexual development.

Biomass saccharification: development of strategies for enzyme recycling

Miguel Gama[1], Ana Cristina Rodrigues[1], Claus Felby[2] and Alexandre Leitão[1]

1 Departamento Engenharia Biológica, Universidade do Minho, Campus de Gualtar 4710-057 Braga, Portugal
2. University of Copenhagen, Faculty of Life Sciences

There is a growing demand for viable alternatives to fossil fuels. In response to the growing global demand the production of biofuels, such as bioethanol, has gained significant notoriety. Biomass conversion may be achieved using cellulolytic enzymes. Herein resides one of the major cost expenditures of the process and so there is a significant need for viable enzyme recycling systems in order to reduce overall ethanol production costs.

Several methods have been proposed in order to minimize enzyme adsorption to lignin. These methods have been put forth in order to ameliorate enzyme recycling through alternative or additional pretreatment of the lignocellulosic biomass. The high stability of cellulolytic enzyme and that most of their activity is retained after desorption from lignin suggests that a cost efficient method of enzyme recycling from the leftover lignin biomass is not only viable but an important in reducing production costs.

In this study we will show that Cel7a retains its activity when adsorbed onto lignin and strategies allowing the reversible conformational and structural changes that allow us to recover a significant amount of this enzyme without it losing activity. Data obtained using circular dichroism and activity measurements using both low molecular weight substrates and insoluble fibres clearly demonstrate that high recovery (up to 70-80%) of cellulases is possible using simple strategies, easy to implement in the large scale process. Studies on the interplay between temperature, conversion yield and the interaction of purified Cel7a with different substrates (purified lignin and cellulose) will be described.

KINETIC DEPENDENCES OF CELLULASE ADSORPTION ON THE BLEACHED PULP

G. Radeva*, I. Valchev and V. Mironova

The speaker's name in bold type followed by other co-authors
University of Chemical Technology and Metallurgy,
8, Kl Ohridski blv., 1756, Sofia, Bulgaria

e-mail:grradeva@abv.bg

Abstract
The enzymatic hydrolysis of cellulose is a complex heterogeneous process including the cellulase adsorption at the cellulose surface as an initial step. The kinetic characteristics of the process are determined by the adsorption step regularities. The aim of this work is to study the kinetics of enzyme complex cellulase adsorption on the bleached pulp.

The enzyme cellulase NS50013 (product of Novozymes AS) of an activity of 700 EGU/g was added at quantity 2% referred to the pulp mass. The temperature varied up to 40^0C, the pH was kept at 5.1 values, while the reaction time is varied up to 30 min. The enzyme adsorption was studied by measuring the residual protein contents with the application of the colorimetric Bradford micromethod.

The adsorption kinetics of celluase on the bleached pulp was described by the exponential kinetic equation. The values of the activation energy and the preexponential factor were estimated. The activation energy is constant during of the adsorption process. This is an indication that the enzyme-pulp system behaves as an energetically homogeneous one.

The preexponential factor accounts for the entropy factors of the system investigated. They are attributed to the spatial orientation of the enzyme molecule and the steric hindrances which accompany the formation of the adsorbed surface compound. The decrease of the preexponential factor in the course of the process can be also explained with the exhaustion of the available active sites on the pulp. The results obtained show that the preexponential factor decrease determines the rate decrease observed.

Enzymatic hydrolysis of different allomorphic forms of microcrystalline cellulose

D. Ciolacu[1,2], S. Gorgieva[1], D. Tampu[2], V. Kokol[1]
[1]University of Maribor, Institute for Engineering Materials and Design, Slovenia

vanja.kokol@uni-mb.si

[2]"Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania,

dciolacu@icmpp.ro

Abstract
Cellulose, the most abundant polysaccharides on earth is an almost inexhaustible renewable raw material with an expanded role in the future. The biological conversion of cellulose materials to nano- and micro-sized materials and glucose has been shown to be a useful method to obtain valuable products, such as nanocomposites, bioethanol and various chemicals. Thus, the complex enzymatic hydrolysis of cellulose has been studied intensively in the last 50 years. However, the effect of the variety of physical structures adopted by the cellulose macromolecules being in different crystalline forms, on their biodegradation were not yet detailed investigated.

In the contribution, the enzymatic hydrolysis of three main allomorphic forms of microcrystalline cellulose (cellulose I, II and III) by cellulases from Trichoderma reesei and Aspergillus niger will be presented. The efficiency of the biodegradation of the cellulosic allomorphs will be estimated by the amount of reducing sugars and the yield of reaction, being established by chemical and HPLC-SEC analysis. The relative susceptibility of different cellulosic substrates to enzymes will be determinate as the modifications in the average particle size of the residues resulted after the hydrolysis process. The changes of the supramolecular structure of cellulosic residues will be observed as alterations of crystalline index and crystallites dimension of the corresponding allomorphic forms by X-ray diffraction method. The ultra-structural aspects of cellulose allomorph residues will be examined by optical and scanning electron microscopies.

This research was supported by the Marie Curie Transfer of Knowledge Fellowship of the EC FP6 under the contract nr. MTKD-CT-2005-029540-POLYSURF.

Towards An assay for glucouronoyl esterase

Hampus Sunner[a,b], Paul Christakopoulosb,[c] and Lisbeth Olsson[a,b]

aDepartment of Chemical and Biological Engineering
Chalmers University of Technology
SE-412 96 Gothenburg, Sweden

bWallenberg Wood Science Center,
Chalmers University of Technology
SE-412 96 Gothenburg, Sweden

cChemical Engineering Department
National Technical University of Athens
GR-15 700 Athens, Greece

The process of separating the different wood polymers is energy-demanding and inherently destructive. An enzyme-assisted separation process could increase yields and allow for tailor-made materials for novel applications. Several of these require the removal of the lignin that is covalently bound to hemicelluloses by a variety of bonds, of which one antipicitated bond is the glucuronoyl ester bond. This bond is believed to be the target of the newly discovered glycuronoyl esterases (Špániková and Biely 2006), which have only been shown to act on synthetic substrates (see figure below). In this work, we use FTIR and alkaline hydrolysis to investigate five different substrates that presumably contain the ester linkages between the 4-O-methyl-d-glucuronic acid (MeGlcA) or d-glucuronic acid of glucuronoxylans and the hydroxyl groups of lignin alcohols. The results indicate this approach can build the basis for a novel glucuronoyl esterase assay using natural substrates by the technique of in situ ester reduction with sodium borohydride, leading to conversion of MeGlcA to 4-O-methyl-D-glucose, which can be detected by ion chromatography. If shown to act on lignin ester bonds, glucuronoyl esterase can be directly applied to produce acetylated hemicelluloses, enabling bio-based materials with new properties.

Figure. The substrates used in the initial discovery of the glucuronoyl esterases. The bond hydrolysed by glucuronoyl esterase is indicated an arrow.

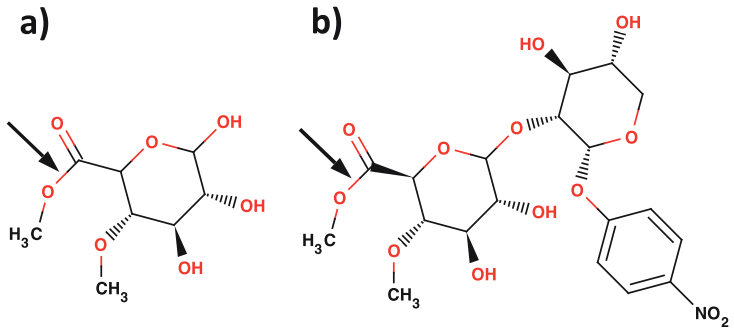

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Figure. The substrates used in the initial discovery of the glucuronoyl esterases. The bond hydrolysed by glucuronoyl esterase is indicated by an arrow.

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Isothermal titration calorimetric studies on the binding of mesophilic and thermophilic feruloyl esterases with linear and branched chain xylooligosaccharides

Evangelos Topakas[1], Athanasios Papadopoulos[2], George Nounesis[2], Paul Christakopoulos[1]

[1]*BIOtechMASS Unit, Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, 9 Iroon Polytechniou Street, Zografou Campus, 15700 Athens, Greece*

[2]*Biomolecular Physics Laboratory, IRRP, National Centre for Scientific Research "Demokritos", 153 10 Aghia Paraskevi, Greece*

Ferulic acid esterases (FAEs, E.C. 3.1.1.73), also known as feruloyl esterases, represent a subclass of the carboxylic acid esterases (E.C. 3.1.1), which catalyses the hydrolysis of the ester linkage of hydroxycinnamic acids (ferulic acid (FA) and *p*-coumaric acid) and diferulates present in plant cell walls. The role of these enzymes in plant biomass degradation but also their use as biosynthetic tools has been studied[1].

It is extremely common for esterases to act on a broad range of substrates. In literature, different types of FAEs exhibit different substrate specificity profiles against various model compounds and natural feruloylated oligosaccharides. In order to probe the substrate specificity of FAEs, it is important to characterize the interactions of branched carbohydrates with the active center of esterases.

In the present study, binding of feruloylated and non-feruloylated oligosaccharides to different types of recombinant FAEs, were examined using isothermal titration microcalorimetry (ITC). In order to avoid hydrolysis of the bound feruloylated substrates, all FAEs investigated were inactivated by replacing the catalytic nucleophile serine of the conserved motif G-X-S-X-G by alanine using site-directed mutagenesis. The results of this study have important implications in understanding the diversity of FAEs and their role in deconstructing plant cell wall material.

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Phenolic Compounds as Laccase Redox Mediators in the Oxidation of Non-Phenolic Compounds

Tânia Rosado[1], Kamila Koci[1], Ana Varela Coelho[1], Maria Paula Robalo[2] and Lígia O. Martins[1*]

[1]Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av da República, 2780-157 Oeiras, Portugal, [2]Departamento de Engenharia Química, ISEL, Rua Cons. Emídio Navarro, 1, 1959-007 Lisboa, Portugal and Centro de Química Estrutural, Complexo I, IST, Av Rovisco Pais, 1049-001 Lisboa, Portugal. E-mail: lmartins@itqb.unl.pt

A few phenolic compounds, related to recurring phenolic structures in lignin, are capable of mediating the oxidation of the non-phenolic lignin units by laccases, with similar or improved performance, as compared with synthetic mediators (1). However, the treatment of lignocellulosic fibers with laccases and phenolic compounds have been shown to result in a variety of reactions (coupling, grafting and polymerization) which are difficult to predict, due to the complexity of the lignocellulosic matrix and the nature of the laccase free radical reactions (2). In the present work we have compared the performance of CotA-laccase from Bacillus subtilis with the commercial fungal laccase from Trametes versicolor in the oxidation of the natural compounds syringaldehyde, acetosyringone and methyl syringate. These enzymes are representative of two major groups of laccases, those with low and with high redox potentials, showing a diverse pH profile for phenolics and structural differences close to the substrate binding site, evident in the 3D-structure. The biotransformation of the non-phenolic substrate veratryl alcohol was mediated by the redox mediators studied, and the enzymatic reactions were followed by HPLC. The information gathered together with the products identification by NMR and MS techniques has elucidated the oxidation mediator-assisted mechanistic pathway of non-phenolic lignin subunits. The results of this study allow understanding the structural features of phenolic substrates and of laccase that are relevant for oxidation efficiency and importantly allowed to devise the phenolic mediators that are better tailored to act as oxidative mediators in the degradation of lignin non-phenolic units.

This work was supported by the project grant BIORENEW (FP6-2004-NMP-NI-4/026456). T. Rosado holds Ph.D. grant (SFRH/BD/65747/2009) from Fundação para a Ciência e Tecnologia, Portugal.

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COMPARISON OF VARIOUS LACCASES IN THE OXIDATION OF LIGNIN MODEL COMPOUNDS IN ORGANIC SOLVENTS

Pekka Maijala1*, Maija-Liisa Mattinen2, Paula Nousiainen3, Jussi Kontro3, Janne

Asikkala3, Jussi Sipilä3, Liisa Viikari1

*1Department of Food and Environmental Sciences, PO box 27, FI-00014 University of Helsinki,

Finland, E-mail: pekka.maijala@helsinki.fi

2VTT Bioprocessing, VTT, Finland

3Department of Chemistry, University of Helsinki, Finland

Stability and reactivity of five different thermostable fungal laccases from species Trametes hirsuta, Melanocarpus albomyces, Thielavia arenaria (two laccases) and Chaetomium thermophilum were investigated in presence of organic solvents. Detailed oxidative reactions of small organic compounds, lignans matairesinol and 7-hydroxymatairesinol as well as synthetic lignin dehydrogenation polymer DHP in aqueous solutions of ethanol and propylene glycol solvents were investigated using oxygen consumption analysis, HPLC and size-exclusion chromatography. The laccases showed variability in their solvent tolerance. Laccase redox potential appeared not to be the main determinant of efficiency of the polymerization reactions of complex phenolic model compounds in aqueous organic solutions. Even in the presence of strongly enzyme activityinhibiting conditions polymerization reactions took place, encouraging enzymatic valorization reactions in presence of organic solvents to be studied further. NMR spectroscopy analysis of DHP, polymerized by laccase in presence of 50% ethanol or in 50% propylene glycol, indicated that the formation of new biphenylic 5-5' structures is favoured in laccase-catalyzed radical coupling reactions over the other possible reaction through the phenolic groups forming new 5-O-4 ether bonds. The results also suggest that the ratio of aliphatic primary and secondary hydroxyls has changed under laccase treatment. Thermostable laccases are excellent candidates for applications in various industries where organic solvent(s) are required for reaction systems. The research was part of the LigniVal project with the BioRefine program funded by the Finnish Funding Agency for Technology and Innovation.

Fungal sterol esterases as biocatalysts for degradation or synthesis reactions

Víctor Barba, Alicia Prieto and María Jesús Martínez

Environmental Biology Department, Centro de Investigaciones Biológicas (CIB-CSIC), Ramiro de Maeztu 9, 28040 Madrid, Spain. mjmartinez@cib.csic.es

The fungus Ophiostoma piceae produces a sterol esterase which efficiently hydrolyzes sterol esters and triglycerides and its use for pitch biocontrol has been reported. This enzyme has been successfully expressed in Pichia pastoris, and the recombinant protein showed improved catalytic properties. In this work we used the native and recombinant enzymes, as well as the commercial lipase from Candida rugosa (with sterol esterase activity) for the esterification of phytosterols with lauric acid to be used as nutraceuticals.

Regarding the esterification assays, the influence of substrate and enzyme concentration, and the effect of different organic solvents in the reactions have been studied. The results showed that O. piceae enzymes seem to be somewhat better than commercial enzyme. These showed an overall production of steryl esters of 75-78% within 48 hours, while C. rugosa enzyme did not exceed 67% when a small excess of substrate was used. In addition, the capability of O. piceae esterase to deacetylate PVAc to its corresponding alcohol derivative has also been confirmed using FTIR spectroscopy and mass spectrometry analysis.

The preliminary results suggest that the O. piceae sterol esterase is a versatile enzyme that can be used in synthesis or hydrolysis reactions of biotechnological interest.

Study of the effects on bast and core flax fibres of laccase-phenols treatments

Amanda Fillat*, M. Blanca Roncero, Teresa Vidal

Textile and Paper Engineering Department. ETSEIAT. Universitat Politècnica de Catalunya. Colom 11. E-08222 Terrassa, Spain. E-mail: amanda.fillat@etp.upc.edu

Flax unbleached pulp is being studied as a raw material target for biotechnology innovation. Laccases have been tested in the presence of several phenolic compounds in order to obtain environmental friendly high-value paper products.

With the aim of better understanding the enzymatic effects of violuric acid (VA) and p-coumaric acid (PCA) on flax, the changes in chemical composition of the two main fibres types that compose this pulp were assessed. After classification of the initial pulp, two fractions according to fibres size were obtained (long and short fibres). There was a significant effect of the fibre dimension on the pulp properties and on the response to the application of different laccase-phenol treatments. PCA and laccase treatments resulted in a high increase in kappa number and darkening in both fibre fractions, indicating a grafting of this compound onto the fibres. Whereas, VA treatments produced long fibres with low lignin content (kappa number of 1.3) and high brightness (5 units higher than the control fraction), proving its bleaching capacity. Both biotreatments resulted in long fibres with high cellulose crystallinity and in a HexA removing in global and short fibres. Furthermore, no morphological changes were observed after laccase treatments, indicating the preservation of fibres integrity. In this study, we demonstrate that laccase acts as polymerization agent for PCA and as a delignification agent for VA and that the action of each enzyme system differs between bast and core fibres.

Aknowledgments

This work was funded as a part of two Spain's MICINN Projects: FUNCICEL CTQ2009-12904 and BIOFIBRECELL CTQ2010-20238-C03-01. The authors are grateful to CELESA (Tortosa, Spain) for supplying the starting pulp and INRA (Marseille, France) for kindly providing the enzyme.

OXIDATION OF LIGNANS AND LIGNIN MODEL COMPOUNDS BY MELANOCARPUS ALBOMYCES LACCASE IN AQUEOUS SOLVENT MEDIA

M-L Mattinen*, P. Maijala, P. Nousiainen, A. Smeds, J. Kontro, J. Sipilä, T. Tamminen,

S. Willför and L. Viikari

VTT Bioprocessing, Tietotie 2, (B.O. Box 1000), Espoo, 02044 VTT, Finland

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The stability and activity of the low redox potential Melanocarpus albomyces laccase in various aqueous organic as acetone, ethanol, propylene glycol and diethylene glycol monomethyl ether media was studied by spectrophotometric means using 2,6-dimethoxyphenol as substrate for the enzyme. Additionally reactivity of the laccase with two lignans as matairesinol and 7-hydroxymatairesinol was studied by oxygen consumption measurements in the most potential aqueous organic solvent mediums. Polymerization of the lignans by Melanocarpus albomyces laccase was confirmed by MALDI-TOF mass spectrometry and size exclusion chromatography. Polymerization of the higher molecular mass lignin model compound as dehydrogenation polymers was verified also by chromatographic means. The functioning of the selected laccase in different aqueous organic media was excellent. Propylene glycol and diethylene glycol monomethyl ether were better solvents than ethanol or acetone in laccase catalysed oxidations. The results support the use of laccase -catalysed reactions in organic solvents to improve the efficiency of lignin oxidation that may be exploited in several technical applications and areas in which the solubility of the reactants or products is a limiting factor. The research was part of the LigniVal project with the BioRefine program funded by the Finnish Funding Agency for Technology and Innovation. In addition financial support from Metso Power Oy, Oy Metsä-Botnia Ab, Stora Enso Oyj, Roal Oy was obtained in addition to the Academy of Finland.

REACTIVITY OF BACTERIAL AND FUNGAL LACCASES ON TECHNICAL LIGNINS IN ALKALINE REACTION CONDITIONS

R. Moya, P. Saastamoinen, M. Hernández, A. Suurnäkki, E. Arias and M.-L. Mattinen*

VTT Bioprocessing, Tietotie 2, (B.O. Box 1000), Espoo, 02044 VTT, Finland

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The ability of Streptomyces ipomoea laccase to polymerize lignan and technical lignins from different plant pieces and isolation processes were examined. In addition reactivity of bacterial laccase was compared to that of low redox potential fungal laccase from Melanocarpus albomyces using small organic phenolic acids as p-coumaric acid, ferulic acid and sinapic acid as well as natural (acetosyringone) and synthetic (TEMPO) mediators as substrates for the enzymes. Oxygen consumption measurement, MALDI-TOF mass spectrometry and size exclusion chromatography were used to follow the enzymatic reactions at pH 7, 8, 9 and 10 at 30°C for comparison. Polymerization of different lignins and lignan by bacterial laccase in extremely alkaline reaction conditions was clear and somewhat enhanced in the presence of acetosyringone mediator. However, reactivity of bacterial laccase on selected phenolic acids was not as good as that of low redox potential fungal laccase even at unoptimal pH (above 7) of the enzyme. In addition to basic research of the mode of action of the laccases, the research results have impact on the enzymatic modification of industrial softwood and hardwood kraft lignins in alkaline reaction conditions. The study was funded by the Autonomous Government of Madrid for the PIA fellowship and the University of Alcalá. In Finland the research was part the LigniVal project in the Tekes (the Finnish Funding Agency for Technology and Innovation) BioRefine program. Also financial support from Metso Power Oy, Oy Metsä-Botnia Ab, Stora Enso Oyj and Roal Oy was obtained.

Laccase catalyzed modification of lignin for enzymatic hydrolysis

Ulla Moilanen*, Tinaïg Le Costaouëc, Miriam Kellock, Liisa Viikari

Department of Food and Environmental Sciences, PO Box 27, 00014 University of Helsinki, Finland

The efficient use of cellulases in hydrolysis of pretreated lignocellulosic biomass is limited due to the presence of lignin. Lignin is known to bind hydrolytic enzymes non-specifically, thus reducing their action on carbohydrate substrates. The composition and location of residual lignin seem therefore to be important for optimizing the enzymatic hydrolysis of lignocellulosic substrates. The use of lignin modifying enzymes, such as laccase, may have potential in the modification or partial removal of lignin from the biomass. In this study, the effect of lignin modification by laccase on the hydrolysis of pretreated spruce and giant reed (Arundo donax) was evaluated. The substrates were first treated with laccase and then hydrolyzed with commercial cellulases. Laccase modification improved the hydrolysis yield of spruce by 12 % but surprisingly, had an adverse effect on giant reed reducing the hydrolysis yield by 17 %. The binding properties of cellulases on the untreated and laccase treated lignins were further studied on isolated lignins. The laccase treatment reduced the binding of enzymes on modified spruce lignin, whereas with giant reed the amount of bound proteins was increased after laccase treatment. Further understanding of the reactions of laccase on lignin will help to control the binding of cellulases on lignin.

Ionic Liquids as Alternative Co-Solvents for Laccases

Tânia Rosado*, Isabel Marrucho, Luís Paulo Rebelo and Lígia O. Martins

Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av da República, 2780-157 Oeiras, Portugal. E-mail: tisrosado@itqb.unl.pt

The pulping industry releases annually, very large amounts of lignin that constitute an immense quantity of biomass partially usable for applied purposes, with the advantage of decreasing the industrial dependence from petrochemical sources. The use of oxidative enzymes can increase the sustainability and performances of the currently available processes and enable the production of new lignin-based products. Laccases show the broadest substrate specificity, among the lignolytic enzymes, extended on substituted phenol, polyphenols, and aromatic amines, requiring only atmospheric oxygen as cofactor. Lignin is a highly branched, irregular three-dimensional polymer with very low solubility in water. Organic solvents have been thoroughly used in bioconversions involving lignin, but the alternative use of ionic liquids is getting increasing attention from both industry and academics. Ionic liquids are well known by their appealing negligible vapor pressure at ambient conditions and excellent solvent quality for many types of compounds.

In this study we have identified ionic liquids that promote solubilisation of lignin model substrates and that simultaneously provide the best conditions for the activity and stability to the bacterial CotA-laccase from Bacillus subtilis and the fungal TvL laccase from Trametes versicolor, two well-characterized laccases with the required robustness for biotechnological applications. A systematic study of several members of the most important IL families was performed and indicated that sulphate-based ILs produced promising results in terms of enzyme activity and stability and hence. The feasibility of ionic liquids as solvent media for mediator assisted reactions was tested using the oxidation of veratryl alcohol to veratryl aldehyde. The outcome of this project will help the set-up of eco-friendly and effective industrial enzymatic processes that would better fit in biotechnological applications.

This work was supported by the project grant BIORENEW (FP6-2004-NMP-NI-4/026456). T. Rosado holds Ph.D. grant (SFRH/BD/65747/2009) from Fundação para a Ciência e Tecnologia, Portugal.

Optimization of a low cost medium for laccase production in Pleurotus ostreatus using response surface methodology: maximum production at minimum cost

*V. Lettera, L. Guarino, G. Sannia

Dipartimento di Chimica Organica e Biochimica,

Università degli Studi di Napoli Federico II

Complesso Monte Sant'Angelo

Via Cinthia, 4

0126 Naples, ITALY

*vincenzo.lettera@unina.it

The present work was carried out responding to the ever-increasing demand of laccases for biodelignification, industrial oxidative and environmental bioremediation processes that requires high production levels of enzymes at low cost. Enzymatic production from hyper producer strain *Pleurotus ostreatus* 5Ax3D was performed under submerged growth in shaken flasks and the interactions between different factors composing fermentation broth were evaluated using response surface methodology (RSM). A low cost medium was formulated using a waste product coming from rapeseed oil extraction processes, a low cost lignosulphonates mixture, copper sulphate, and yeast extract as raw materials. Optimization of production conditions by coupling both regression equations of enzymatic production and medium formulation costs yielded over 190.000 unit of laccases with a cost of 0,157 euro per liter of broth. The optimized medium was also tested in submerged growth performed in a stirred tank bioreactor, reaching a titer value of laccase activity still suitable for the enzymatic application in bioprocessing at industrial scale.

Extracellular ligninolytic enzyme activities in yeast isolates from wastewater treatment plants

A. R. Silva, P. R. Moreira* and M. Pintado

Escola Superior de Biotecnologia, Universidade Católica Portuguesa

R. Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal
*prmoreira@esb.ucp.pt

Many microorganisms have been found to be capable of degrading dyes; these include bacteria, filamentous fungi, yeasts, actinomycetes and algae. When compared to bacteria and filamentous fungi, yeasts present advantages; they grow rapidly and have the ability to resist unfavourable conditions. However, degradation of synthetic dyes by yeasts has not been extensively reported, namely its relation with extracellular ligninolytic enzymes.

Forty six yeast strains isolated from two wastewater treatment stations along with other 81 cheese isolates were compared on their ability to decolorize five textile dyes in solid media.

After a screening methodology that included liquid culture decoulorisation ability evaluation, yeasts isolates, LIII S 36 and L III ST 7 presented the best performance in the decolourisation for the five dyes tested: Remazol Black B-A, Remazol Yellow RR, Levafix Blue CA, Remazol Brilliant Blue R and Levafix® Red CA).

As an attempt to understand the mechanism of decolourisation of the strains, L III ST 7 and L III S 36, spectral scanning and enzymatic activity assays were performed. It was possible observe that, depending on the dye, decolourisation might be achieved through mechanisms of either adsorption or true degradation. The presence of extracellular ligninolytic manganese peroxidase activity was detected in strains L III ST 7 and L III S 36 with an average of 2.30 and 2.06 IU. l^{-1}, respectively; this enzymatic activity rarely demonstrated in yeasts might be related to the mechanism of true degradation as already proven for several filamentous fungi.

Fungal pretreatment of wheat straw for second-generation ethanol production

Davinia Salvachúa, Alicia Prieto, Ángel T. Martínez, and María Jesús Martínez

Environmental Biology Department, Centro de Investigaciones Biológicas (CIB-CSIC), Ramiro de Maeztu 9, 28040 Madrid, Spain. mjmartinez@cib.csic.es

Ethanol production from lignocellulosic biomass is one of the most promising alternatives to avoid the use of food-related raw materials for energy production. The main drawback of cellulosic ethanol comes from the high costs associated to its production, arising mainly from the need of pretreating feedstocks to disrupt the lignocellulose structure. Nowadays, acidic steam explosion is the most effective pretreatment for this purpose. Nevertheless, this approach is expensive, since requires high pressures and temperatures and generates by-products that adversely affect subsequent steps of the process. In this work, the potential of combining a fungal pretreatment with a mild alkali treatment for ethanol production from wheat straw, as a potential alternative to steam explosion, has been investigated.

Twenty one strains of basidiomycetes were tested for their ability to grow on wheat straw under solid state fermentation (SSF) conditions. Changes in substrate composition, secretion of ligninolytic enzymes, enzymatic hydrolysis efficiency and ethanol production yield after 7, 14 and 21 days of each biopretreatment were evaluated. Most fungi degraded lignin with variable selectivity degrees, although only eight of them improved sugar recovery compared to untreated samples. Glucose yields after 21 days of pretreatment with Poria subvermispora and Irpex lacteus reached 69% and 66% of cellulose available in the initial straw, respectively, with an ethanol yield of 62% in both cases. Conversions from glucose to ethanol reached around 90%, showing that no inhibitors were generated during this pretreatment. No close correlations were found between ligninolytic enzymes production and sugar yields.

The Inbicon process - Thermal pretreatment with no addition of chemicals

Kit Kellebjerg Mogensen, Mai Østergaard Haven, Martin Dan Jeppesen, Laila Thirup

Inbicon A/S, Klippehagevej 22, 7000 Fredericia, Denmark, Email: kitmo@inbicon.com

Inbicon A/S (a DONG Energy subsidiary) has been operating a large-scale pilot plant (100-1000 kg/h) for the conversion of wheat straw to ethanol since 2005 and a demonstration plant (4000 kg/h) since 2009. The focus in process development has been on sustainability and energy efficiency. Therefore all process steps are carried out at high dry matter content (above 25 % water insoluble solids) and without addition of chemicals. To further increase energy efficiency the production of bioethanol is integrated with a power plant.

The process converts cellulose into bioethanol. Lignin is converted into a high-quality solid biofuel which supply the process energy as well as a surplus of heat and power. Hemicellulose is used as a feed molasses but could in the future also be used for additional ethanol production or other valuable products.

This poster compares pretreatment results from different biomasses treated with the Inbicon technology at a series of different pretreatment conditions. The results show that different biomasses require varying pretreatment strategies for optimal overall yield. The pretreatment data will be supplemented with data for enzymatic conversion for selected biomasses.

Studies of enzymatic hydrolysis and fermentation of lignocellulosic biomasses at high solids concentration

David Cannella*, Claus Felby, Henning Jørgensen

*Presenting author/ corresponding e-mail: dac@life.ku.dk

Faculty of Life Sciences, University of Copenhagen, Rolighedsvej 23, DK-1958 Frederiksberg C, Denmark

The future of second generation bioethnaol is to become an economically viable renewable biofuel. The key to overcome this barrier is the founding of a technology able to obtain an efficient breakdown of lignocellulosic biomass, working at very high solids concentration in order to improve product concentrations, plant productivity, and decrease energy input and final costs. Most of available data in literature regarding the high solids concentration refer to 10-15% of water insoluble solids (WIS). In this work solids concentration in the range of 20 to30% WIS are applied. There are several problems associated with the operation of lignocellulose conversion at high solids: i) the enzyme performance expressed as released sugars from cellulose and hemicellulose; ii) mass transport of enzyme and soluble glucose reflecting the mixing efficiency of biomass in water; iii) the tolerance of yeast at high concentrations of compounds such as sugars, ethanol and toxicants. Understanding the balance of operative parameters between hydrolysis and fermentation in high solids concentration is crucial to obtain an efficient saccharification and simultaneous or separate fermentation.

In this presentation we will show how the conversion yield and fermentation yield is effected by using various process configurations. The results were obtained on wheat straw (hydrothermally pretreated) and spruce (pretreated with sulphuric acid) at high solids concentration. For the hydrolysis the latest commercially available cellulase enzymes were used, and for fermentation a commercial Saccharomyces cerevisiae strain (Thermosacc) was studied.

BIOENERGY FROM WHEAT STRAW : PREATREATMENT,ENZYMATIC HYDROLYSIS AND ETHANOL PRODUCTIVITY

Luís Duarte, B. Ribeiro, S. M. Paixão, L. Baeta-Hall, M. C. Sàágua and José C. Duarte*

LNEG – Laboratório Nacional de Energia e Geologia, I.P,

Bioenergy Unit: Estrada do Paço do Lumiar, 22, 1649-038 Lisboa, Portugal.

Biovalorization of wheat straw, a low-cost lignocellulosic biomass into valuable compounds was studied. For bioethanol conversion there are mainly three processes involved: (i) pretreatment for hemicellulose removal from lignocellulosic biomass, (ii) hydrolysis of cellulose to produce reducing sugars, and (iii) fermentation of the sugars to ethanol using an ethanologenic microorganism. Wheat straw biomass pre-treatments were applied: autohydrolysis and acid hydrolysis were compared. Enzymatic hydrolysis was studied using a commercial cellulase mixture (Celluclast 1.5 L and Novozym 188, Novozymes A/S, Denmark) at different conditions. The influence of different parameters such as temperature (35-60ºC), incubation time (1-7 days) and enzyme loading (10-15 FPU/g and 0.2-0.4 mL/g polysaccharides) during the hydrolysis process was evaluated. The optimisation criterion was the fermentable sugar yields, which were analysed by HPLC. The best results for the wheat straw hydrolysis were obtained for the enzymatic loading: Celluclast 10 FPU/g polysaccharides + Novozym 188, 0.2 ml/g polysaccharides at 55ºC for 48 h. These optimal saccharification conditions for wheat straw pretreated (WSP) allowed a final process yield of ~60% in hydrolisate sugar content.

The potential for ethanol production by fermenting wheat straw hydrolisate (WSH) with two yeast strains of S. cerevisiae (strains F and K from our collection) and a bacterial strain of Z. mobilis (strain CP4 a gift from LO Ingram) was evaluated. Batch fermentation tests of the WSH showed an ethanol yield of 74%, 79% and 58%, respectively. Supplementing the WSH with peptone and/or yeast extract had no effect on yield for yeast fermentation. However, the ethanol production by Z. mobilis was highly increased when yeast extract was added to the hydrolisate, corresponding to an ethanol yield of 98%.

Moreover, further pretreatment optimization of lignocellulosic materials to remove lignin can significantly enhance the hydrolysis of cellulose improving the bioethanol yield and in addition the recovered lignin can also be converted into valuable compounds.

These results show that an integrated exploitation of this by-product from agricultural wheat production is economically possible and highly advantageous for energy and chemicals production.

Rye straw: a suitable feedstock for oligosaccharides and glucose production

Beatriz Gullón*, Remedios Yáñez, Belén González, José Luis Alonso and Juan Carlos Parajó

Lignocellulosic materials (LCMs) can be used as a feedstock for variety of industrial processes, including those based on the “biorefinery” approach. According to this phylosophy, LCMs can be sequentially fractionated to obtain the main components (cellulose, hemicelluloses and lignin) or their derivatives in separated streams, achieving an integrated process for the production of food, feed, chemicals, materials, goods, and fuels for the future.

In this context, autohydrolysis could be a suitable alternative as the first fractionation stage of a biorefinery. Starting from a xylan-containing LCM, the breakdown of hemicellulose chains leads to xylooligomers with food, medical, and pharmaceutical applications. As food ingredients, XOS may affect the human gastrointestinal tract beneficially through the modulation of colonic microbiota, especially Bifidobacteria and Lactobacilli. Solids (mainly containing lignin and cellulose) can then be further processed to obtain sugar-containing solutions.

This work deals with production of xylooligosaccharides and sugars by autohydrolysis of rye straw (a feedstock with high xylan content) followed by enzymatic hydrolysis of the resulting solids. When samples were treated at 208 ºC, 69.2% of the initial xylan was converted into xylooligosaccharides, leading to reaction liquors containing up to 22.4 g substituted oligosaccharides/L, additionally when the solid was subjected to enzymatic hydrolysis, 70.6% of cellulose and 63.8% of xylan were saccharified after 48 h.

Obtaining food ingredients from waste solids containing barley hulls

Patricia Gullón*, María Jesús González-Muñoz, Juan Carlos Parajó

Chemical Engineering Department, Polytechnical Building, University of Vigo (Campus Ourense) , As Lagoas, 32004 Ourense, Spain

INTRODUCTION

When a xylan-containing material (for example, barley hulls) is processed under suitable operational conditions, xylan can broken down to soluble xylooligosaccharides (XOS). Simultaneously, other effects (including extractive removal and side-reactions) may occur in the reaction media, resulting in the presence of undesired products in liquid phase. The interest in producing XOS lies mainly on their utilization as food ingredients, which require high purity products. XOS purification can be achieved by separation technologies.

METHODS

Samples of industrial solid wastes containing barley hulls were assayed for composition and subjected to aqueous processing using a pressurized, batch reactor, operating under optimized conditions. Hemicellulose-derived products were quantified by HPLC before and after quantative posthydrolysis. Oligosaccharides derived from hemicelluloses were purified using membrane technologies and ion exchange.

RESULTS

Liquors hydrothermal treatment of the feedstock were refined by membrane processing and ion exchange. The the final concentrate, mainly made up of XOS, was assayed for composition and as a substrate for supporting the in vitro growth of intestinal bacteria coming from faecal inocula. After 48 h, 91.5% of XOS were consumed, resulting in the production of Short Chain Fatty Acids (formiate, acetate, propionate, and butyrate). This finding confirmed the prebiotic potential of the purified XOS.

ACKNOWLEDGEMENTS

The authors are grateful to the Spanish Ministry of Science and Innovation for supporting this study, in the framework of the research Project AGL2008-02072, which was partially funded by the FEDER Program of the European Union.

EFFECT OF STEAM EXPLOSION PRE-TREATMENT ON ENZYMATIC SACCHARIFICATION OF LIGNOCELLULOSIC MATERIAL

Laurence Meyer[1], Nicolas Jacquet[1], Caroline Vanderghem[1], Christophe Blecker[2], Michel Paquot[1]

[1]Department of Industrial Biological Chemistry, Gembloux Agro-Bio Tech – Université de Liège, Gembloux, Belgium
[2]Department of Bio-Ressources Valorisation, Gembloux Agro-Bio Tech – Université de Liège, Gembloux, Belgium

Taking into account the sharp rise in prices and the depletion of resources of petroleum, an alternative to fossil resources is needed. A probable alternative is the use of lignocellulosic raw material to produce biofuels. The “first generation” biofuels are highly controversial because of the use of food plant material. The aim of the “second generation” biofuels is to take lignocellulosic non-food plant material as raw material.

Lignocellulosic biomass has a very complex structure made of linkages between lignins, cellulose and hemicelluloses. The saccharification of these lignocellulosic materials requires the fractionation of its constituents. Research has lead to many lignocellulosic biomass fractionation pre-treatments. This study particularly focuses on the steam explosion pretreatment followed by an enzymatic saccharification. Steam explosion is a thermomechanical process which allows the breakdown of the lignocellulosic material structure by the combined action of steam heating, hydrolysis induced by the organic acids formed during the process and shear stress resulting from the pressure rough drop. This treatment leads to modification of the physical parameters such as water retention capacity, cristallinity rate of the cellulosic fraction, hydrolysis of the hemicellulosic fraction and rearrangement in the lignin structure.

Such modifications are supposed to make cellulose enzymatic hydrolysis from complex lignocellulosic material easier. In order to verify this hypothesis, different lignocellulosic raw materials have been pre-treated by steam explosion. These materials were sugar beet pulp, corn straw and miscanthus. In order to check the effect of steam explosion pre-treatment on cellulose, a microcrystalline cellulose was also treated.

Steam explosion was performed at a vapor pressure of 18 bars and with a retention time of 2 minutes. The steam exploded lignocellulosic materials and the untreated one were submitted to a hydrolysis with a mixture of enzymes composed of cellulases and cellobiase activities during 24 hours. The quantification of glucose in the hydrolysates at different times was performed by HPAEC-PAD. Rate of cellulose converted into glucose were better with steam exploded raw material showing that steam explosion allows improvement of lignocellulosic material for enzymatic saccharification.

Acknowledgement: Authors are grateful for the financial support of the Walloon Region in the frame of the LIGNOFUEL Program (Convention n°716721)

COMPARATIVE CONSIDERATION OF THE PRETREATMENT METHODS USED IN BIOETHANOL PRODUCTION ON THE BASIS OF WHEAT STRAW AND MAIZE STALKS

P. Tsekova*, S. Petrin, I. Valchev, S. Nenkova, V. Blyahovski and G. Radeva

University of Chemical Technology and Metallurgy,
8, Kl Ohridski blv., 1756, Sofia, Bulgaria
e-mail: ivoval@uctm.edu

ABSTRACT

Industrial and agricultural lignocellulosic wastes are potential sources for the production of ethanol, amino acids, and other products of practical importance. Furthermore this available lignocellulosic biomass is inexpensive, renewable, and environmentally friendly.

The aim of the present work is to provide a comparative consideration of different pretreatment methods applied in case of bioethanol production on the ground of agricultural lignocellulosic raw materials. The latter are derived from wheat straw and maize stalks.

The pretreatment methods used included hydrothermal hydrolysis, dilute acid hydrolysis and steam explosion. Their application was followed by enzyme hydrolysis with (i) Novozymes AS cellulase enzyme complex NS 50013 combined with β-glucosidase NS 50010 and with (ii) the cellulase product Celluclast 1.5L mixed with Novozym 188 enzyme. HPLC and spectrophotometric analysis were carried out.

The time- and temperature-effect on the processes of hydrothermal and acid hydrolysis were followed. The results obtained provided to determine the optimal hydrolysis conditions. The subsequent experiments carried out showed that the total amount of reducing sugars was significantly greater in case of dilute acid hydrolysis followed by enzyme treatment when compared with that obtained in the other two cases. The effect observed was attributed to the fact that the hemicellulose sugars were better preserved. Furthermore, only traces of furfural and HMF were detected. This was not valid for the hydrothermal hydrolysis and steam explosion pretreatment. In the latter cases the furfural and hydroxymethylfurfural quantities were much greater.

It can be concluded that the dilute acid hydrolysis pretreatment has a definite advantage in case of bioethanol production on the ground of wheat straw and maize stalks.

COMPARING OF PRETREATMENT METHODS FOR BIOETHANOL PRODUCING FROM WHEAT STRAW AND MAIZE STALKS

P. Tsekova*, S. Petrin, I. Valchev, S. Nenkova V. Blyahovski and G. Radeva

University of Chemical Technology and Metallurgy,
8, Kl Ohridski blv., 1756, Sofia, Bulgaria
e-mail: ivoval@uctm.edu

Industrial and agricultural lignocellulosic wastes are potential sources for the production of ethanol, amino acids, and other useful products. Lignocellulosic biomass is inexpensive, renewable, widely available and environmentally friendly.

The aim of this work is to compare the different methods of pretreatment of agricultural lignocellulosic raw materials from wheat straw and maize stalks to producing of reducing sugars for bioethanol.

Hydrothermal hydrolysis, dilute acid hydrolysis and steam explosion are used as pretreatment methods. These treatments lead to separation of a large quantity of sugars, which could be used for producing ethanol.

After the pretreatment methods the enzyme hydrolysis was carried out. The enzyme treatment was performed with Novozymes AS cellulase enzyme complex NS 50013 in combination with β-glucosidase NS 50010 and with cellulase product Celluclast 1.5L mixed with the enzyme Novozym 188. The reducing substances were measured by HPLC and UV Spectrophotometric analysis.

It was studied temperature-time dependence on the process of hydrothermal hydrolysis and acid hydrolysis. The optimal hydrolysis conditions were determined. More sugars were obtained after the acid hydrolysis and were not detected any amount of furfural and HMF in compare to steam explosion and hydrothermal hydrolysis.

PAULOWNIA BIOMASS AS A PROMISING SOURCE OF REDUCING SUGARS

S. Petrin*, P. Tsekova, I. Valchev, S. Nenkova and G. Radeva

University of Chemical Technology and Metallurgy,
8, Kl Ohridski blv., 1756, Sofia, Bulgaria
e-mail: ivoval@uctm.edu

ABSTRACT

Biomass from plantations of fast growing tree species is suitable for processing to bioethanol. Paulownia is a deciduous tree capable of achieving very high growth rates under favourable conditions.

The aim of this work is to investigate the potential of paulownia biomass for the bioethanol production and to determinate the optimal pretreatment hydrolysis conditions of the process.

Pretreatment of lignocellulosic biomass in order to fractionate the lignocellulosic structure into its consituents is one of the key steps during the transformation of lignocellulosic biomass into value added products. The several pretreatment methods for investigations are used: dilute acid hydrolysis, hydrothermal hydrolysis (autohydrolysis) and steam explosion method. The enzyme hydrolysis of the paulownia biomass was carried out after the pretreatment method. The enzyme treatment was performed with Novozymes AS cellulase enzyme complex NS 50013 in combination with β-glucosidase NS 50010. The reducing substances were measured by HPLC and UV Spectrophotometric analysis.

The temperature and time dependencies on the hydrothermal and acid hydrolysis were investigated and the kinetics of the process was studied. The optimal hydrolysis conditions of the pretreatment methods were defined.

The amount of reducing sugars obtained after the acid hydrolysis is more compared to the resulting amount after the hydrothermal hydrolysis.

On the basis of the obtained experimental results an original technology will be developed that will allow the designing of an industrial plant for production of second generation bioethanol.

Improving enzymatic hydrolysis of alkali-extruded barley straw by varying the dosage and ratio of hemicellulases and cellulases

Zsolt Barta[a]*, Aleta Duque[b], José Miguel Oliva[b], Paloma Manzanares[b], Alberto González[b], Mercedes Ballesteros[b], Kati Réczey[a]

[a]Budapest University of Technology and Economics Department of Applied Biotechnology and Food Science Szt. Gellért tér 4., 1111 Budapest, Hungary

[b]CIEMAT

Renewable Energies Division Biofuels Unit

Avda. Complutense n° 22, 28040 Madrid, Spain

*corresponding author: zsolt_barta@mkt.bme.hu

The cellulose and hemicellulose fractions of lignocelluloses are not readily available for enzymatic hydrolysis. Therefore, the pretreatment of the material becomes inevitable prior to hydrolysis to open up the structure and to make the biomass more accessible for enzymes. Various pretreatment methods have been investigated, and each has advantages and disadvantages. Extruding the biomass at relatively low temperature is a promising alternative to other pretreatments requiring high energy input, since due to the low temperature degradation of carbohydrates and lignin does not occur. Extruder has the ability to provide high shear forces, rapid heat transfer and effective mixing. As it is a widely used method in the food and plastic industries, the scale up of the equipment is not a limiting factor.

The enzymatic digestibility of the material submitted to extrusion can be improved by adding catalyst in the extrusion process. In this study sodium hydroxide was used as catalyst at low dosage (6 g/100 g barley straw dry matter), which resulted in moderate digestibility of extruded barley straw. Our aim was to improve the conversion of carbohydrates in the enzymatic hydrolysis by varying the dosages and ratios of the hydrolytic enzymes.

Barley straw in a particle size of 5 mm was pretreated in a twin-screw extruder. The temperature was maintained at 68ºC, and the pretreated material was neutralized by adding phosphoric acid. Enzymatic hydrolysis of the alkali-extruded material was carried out in shake flasks at various dry matter contents using commercial enzyme preparations (Cellic CTec2 – cellulase complex, Cellic HTec2 – hemicellulase preparation, both from Novozymes A/S, Bagsværd, Denmark) at different dosages and ratios.

Acknowledgements: This work has been partially funded by the European Community Seventh Framework Programme under Grant agreement reference n° 227498 and the Regional Government of Madrid under the R&D Technology Programme/2009 (Project Ref. nº P2009/ENE-1743).

PECTINASE BOOSTED ENZYMATIC HYDROLYSIS OF STEAM AND ALKALI TREATED FRESH AND ENSILED HEMP MATERIAL.

Annukka Pakarinen*, Tuomas Brock, Liisa Viikari

University of Helsinki, Department of Food and Environmental Sciences, PO Box 27, FI-00014 University of Helsinki, Finland

*contact person e-mail: annukka.z.pakarinen@helsinki.fi

Many cellulose rich crops contain significant amounts of pectin. The knowledge of the effect of pectic substances, abundant in the cell wall and the middle lamella is limited but it can be assumed that the location and structure of pectin hinders the accessibility of lignocellulolytic enzymes. Therefore, the removal of pectin in pretreatments or the addition of pectinases in the hydrolysis might enhance the conversion of recalcitrant crops by changing the structure of the substrate.

In this study, the industrial fiber hemp was investigated as a potential green energy crop. Galacturonic acid amounts to almost 6% of the raw material, and comprises about 10% of total carbohydrates. The fresh untreated hemp was hydrolyzed poorly (45-55% from the theoretical carbohydrates) with commercial cellulases but increased after steam explosion and alkaline to 75% and 69% from the theoretical carbohydrates, respectively. It was noteworthy that especially the conversion of xylose was significantly enhanced after alkali treatment as compared to the fresh material. The hydrolysis of the fresh and pre-treated materials was further boosted with an additional dosage of pectinase. The hydrolysis yield of fresh hemp increased by 8% with pectinase addition and even more for acid ensiled material. After steam explosion or treatment with 1% NaOH at 120ºC part, or even all of the uronic acids were dissolved and therefore the effect of the pectinase was not obvious anymore. Optimizing the ensilage conditions, pretreatments and enrichment of the enzyme mixture with pectinases can significantly improve the conversion processes of pectin rich annual green crops.

The Impact of Pulp Properties on the Performance of Enzymatic Hydrolysis

M. Hundt1, K. Schnitzlein1,2

1Brandenburg University of Technology, Department of Chemical Reaction Engineering,

Burger Chaussee 2, 03044 Cottbus, Germany; e-mail: M.Hundt@rt.tu-cottbus.de

2NatureChem.EU, 95213 M¨unchberg, Germany

The hydrolysis of cellulose is one of the most important steps in lignocellulose based biorefinery. The splitting of polymer chains into glucose units can be catalyzed by acids as well as enzymes. Making the application of enzymatic hydrolysis profitable is still a big challenge for example because of cellulase costs and decreasing reaction rates at high conversions. However, entailing deciding advantages like lower utility and disposal costs, no corrosion issues or equipment and high selectivity it is the convenient process to provide monomeric sugars as precursors for subsequent conversions. It is well known that in addition to reactor characteristics, reaction conditions and enzymatic activity the substrate properties have a great impact on reaction rate and maximum conversion. Pulp properties are in turn greatly influenced by pulping conditions. As will be shown in this contribution the performance of enzymatic hydrolysis is strongly determined by the cellulose properties, e.g. kappa number, cellulose crystallinity, residual lignin content and separation processes (e.g. see Fig. 1).Hydrolysis experiments were carried out in closed 100 ml glas bottles placed in a tempered water bath. Ten reactions were run in parallel. Mixing was realized by a multipoint magnetic stirrer. Before adding the enzymes (either cellulase from Trichoderma reesei and cellobiase from Aspergillus niger, both Novozymes or cellulase mix from Penicillium janthinellum, University of Applied Science Senftenberg, Germany) the mixture of buffer and pulp was agitated and preheated to reaction temperature. In the course of reaction several samples are withdrawn to obtain the reaction kinetics. Sugar concentrations were determined via HPLC. At the end of reaction the solid residues were filtered off, rinsed with water, dried and weighted.

These investigations are part of a new lignocellulose based biorefinery concept. The biomass is fractionated by the AlkaPolP™ process developed by NatureChem. EU yielding the three main product fractions cellulose, hemicellulose and lignin [1]. The cellulosic pulp fraction was used as substrate for the enzymatic hydrolysis experiments. Due to the high flexibility of the pulping process reaction conditions can be varied over a wide range resulting in diverse pulp properties beeing decisive for its hydrolyzability. The target variables of the pulping process are not only the pulp characteristics but predominantly the conversion and enzyme consumption of subsequent hydrolysis. As a result of this feedback between downstream and pulping process respective cellulose properties can be obtained maximizing the glucose yield.

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Separation and characterization of products from the hydrolytic processing of *Pinus pinaster* wood

Sandra Rivas, María Jesús González-Muñoz*, Valentín Santos, Juan Carlos Parajó

Chemical Engineering Department, Polytechnical Building, University of Vigo (Campus Ourense) , As Lagoas, 32004 Ourense, Spain

INTRODUCTION

Hemicelluloses of *Pinus pinaster* wood are mainly made up of galactomannans and glucomannans, with minor amounts of arabinoglucuronoxylans, arabinogalactans and xyloglucans. Mannans have commercial interest, as they are used in a number of applications.

In this work, hemicelluloses from *Pinus pinaster* wood were selectively separated from cellulose and lignin by an aqueous treatment (autohydrolysis), which solubilizes hemicelluloses and leads to exhausted solids with enhanced contents of cellulose and lignin.

METHODS

Samples of *Pinus pinaster* wood were assayed for composition and subjected to aqueous processing using a pressurized, batch reactor, operating under optimized conditions. Hemicellulose-derived products were quantified by HPLC before and after quantative posthydrolysis. Oligosaccharides derived from hemicelluloses were purified using membrane technologies.

RESULTS

The liquors from hydrothermal processing contained oligosaccharides as major reaction products, together with monosaccharides in lower concentrations. Oligosaccharides, mainly made up of mannosyl units, presented a wide molecular weight distribution. By means of a sequence of diafiltration and concentration steps using ultra- and nano-filtration membranes, several fractions of oligosaccharides with a narrower molecular weight distribution were obtained. These fractions were subjected to furher analysis by HPLC, HPSEC and HPAEC to determine their compositions, molecular weight distribution and structure.

ACKNOWLEDGEMENTS

The authors are grateful to the Spanish Ministry of Science and Innovation for supporting this study, in the framework of the research Projects AGL2008-02072 and AGL2010-16525, which were partially funded by the FEDER Program of the European Union.

Effect of Different Temperatures on Ethanol Recovery using Pervaporation Unit

Imamoglu, Esra and Vardar-Sukan, Fazilet

Ege University, Faculty of Engineering, Department of Bioengineering, Izmir, 35100, Turkey

e-mail: esraimamoglu@yahoo.com

Pervaporation is one of the techniques employed in order to remove ethanol from the post-fermentation liquid. When used on an industrial scale, pervaporation appears to be a competitive approach in relation to traditional methods (Kujawski & Lewandowska, 2005). Ethanol fermentation was achieved by E. coli mutant from dilute acid hydrolysates of rice hulls. Cells were harvested by centrifugation (6,000 g, 5 min, 5°C) and pervaporation (Lab Test Cell Unit, Sulzer Chemtech) was used for the recovery of ethanol from fermentation broth. The feed was heated in a 2 L stainless steel tank and circulated over the membrane by a centrifugal pump with a feed flow rate of 100 L/h. Every 60 min, permeate was collected in a glass trap cooled in a Dewar flask containing liquid nitrogen. The vacuum level was maintained below 8 mbar by a two-stage vacuum pump. Flat sheet membrane with an active membrane area of 172 cm^2 was used. The experiments were performed at three different temperatures; 60, 65 and 70 °C for 10 h of pervaporation period each. In this study, the ethanol concentration was increased from 1.95% to 4.71% m/v with the highest membrane flux of 2.897 $kgm^{-2}h^{-1}$ at the feed temperature of 70 °C.

Acknowledgements We wish to thank The Scientific and Technical Research Council of Turkey (TUBITAK) for the financial support.

BIOETHANOL PRODUCTION FROM MIXTURE OF VEGETABLE STALKS

Yalçın Çöpür, Ayhan Tozluoglu**Errore. Il segnalibro non è definito.** and Ömer Özyürek

Abstract

Turkey, producing almost 26 million tons of vegetables is the third biggest vegetable producer. Vegetable stalks with no economic value, burnt or left in the field after harvest creates environmental pollution. The aim of this study was to examine the use of the mixture of tomato, pepper and eggplant stalks as renewable resources for bio-ethanol production. Integrated steam explosion/dry-milling and chemical pretreatment to stalks were applied before enzymatic hydrolysis. Stalks were steam exploded at 198-200ºC at 15psi for 5 minutes and dry milled. Subsequently, each was pretreated with chemicals at a solid loading of 10% (w/v) of sodium borohydrate ($NaBH_4$), sodium hydroxide (NaOH), and sulfuric acid (H_2SO_4). The concentration was of 2% (w/v) and treatment temperatures of 90ºC and at 15 psi were examined for residence time of 30 and 90min. for each sample to evaluate comparative performance data. Results showed that the after enzymatic hydrolysis of Celluclast (50 FPU/ml) and Novozym 188 (6360 nkat/ml) mixture, the highest theoretical sugar yields of 69.3% and 79.0% was observed when the samples were steam exploded and milled then treated with $NaBH_4$ for 30 and 90min., respectively. Mixture of vegetable stalks had high enough fermentable sugars and is suitable for subsequent fermentation process to produce ethanol.

[1] Düzce Üniversitesi Orman Fakültesi, Orman Endüstri Mühendisliği Bölümü, Konuralp Yerleşkesi, 81000, Düzce, Türkiye
[1]Corresponding author: Tel: +90 380 5421137
Email: yalcincopur@duzce.edu.tr

COMPARING OF PRETREATMENT METHODS FOR BIOETHANOL PRODUCING FROM WHEAT STRAW AND MAIZE STALKS

P. Tsekova*, S. Petrin, I. Valchev, S. Nenkova V. Blyahovski and G. Radeva

University of Chemical Technology and Metallurgy,
8, Kl Ohridski blv., 1756, Sofia, Bulgaria
e-mail: ivoval@uctm.edu

ABSTRACT

Industrial and agricultural lignocellulosic wastes are potential sources for the production of ethanol, amino acids, and other useful products. Lignocellulosic biomass is inexpensive, renewable, widely available and environmentally friendly.

The aim of this work is to compare the different methods of pretreatment of agricultural lignocellulosic raw materials from wheat straw and maize stalks to producing of reducing sugars for bioethanol.

Hydrothermal hydrolysis, dilute acid hydrolysis and steam explosion are used as pretreatment methods. These treatments lead to separation of a large quantity of sugars, which could be used for producing ethanol.

After the pretreatment methods the enzyme hydrolysis was carried out. The enzyme treatment was performed with Novozymes AS cellulase enzyme complex NS 50013 in combination with β-glucosidase NS 50010 and with cellulase product Celluclast 1.5L mixed with the enzyme Novozym 188. The reducing substances were measured by HPLC and UV Spectrophotometric analysis.

It was studied temperature-time dependence on the process of hydrothermal hydrolysis and acid hydrolysis. The optimal hydrolysis conditions were determined. More sugars were obtained after the acid hydrolysis and were not detected any amount of furfural and HMF in compare to steam explosion and hydrothermal hydrolysis.

Impact of acid and alkali pretreatment of Miscanthus *giganteus* on cell-wall lignin and hydroxycinnamic acids in relation to degradation by polysaccharide-active enzymes

BELMOKHTAR N.[1.2.], HABRANT A[1], LOPES-FERREIRA N.[2], CHABBERT B.[1]

[1]UMR FARE, INRA-URCA, BP 224, 51686 Reims cedex France

[2]IFP Energies nouvelles, 1&4 avenue du Bois Préau, 92500, Rueil-Malmaison France

Corresponding author: Nassim.Belmokhtar@reims.inra.fr

Miscanthus giganteus is perennial rhizomatous and herbaceous energy crop that gives rise to great biomass yields. However, efficient enzymatic saccharification of cellulose is hindered by many physicochemical, structural, and compositional features. Grass cell walls are characterized by the presence of hydroxycinnamic that play a significant role in cross-linking wall polymers into a cohesive network. Notably, ferulic acid is reported to cross-link hemicellulose and lignin whereas *p*-coumaric acid mostly esterifies lignin. Generally speaking lignin and phenolic acids are known to hamper bioconversion of polysaccharides. Pretreatments are thus required to overcome the recalcitrance of this lignocellulosic network.

In the scope of a better identification of the main limiting factors to saccharification we have compared the effect of dilute acid and ammonium hydroxide pretreatments on the efficiency of a cellulase cocktail produced by *Trichoderma reesi* on small fragments isolated from miscanthus. To this end, the cell wall phenolic component was addressed at the cell level.

Following a 144h enzyme incubation of untreated plant specimen, no alteration of the tissues was evidenced in contrast to acid or alkali pretreated samples. Detailed investigation of lignin and phenolic acids distribution in individual secondary cell walls of epidermic and vascular sclerenchyma, vessels and parenchyma was assessed using UV micro spectrophotometry Comparing UV absorbance spectra between untreated and pretreated substrates shows a reduction in lignin and phenolic components in all tissues except in parenchyma where phenolic acids were reduced. The heterogeneity of phenolic composition according to the cell types may thus impacts on the efficiency of pretreatments aiming at improving lignocellulose saccharification.

ENHANCEMENT OF THE ENZYMATIC HYDROLYSIS OF SUGARCANE BAGASSE BY GLYCEROL PRETREATMENT

C. Martín[1,3], J. Puls[1], B. Saake[2] and A. Schreiber[2]

The speaker's name in bold type followed by other co-authors

[1]vTI-Institute of Wood Technology and Wood Biology, Hamburg, Germany

[2]Hamburg University, Wood Science Centre, Chemical Wood Technology, Hamburg, Germany

[3]*University of Matanzas, Matanzas, Cuba*

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ugarcane bagasse in order to activate cellulose towards enzymatic hydrolysis. Three different glycerol concentrations (40, 60 and 80% (w/w)) and four different pretreatment times (1, 2, 3 and 4 h) were investigated. The temperature was 190^{o}C, and the liquid-to-solid ratio was 10:1. For all the conditions, the pretreatment improved the enzymatic hydrolysis of cellulose. The best activation of the fibres was achieved in the H_2SO_4-assisted pretreatment, which allowed the enzymatic hydrolysis of nearly 100% of the cellulose contained in the pretreated material. However, the highest overall cellulose convertibilities (85-94%), based on the cellulose contained in the raw bagasse, were achieved in the pretreatments without chemicals. The convertibility decreased with time for the acid-assisted pretreatment, and increased slightly for the other ones. Partial hydrolysis of the xylan remaining in the pretreated fibres was also observed.

PROCESSING OF ARTISAN RICE HULLS BY COMBINING DILUTE-ACID HYDROLYSIS, ALKALINE DELIGNIFICATION, NMMO TREATMENT AND ENZYMATIC HYDROLYSIS

Y. López[1,2], K. Karimi[2,3], M.J. Taherzadeh[2] and **C. Martín**[1,4]

The speaker's name in bold type followed by other co-authors

[1]*University of Matanzas, Matanzas, Cuba*
[2]*University of Borås, Borås, Sweden*

[3]*Isfahan University of Technology, Isfahan, Iran*

[4]*vTI-Institute of Wood Technology and Wood Biology, Hamburg, Germany*

Because of their particular composition, artisan rice hulls, which are produced in small-scale mills that are common in rural areas in Cuba, are a promising raw material for ethanol production. In addition to cellulose and hemicelluloses, artisan rice hulls contain up to 15% (DM) starch, which can be hydrolysed and converted to ethanol. However, starch is a challenge to the hydrolytic process, since the glucose resulting from its hydrolysis is susceptible to be destroyed under standard pretreatment conditions. In the current work, the optimization of dilute-acid hydrolysis of starch was performed using a central composite experimental design. The investigated factors were temperature (70 – 150ºC), biomass load (5 – 15 %), acid concentration (0 – 2%) and reaction time (30 – 90 min). The maximum hydrolytic conversion (97.02%) was reached at 125ºC, 1.36% (v/v) sulphuric acid, 8.04% of solids, and during 74 min of reaction time. The resulting cellulignin was either delignified with NaOH or submitted to treatment with N-methylmorpholine-N-oxide (NMMO), and then hydrolysed with commercial cellulases. The highest enzymatic convertibility (84.6%) was achieved in the NMMO-treated cellulignin. On the other hand, direct NMMO pretreatment was not effective for improving the enzymatic hydrolysability of artisan rice hulls.