BUDAPEST UNIVERSITY OF TECHNOLOGY AND ECONOMICS
FACULTY OF CHEMICAL TECHNOLOGY AND BIOTECHNOLOGY
OLÁH GYÖRGY DOCTORAL SCHOOL

EXPERIMENTAL AND TECHNO-ECONOMIC APPROACHES IN IMPROVEMENT OF THE LIGNOCELLULOSIC-ETHANOL PROCESS

THESIS BOOK

Zsolt Barta

Supervisor: Dr. Kati Réczey
Associate professor

Department of Applied Biotechnology and Food Science
Non-Food Research Group

2011

1 INTRODUCTION AND AIMS

Increasing awareness that fossil fuel resources are limited has prompted the research for alternative fuels. Ideally, alternative fuels should not only be ecologically sustainable, but also cheaper than fossil fuels. Ethanol is one of the leading candidates with respect to replacing some fraction of fossil fuels. It can be produced in large quantities from renewable raw materials at a relatively low cost. Its introduction to the fuel market is facilitated by the following features: ethanol can be handled and distributed similarly to petrol and diesel, it is completely miscible with petrol, and ethanol-petrol mixtures with ethanol content up to 20% can be used in current spark-ignited combustion engines without any major modification.

The majority of governments also support the replacement of conventional fossil fuels in the transportation sector with alternative ones by means of directives. The European Commission plans to progressively replace 20% (on energy basis) by 2020, and ethanol is expected to be one of the main means of achieving this goal.

According to maturity and raw materials of the biochemical processes first- and second-generation ethanol production technologies can be distinguished. For the former, sugar substances and grains serve as feedstocks and these technologies already exist on a large scale, while the latter utilises lignocellulosic materials and involve more complex technologies that have not yet been demonstrated on an industrial scale. Whereas sugar and starch-containing feedstocks are limited, relatively expensive and supply the food industry as well, lignocellulosic biomass is abundant, available at low cost and largely unused. However, currently the two major feedstocks used on a full scale are corn grain and sugar cane.

The general aim of the thesis was to add some novel knowledge to the lignocellulose-to-ethanol process by gathering information either by performing experiments regarding certain process steps or by constructing and evaluating techno-economic models of the whole process. Based on the two-way approach, the present work contains the results of the following studies:

1) Optimisation of steam pretreatment of hemp hurds
2) Integrated ethanol production from wheat straw and wheat meal
3) Modelling of on-site enzyme fermentation
4) Modelling of alternative stillage treatment and production of various co-products

2 BACKGROUND

Lignocelluloses are complex materials consisting of cellulose (linear glucose homopolymer), hemicellulose (branched sugar heteropolymer) and lignin (polymer of phenyl propane units).

The schematic overview of the enzymatic route of lignocellulosic ethanol production is illustrated in Figure 1. The raw material is pretreated and hydrolysed by cellulase and hemicellulase enzymes to monomer sugars that are fermented to ethanol by a microorganism, such as yeast. Both cellulase enzyme production and propagation of the fermenting organism can be carried out using the sugar-containing liquid fraction of the pretreated material. Ethanol is recovered by means of distillation, and various types of co-product can be produced, including pelletised solid fuel, biogas, electricity and district heat.

Pretreatment generally refers to the disruption of the resistant carbohydrate-lignin shield that limits the accessibility of enzymes to cellulose and hemicellulose. Steam pretreatment (formerly referred to as steam explosion) is one of the most widely investigated and used methods for pretreatment of lignocelluloses.

Cellulase enzymes can be purchased from enzyme producers or can be fermented on site. The former has the advantage of large-scale production, maintenance of latest technology, while the latter can provide with tailor-made solution, simplified logistics, quick and flexible supply. Moreover, on-site enzyme production eliminates costs of enzyme stabilisation, formulation and transportation and there are potential synergies (e.g. sharing utilities) between the enzyme fermenting step and the main, lignocellulose-to-ethanol process.

Enzymatic hydrolysis, where various hydrolytic enzymes such as cellobiohydrolases, endoglucanases, hemicellulases and β-glucosidases degrade cellulose and hemicellulose into monomer sugars, is generally the rate limiting step in the process.

The sugars released in the pretreatment and enzymatic hydrolysis are fermented to ethanol by yeast or bacteria. However, so far *Saccharomyces cerevisiae* has almost exclusively been employed in industrial ethanol production, since it has been robust enough to perform well under large-scale conditions. During pretreatment, at elevated temperature, various kinds of compounds form, such as sugar and lignin degradation products and organic acids, which inhibit the fermenting microorganism by affecting its metabolism in various ways, including extension of lag phase and reduction of growth rate, ethanol yield, specific ethanol productivity and cell viability.

Finally, ethanol is purified and concentrated to 96 v/v% by distillation. When water-free ethanol is required, an additional step is performed, e.g. azeotropic distillation, molecular sieve adsorption, pervaporation or other membrane-based operations.

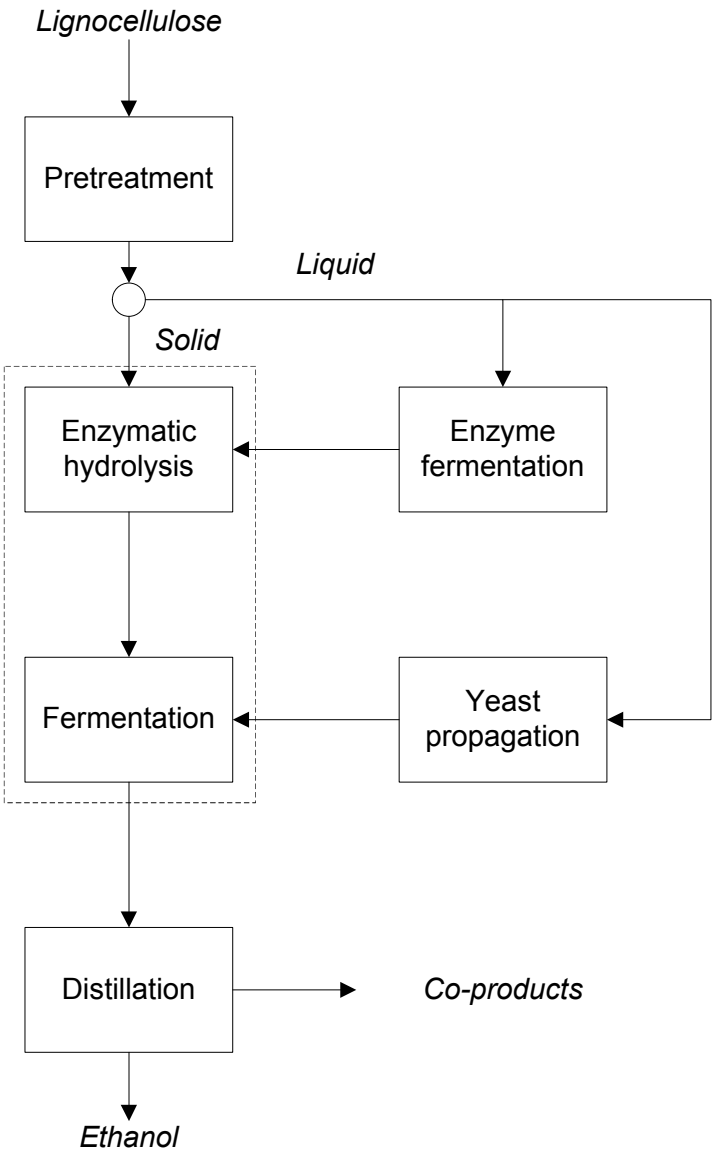

Figure 1 Enzymatic route of the lignocellulosic ethanol process.
The dashed rectangle represents simultaneous saccharification and fermentation (SSF).

3 MATERIALS AND METHODS

Raw materials
In the experimental work hemp hurds, wheat straw and wheat meal were used as polysaccharide containing raw materials. In the techno-economic studies spruce-based ethanol processes were investigated.

Steam pretreatment
At the Biomass Unit of CIEMAT (Madrid, Spain) five pretreatments of hemp hurds were performed for 10 min without impregnation at various temperature and reactor size. Steam pretreatment of wheat straw impregnated with sulphuric acid was carried out at 190°C for 10 min at the Department of Chemical Engineering, Lund University (Lund, Sweden).

Enzymes
α-Amylase (Termamyl SC) and amyloglucosidase (Spirizyme Fuel) amylolytic enzymes were used for starch liquefaction and saccharification, respectively. For hydrolysis of lignocelluloses, cellulase (Celluclast 1.5 L and NS50013) and β-glucosidase (Novozym 188 and NS50010) enzyme preparations were used. Each enzyme preparation was produced by Novozymes A/S (Bagsværd, Denmark).

Enzymatic hydrolysis
Enzymatic hydrolysis of the washed solid fraction of hemp hurds was carried out in shake flasks in a reaction volume of 25 mL at a water-insoluble solid (WIS) content of 5% at 50°C, pH 5.0 and 150 rpm using NS50013 and NS50010 enzymes. Enzyme loading of NS50013 was 15 filter paper unit (FPU)/g WIS and the volumetric ratio of NS50010 to NS50013 was 0.1. Released sugars were analysed by high-performance liquid chromatography.

Ethanol fermentation
Non-sterile simultaneous saccharification and fermentation (SSF) of washed fibre fraction of pretreated hemp hurds was performed in shake flasks in a volume of 50 mL at a WIS content of 10% by applying commercial enzyme preparations (NS50013, NS50010) and hexose fermenting *Saccharomyces cerevisiae* at 32°C. Presaccharified wheat meal, slurry of steam pretreated wheat straw and various mixtures of these were used as substrates in SSF experiments carried out in 2-L laboratory fermentors at a total WIS content of 5%. Ethanol was measured either by gas chromatography or by high-performance liquid chromatography.

Methodology of techno-economic analysis
In the process model, mass and energy balances were solved using the commercial flowsheeting program Aspen Plus 2006.5 (Aspen Technology, Inc., Cambridge, MA). Aspen HX-Net 2006.5 was used to design a near-optimal heat exchanger network that was implemented in the process model in Aspen Plus. Fixed capital investment costs were estimated either with Aspen Icarus Process Evaluator 2006.5 based on costs for 2009 or from vendor quotation.

4 RESULTS AND DISCUSSION

Hemp hurds as potential raw material for ethanol production

Steam pretreatment of non-impregnated hemp hurds was investigated at two reactor scales (2 and 10-L) by varying the temperature from 200-230°C. Glucan recoveries were relatively high (> 82% of original content), while xylan recoveries ranged from 18-66% of original. Conversions of glucan and xylan in enzymatic hydrolysis varied between 62-83% and 46-96%, respectively, based on glucan and xylan contents of the substrate. Ethanol yields in simultaneous saccharification and fermentation ranged from 38-70%, based on glucan and mannan contents of the substrate. The highest overall glucose yield was 336 g/kg dry hurds (75% of theoretical) obtained at 210°C, whereas the maximum sugar yield (glucose + xylose), 414 g/kg dry hurds (63%), was achieved at 200°C. The highest ethanol yield, 141 g/kg dry hurds (60%), was obtained at 210°C.

Integration of cellulose- and starch-based ethanol production from wheat straw and wheat grain

Integration of ethanol production of first and second generation can facilitate the introduction of the latter technology. Simultaneous saccharification and fermentation of mixtures of steam-pretreated wheat straw (SPWS) and presaccharified wheat meal (PWM) were investigated. Both the ethanol concentration and the ethanol yield increased with increasing amounts of PWM in the mixture. The maximum ethanol yield (99% of theoretical) was obtained with a mixture of SPWS containing 2.5% water-insoluble solids (WIS) and PWM containing 2.5% WIS, resulting in an ethanol concentration of 56.5 g/L. This yield was higher than those obtained with SSF containing SPWS (68%) or PWM (91%) exclusively.

Techno-economic analysis of on-site enzyme production

On-site cellulase enzyme fermentation in a softwood-to-ethanol process, based on SO_2-catalysed steam pretreatment followed by simultaneous saccharification and fermentation, was investigated from a techno-economic aspect. The effect of varying the carbon source of enzyme fermentation, at constant protein and mycelium yields, was monitored through the whole process. Enzyme production step decreased the overall ethanol yield (270 L/dry tonne of raw material in the case of purchased enzymes) by 5-16 L/tonne. Capital cost was found to be the main cost contributor to enzyme fermentation, constituting to 60-78% of the enzyme production cost, which was in the range of 0.42-0.52 Swedish kronor (SEK)/L ethanol (1 € ≈ 10.5 SEK, 1 US$ ≈ 7.3 SEK, 1 SEK ≈ 30 Hungarian forint, in 2010). The lowest minimum ethanol selling prices (4.71 and 4.82 SEK/L) were obtained in those scenarios, where pretreated liquid fraction supplemented with molasses was used as carbon source. In some scenarios, on-site enzyme fermentation was found to be a feasible alternative. However, the feasibility of including enzyme production in the lignocellulosic ethanol process highly depends on the full-scale price of commercial cellulase enzyme preparation, which is still very uncertain.

Techno-economic evaluation of alternative stillage treatment

Replacing the energy-intensive evaporation of stillage by anaerobic digestion is one way of decreasing the energy demand of the lignocellulosic biomass to ethanol process. Various configurations of processing the stillage with anaerobic digestion into different combinations of co-products were compared in a spruce-to-ethanol process, based on SO_2-catalysed steam pretreatment followed by SSF (Table 1). In scenarios A only the liquid fraction of the stillage was subjected to anaerobic digestion, whereas in scenario B the whole stillage stream.

Table 1 Summary of the scenarios investigated

Case	Pellet	Biogas upgrading	Co-products	Burnt on-site
A1	Part of solid fr.	Yes	Biogas, pellet, electricity	Part of solid fraction
A2	Solid fraction	No	Pellet, electricity, heat	Biogas
A3	-	Yes	Biogas, electricity, heat	Part of solid fraction
A4	-	No	Electricity, heat	Solid fraction., biogas
B	-	Yes	Biogas, electricity, heat	Pressed anaerobic residue

The scenarios were evaluated in terms of energy efficiency and ethanol production cost versus the reference case of evaporation. Anaerobic digestion of the stillage showed significantly higher overall energy efficiency (87-92%), based on the lower heating values, than the reference case (81%). Although the amount of ethanol produced was the same in all scenarios, the production cost varied between 4.00 and 5.27 SEK/L (Figure 2).

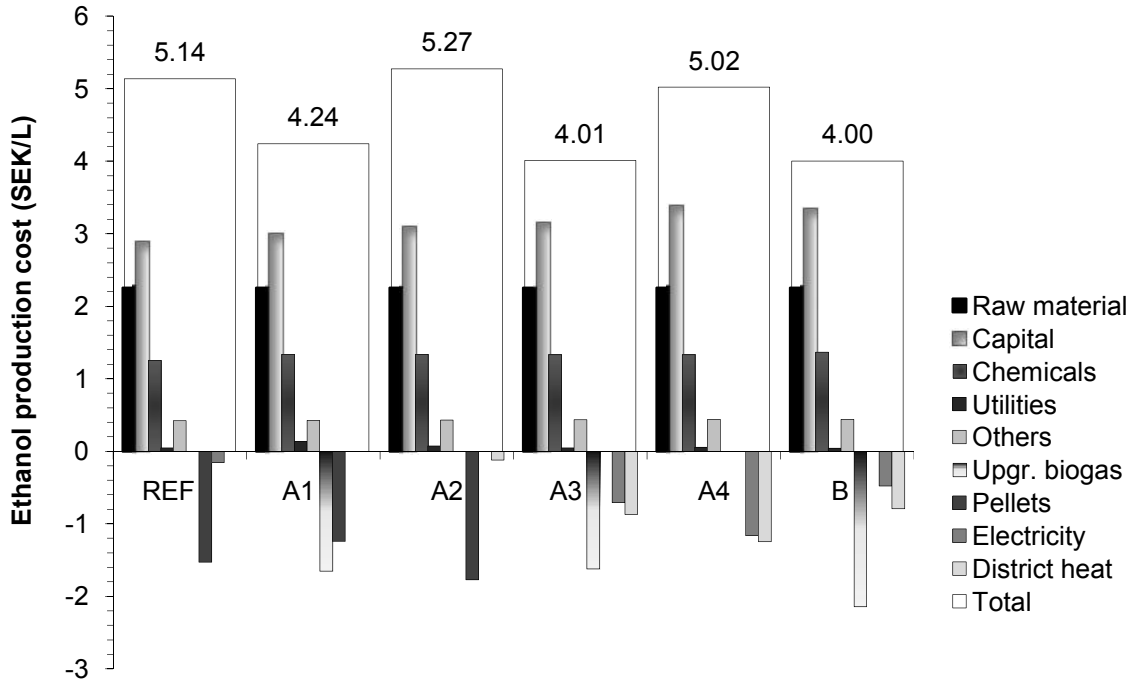

Figure 2 Breakdown of ethanol production cost in Swedish kronor per litre (SEK/L). Chemicals include enzymes. 'Others' refers to the cost of labour, insurance and maintenance. REF: reference case; Upgr: upgraded; 1 € ≈ 10 SEK.

5 NEW SCIENTIFIC FINDINGS

1. The optimal temperature of steam pretreatment (performed without catalyst addition, for 10 minutes, in a reactor volume of 10 L) of hemp hurds for releasing fermentable sugars in enzymatic hydrolysis and for subsequent ethanol fermentation, is 210°C in terms of overall glucose (336 g/kg dry hurds, 75% of theoretical) and ethanol yield (141 g/kg dry hurds, 60%). However, the maximum sugar yield (414 g/kg dry hurds, 63%), considering glucose and xylose, can be obtained at lower temperature (200°C) (Paper I).

2. Simultaneous saccharification and fermentation of a mixture containing equal amounts of presaccharified wheat meal and steam pretreated wheat straw (on a water-insoluble solids basis) results in higher ethanol yield (99% of theoretical) than the simultaneous saccharification and fermentation of presaccharified wheat meal alone (91%) (Paper II).

3. In the case of on-site enzyme production integrated in a lignocellulose-to-ethanol process, the productivity of cellulase fermentation affects the specific enzyme cost (related to unit ethanol produced). Providing the same enzyme fermentation configuration, the decrease of enzyme cost is determined by the extent of productivity increment and not by the origin of the increment (Paper III).

4. In enzyme fermentation pentose sugars are utilised together with hexose sugars. In the model of Paper IV, even including on-site enzyme production, the major part of pentose sugars (87%≤) is burnt or dried into solid fuel. The amount of hexose sugars consumed during enzyme fermentation is small (≤6% of the total amount present in the pretreated material).

5. The main contributor to specific enzyme cost is the capital investment cost. On-site enzyme fermentation contributes to 9-11% of the ethanol production cost (Paper IV).

6. Anaerobic digestion of the stillage decreases the heat duty of the process considerably, compared to the evaporation of the stillage (from 27.8 MW to 17.1-17.9 MW). Consequently, the energy efficiency increases (from 81% to 87-92%, based on lower heating values) (Paper V).

7. Anaerobic digestion of the stillage results in lower ethanol production cost than evaporation of the stillage in some cases. The favourable combinations for the co-products of the stillage treatment with anaerobic digestion are: (i) upgraded biogas and pellets, and (ii) upgraded biogas, electricity and district heat. These combinations remain favourable by changing the price of one co-product at a time between -40% and +40%. The difference in the production cost of ethanol between using the whole stillage or only the liquid fraction in anaerobic digestion is negligible for the combination of co-products including upgraded biogas, electricity and district heat (Paper V).

6 CONCLUSION, POTENTIAL APPLICATIONS

Ethanol, an important alternative fuel, can be produced from lignocelluloses in biochemical way, however, the conversion of carbohydrates into fermentable sugars and then into ethanol, i.e. the pretreatment, the enzymatic hydrolysis and the fermentation, still needs to be improved before commercialisation of the process. Regarding the steam pretreatment, besides achieving appropriate cellulose digestibility, high recovery of hemicellulose is also important. The low xylan recovery obtained at hemp hurds pretreatment can be improved by adding catalyst, or by performing two-step steam pretreatment. It must be verified, however, that the increased operating and capital costs, respectively, are compensated by the yield increment. Integrated simultaneous saccharification and fermentation of steam pretreated wheat straw and presaccharified wheat meal is an excellent alternative for obtaining industrially feasible ethanol concentration together with an ethanol yield close to the theoretical maximum. The ethanol concentration can be increased further by applying higher dry matter concentration, providing that the ethanol yield does not decrease, and by applying hexose and pentose co-fermenting microorganisms. The economics of a lignocellulose-to-ethanol process including on-site enzyme fermentation can be improved by increasing the productivity of enzyme fermentation. This can be achieved by strain development, by increased sugar concentration applying catabolite derepressed mutants, and by fed-batch fermentation technique. Anaerobic digestion of the stillage with biogas upgrading was proved to be an energy- and cost-effective way of stillage processing, therefore further research activity is advised in this field in the future.

7 PUBLICATIONS

Papers on which the thesis was based

I. **Barta, Zs.**, Oliva, J.M., Ballesteros, I., Dienes, D., Ballesteros, M., Réczey, K. (2010) Refining hemp hurds into fermentable sugars or ethanol. Chemical and Biochemical Engineering Quarterly. 24 (3), 331-339. IF: 0.35

II. Erdei, B., **Barta, Zs.**, Sipos, B., Réczey, K., Galbe, M., Zacchi, G. (2010) Ethanol production from mixtures of wheat straw and wheat meal. Biotechnology for Biofuels. 2010, **3:**16, doi:10.1186/1754-6834-3-16. IF: 4.12

III. **Barta, Zs.**, Sassner, P., Zacchi, G., Réczey, K. (2008) Techno-economic aspects of on-site cellulase production. Hungarian Journal of Industrial Chemistry Veszprém. 36 (1-2), 5-9.

IV. **Barta, Zs.**, Kovács, K., Réczey, K., Zacchi, G. (2010) Process design and economics of on-site cellulase production on various carbon sources in a softwood-based ethanol plant. Enzyme Research. 2010, 734182, doi:10.4061/2010/734182.

V. **Barta, Zs.**, Réczey, K., Zacchi, G. (2010) Techno-economic evaluation of stillage treatment with anaerobic digestion in a softwood-to-ethanol process. Biotechnology for Biofuels. 2010, 3:21 doi:10.1186/1754-6834-3-21. IF: 4.12

Oral presentations

Barta, Zs., Réczey, I., Zacchi G. What kind of co-products makes a lignocellulose based ethanol plant the most feasible? (Milyen melléktermékek esetén a leggazdaságosabb egy lignocellulóz alapú alkoholgyár?) *Forum of PhD Students*. Debrecen, November 4, 2010.

Barta, Zs., Dienes, D., Réczey, I. How can a sweet sorghum based small-scale ethanol plant be economically feasible? (Hogyan tehető gazdaságossá egy cukorcirok alapú alkoholgyártó kisüzem?) *LI. Georgikon Days*. Keszthely, October 1-2, 2009.

Barta, Zs., Oliva, J.M., Ballesteros, I., Dienes, D., Ballesteros, M., Réczey, I. Refining hemp hurds into fermentable sugars and ethanol (Kenderpozdorja feldolgozása fermentálható cukrokká és etanollá) *336. Scientific Colloquy (KÉKI).* Budapest, September 18, 2009.

Barta, Zs., Dienes, D., Réczey, K. Kinetic study on enzymatic cellulose hydrolysis of agricultural residues (Mezőgazdasági melléktermékeken végzett enzimes cellulózhidrolízis kinetikai leírása) *Polysaccharide-chemistry Workshop*. Budapest, September 29, 2008.

Barta, Zs., Sassner, P., Zacchi, G., Réczey, K. Techno-econimic analysis of enzyme production (Celluláztermelés technológiai és gazdasági elemzése) *Technical Chemical Days'08*. Veszprém, April 22-24, 2008.

Barta, Zs. Ethanol production from lignocelluloses – Pretreatment, fermentation and modelling the downstream process in Aspen Plus (Etanol előállítás lignocellulóz

szénforrásból - Előkezelés, fermentáció és a downstream műveletek modellezése Aspen Plus-szal) *Polysaccharide-chemistry Workshop*. Budapest, November 7, 2007.

Poster presentations

Barta, Zs., Réczey, K., Zacchi, G. Improving the energy efficiency and economics of a softwood-based ethanol process by producing biogas from the stillage. *The 4th Annual Workshop of COST FP0602, Biotechnical processing of lignocellulosic raw materials.* Cesme, Turkey, September 21-24, 2010.

Barta, Zs., Réczey, K., Zacchi, G. Techno-economic evaluation of biogas production from stillage in a spruce-to-ethanol process. *32nd Symposium on Biotechnology for Fuels and Chemicals*. Clearwater Beach, FL, USA, April 19-22, 2010.

Gyalai-Korpos, M., Mangel, R., **Barta, Zs.**, Alvira, P., Dienes, D., Ballesteros, M., Réczey, K. Cellulase production using different streams of wheat grain and wheat straw based ethanol processes. *32nd Symposium on Biotechnology for Fuels and Chemicals.* Clearwater Beach, FL, USA, April 19-22, 2010.

Barta, Zs., Oliva, J.M., Ballesteros, I., Dienes, D., Ballesteros, M., Réczey, K. Steam pretreatment of hemp hurds for bioethanol production. *Third European Workshop on Biotechnology for Lignocellulose Biorefinerie*s. Varenna, Italy, September 3-4, 2009.

Barta, Zs., Deák, A., Sipos, B., Réczey, K. Modelling a sweet sorghum processing bioethanol plant – whole crop utilization. *Second European Workshop on Biotechnology for Lignocellulose Biorefinerie*s. Biel, Switzerland, December 4-5, 2008.

Sipos, B., **Barta, Zs.**, Gyalai-Korpos, M, Sassner, P., Réczey, K. Can sweet sorghum be a feasible raw material for ethanol production in Hungary? *16th European Biomass Conference & Exhibition*. Valencia, Spain, June 2-6, 2008.

Gyalai-Korpos, M., **Barta, Zs.**, Sipos, B., Réczey, K. Looking for feedstock – bioethanol potential in Hungary. *16th European Biomass Conference & Exhibition*. Valencia, Spain, June 2-6, 2008.

Barta, Zs., Dienes, D., Réczey, K. Kinetic modelling of enzymatic hydrolysis of agricultural residues. *Era-chemistry workshop*. Krakow, Poland, April 13-16, 2008.

Barta, Zs., Dienes, D., Réczey, K. Kinetic modelling of enzymatic hydrolysis of agricultural residues. *First European Workshop on Biotechnology for Lignocellulose Biorefinerie*s. Copenhagen, Denmark, March 27-28, 2008.

Conference proceedings

Barta, Zs., Dienes, D., Réczey, I. (2009) How can a sweet sorghum based small-scale ethanol plant be economically feasible? (Hogyan tehető gazdaságossá egy cukorcirok alapú alkoholgyártó kisüzem?) Conference Proceedings of LI. Georgikon Days (ISBN: 978-963-9639-35-5) 73-82.

Barta, Zs., Sassner, P., Zacchi, G., Réczey, K. (2008) Techno-econimic analysis of enzyme production (Celluláztermelés technológiai és gazdasági elemzése) Conference Proceedings of Technical Chemical Days ’08 (ISBN 978-963-9696-36-5), 31-36.