FINAL REPORT OF THE NKFIH PROJECT 108389 ENTITLED "INVESTIGATION OF PLANT-BIOMASS-BASED BIOREFINERY PROCESSES"

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1A. Fractionation with ionic liquids

In the ionic liquid pretreatment experiments Avicel PH-101, which is a pure cellulose of a particle size 50 micron, was dissolved in 1-butyl-3-methylimidazolium acetate. After complete dissolution distilled water was added as anti-solvent to precipitate the cellulose. However, we could not separate the cellulose from the ionic liquid entirely, even if large amount of hot washing water was added. According to the performed enzyme activity measurement the ionic liquid residues trapped on the surface of cellulose particles strongly inhibited the action of cellulolytic enzymes. Hence, in the hydrolysis of ionic liquid pretreated cellulose powder low cellulose conversion was obtained. Using ethanol as washing liquid resulted in increased ionic liquid removal from the cellulose, however, the hydrolysis efficiency (cellulose conversion) was still low. In general, the cellulose conversion was only slightly higher than in the case of the untreated cellulose powder.

As the ionic liquid experiments did not provide publishable results in the first and second years of the project, in the third year selective arabinose separation was thoroughly investigated in Task 1A using corn stover. This change was approved by the NKFIH. In this study hydrochloric acid resulted in the highest, 88.8% xylose yield of theoretical under the condition of 2% (w/w) hydrochloric acid concentration, 40 minute reaction time, 10% (w/w) dry matter, at 120°C. Sulfuric acid experiments resulted in 81.9% xylose yield of theoretical by using 1.5% (w/w) sulfuric acid, 60 minute reaction time at 140°C, 7% (w/w) dry matter. Acidic hydrolysis at low dry matter content resulted in relatively low sugar concentrations. To concentrate xylose in the hydrolyzate, corn stover was hydrolyzed with a recycled hydrolysis solution. Hydrolyzate recycling concentrated xylose to three-times, while the recycling does not decrease the xylose yields. Corn stover pre-hydrolysis with sulfuric acid (<0.3%, w/w) is appropriate for the arabinose removal. However, the following xylose hydrolysis does not result in higher xylose purity in the solution. We showed that the pseudo first-order and biphasic kinetic models of the acidic hydrolysis could be based on total sugar concentrations.

1B. Fractionation of corn fibre

The composition of corn fibre obtained from Hungrana Ltd. was determined as follows: starch 14.6%, cellulose 17.1%, hemicellulose 32.5%, lignin 7.5%, ashes 0.8%, ethanol extractives 1.0%. As chemical pretreatment method ammonia steeping of destarched corn fibre (soaking in aqueous ammonia – SAA) was performed at 10% dry matter content in closed glass-flasks using 15% (w/w) ammonia solution for 6 or 24 h at 55 ∘C in a rotary shaker (175 rpm). The enzyme activity

measurements showed that Hemicellulase NS22002 (Novozymes) had a relative xylanase activity of 1% (of measured highest) and 9%, and a relative arabinoxylan-arabinofuranohydrolase (AX-AFH) activity of 71% and 100% at pH 3 and 4, respectively. At pH 6 its relative xylanase and AX-AFH activities were 93% and 52%, respectively. Hydrolyses of SAA pretreated destarched corn fibre with Hemicellulase NS22002 at pH 4 and 6 resulted in a large amount of hemicellulosic oligomers, considerable amount of monomer arabinose and negligible amount of monomer xylose and galactose in the supernatants. During the hydrolysis at pH 3 monomer sugars were not released and low amounts of hemicellulosic oligomers were solubilized. SAA pretreatment was found to be an appropriate method to make the structure of destarched corn fibre accessible for hemicellulose-degrading enzymes. Hemicellulase NS22002 had high endo-xylanase activity over a broad pH range, and its alpha-L-arabinofuranosidase could release arabinose from solubilized hemicellulosic oligomers derived from corn fibre. Enzymatic hydrolysis of SAA pretreated destarched corn fibre using Hemicellulase NS22002 was suitable to solubilize the hemicellulose fraction, however it could not selectively release arabinose monomers.

Model equations were developed to predict the yields of monomer and total sugars and to evaluate the selectivity of hydrolysis under the conditions investigated during the acidic treatments of raw corn fibre and destarched ground corn fibre. At appropriately mild conditions moderate arabinose yields were obtained with good hydrolysis selectivity, however at high arabinose yields significant amounts of other sugars were also solubilized. During most of the mild acid hydrolysis experiments the amount of oligosaccharides were comparable with the amount of monosaccharides. The major part of the arabinose was released in monomeric form – except when using ammonia-pretreated corn fibre – when other hemicellulosic sugars were mostly present in oligosaccharides. The effects of acid concentration and reaction time on the sugar yields and other hemicellulosic sugars/arabinose values were evaluated by statistical analysis, and model equations were established. Under the reaction conditions investigated in the case of destarched ground corn fibre, the sugar yields increased linearly as a function of reaction time. However, acid concentration had the main effect on sugar yields. Up to a limit the sugar yields depended linearly on the acid concentration, however, above that they changed according to a quadric function of acid concentration with declining slope of the curve. The limits of sugar yield were found to be different for arabinose and other hemicellulosic sugars. Both the monomer and total arabinose yields changed linearly until around 30%, while the monomer and total other hemicellulosic sugars yields increased linearly until around 60%. In the case of acid hydrolysis of raw corn fibre similar trends were obtained except that increasing the acid concentration increased the other hemicellulosic sugars yields as a second-order function with increasing slope under the conditions examined. Glucose was released in almost all acidic treatments of destarched ground corn fibre implying the existence of a non-starch glucan fraction, which could be easily hydrolyzed by dilute acid treatments.

2. Xylitol fermentation

During the fermentative production of xylitol the following environmental parameters have a controlling effect on the xylitol yield: concentration of monosaccharides, temperature, aeration and pH. The purpose of our first study was to evaluate xylitol production by four yeast strains at different pH values and oxygen transfer rates (OTRs). In shake-flask experiments the highest xylitol yields were obtained under the following conditions: Candida parapsilosis: pH 5.0, OTR 6.1 mmol L^{-1} h^{-1}; Candida guilliermondii: pH 4.5, OTR 5.7 mmol L^{-1} h^{-1}; Candida boidinii: pH 6.0, OTR 5.7 mmol L^{-1} h^{-1}; Hansenula anomala: pH 4.5, OTR 2.8 mmol L^{-1} h^{-1} using 50 g L^{-1} initial xylose concentration in semi-defined medium.

Biopurification of hemicellulosic hydrolysate is an interesting and inexpensive strategy to produce pure arabinose solution through the depletion of other sugars (e.g. glucose, xylose, galactose) beside arabinose using the adequate microorganism. Hemicellulosic hydrolysate is also a promising raw material for microbial xylitol production, that is becoming more attractive, since the downstream processing is expected to be cheaper than that of the chemical route and mild reaction conditions are required.

Two-step acidic fractionation of corn fibre was developed to produce a glucose- and arabinose-rich hydrolysate and a xylose-rich hydrolysate. An integrated process of arabinose biopurification on the glucose- and arabinose-rich hydrolysate and xylitol fermentation on the xylose-rich hydrolysate using C. boidinii was introduced, in which cell mass produced in arabinose biopurification was used as inoculum in the xylitol fermentation. Aerobic biopurification resulted in an arabinose solution containing 9.2 g L^{-1} of arabinose with a purity of 90 %, based on total sugars. Xylitol fermentation under microaerobic conditions resulted in a xylitol yield of 53 % of theoretical and a xylitol concentration of 10.4 g L^{-1} in three days. Hence, an integrated biorefinery process of hemicellulosic hydrolysates was developed based on the diverse action of C. boidinii to purify arabinose and produce xylitol.

Ogataea zsoltii was also investigated in shake-flask experiments in terms of xylitol fermentation on semi-defined medium and arabinose biopurification on hemicellulosic hydrolysate derived from acidic fractionation of corn fibre. O. zsoltii was found to be promising microorganisms for arabinose biopurification under aerobic conditions and for xylitol fermentation under micro-aerobic conditions. Xylitol fermentation on semi-defined medium using O. zsoltii resulted in a xylitol yield of 68% of theoretical and a xylitol volumetric productivity of 0.2 g/(L*h). Biopurification of the hemicellulosic hydrolysate of corn fibre resulted in an arabinose solution with arabinose purity of 90% regarding total sugars.

Fermentations were also carried out in a pilot fermentor to study the correlation between xylitol yield and composition of carbon source, and C. boidinii was used as fermenting microorganisms. The experiments were carried out on semi-defined medium and hydrolysate of corn fibre. The fermentation could be divided into: a) the growth of yeast cells, and b) the conversion of xylose into xylitol. During the first phase aerobic conditions (2 VVM - volume of air/volume of fermentation broth/minute, 400 rpm) were applied to obtain 4-6 g/l cell concentration after 6-7 hours. The length of the second phase depended on the obtained cell mass concentration, and the remaining concentration of xylose. At this stage aeration and stirring were reduced to 0.8 VVM and 150 rpm, respectively, to ensure microaerobic conditions. During both phases pH 5.5 and 30°C were maintained. Xylose consumption and formation of xylitol and by-products were monitored in the process. In the case of semi-defined medium a yield of 0.90 g xylitol/g xylose consumed and a productivity of 0.18 g xylitol/(L*h) were achieved, while in the case of hydrolysate of corn fibre a yield of 0.55 g xylitol/g xylose consumed and a productivity of 0.18 g xylitol/(L*h) were obtained. The results showed that the inhibitory components of the corn fibre hydrolysate decreased the xylitol yield significantly, however the productivity was not affected.

3. Kinetic study of enzymatic hydrolysis

A classification scheme for quantitative models of enzymatic hydrolysis of cellulose is proposed by Zhang and Lynd (Biotechnology and Bioengineering, 88, 7, 797-824, 2004). They use the term 1) nonmechanistic models for models based on data correlation without an explicit calculation of adsorbed cellulase concentration. Models featuring an adsorption model, which are based on

concentration as the only variable describing the state of the substrate and/or are based on a single cellulose hydrolyzing activity are termed 2) semimechanistic. Semimechanistic models can be useful in the context of including the minimal information necessary for descriptive purposes. Models featuring an adsorption model, substrate state variables in addition to concentration, and multiple enzyme activities are denoted 3) functionally based models. Functionally based models are particularly useful for developing and understanding at the level of substrate features and multiple enzyme activities, including identification of rate-limiting factors. Models based on structural features of cellulase components and their interaction with their substrates are termed 4) structurally based models. As our aim in the current project was to incorporate the fundamentals of enzymatic action, thermal effects and end-product inhibition, we gathered the nonmechanistic and semimechanistic models available in the literature, and validated these models using experimental data obtained in our laboratory on wheat straw and corn stover. The semimechanical model of Zhang et al. (Bioresource Technology, 101, 8261-8266, 2010) was found to be suitable to predict the glucose concentration during the hydrolysis of pretreated wheat straw and corn stover using three model parameters determined from binary nonlinear regression analysis of our experimental data.

The enzymatic hydrolysis of cellulose to glucose by enzymes is one of the major steps in the conversion of lignocellulosic biomass. In the experimental work our aim was to improve the efficiency of enzymatic hydrolysis of alkali-extruded wheat straw. The hydrolysis experiments were carried out in a working volume of 50 mL at constant temperature (50°C) and stirring (150 rpm) for 72 hours in 0.05 M acetate buffer solution (pH 5). Water-insoluble solid (WIS) concentrations were the following: 2.5, 5 and 7.5% in the case of washed solid fraction, and 3, 5 and 7% when the whole slurry was used. At each substrate concentration cellulase enzyme dosages of 2.8, 4.6 and 5.2 FPU (filter paper unit)/g WIS were applied. The concentrations of the sugars produced were quantified by high performance liquid chromatography (HPLC) analysis. These values were used for the kinetic modelling. In the developed model the independent variable was the hydrolysis time, the dependent variable was the glucose concentration, while the substrate concentration and the enzyme dosage were the inputs of the model. The model contained three constants, which were regressed using Berkeley Madonna and Matlab 7.1. The predicted values of the model were compared with the glucose concentrations measured by HPLC (samples at 0, 4, 8, 24, 48 and 72 h), and the adequacy of fit was found to be good.

4. Design of energy-efficient processes

The designed process of a corn-fibre-based biorefinery consisted of the following process steps: fractionation, enzymatic hydrolysis and ethanol fermentation, distillation and dehydration, anaerobic digestion, biogas upgrading, aerobic waste water treatment, combined heat and power production, xylitol fermentation and recovery. The ethanol production in the scenarios investigated was low. One reason of that was the step of separation and washing between the pretreatment and the hydrolysis. In the future, more experiments should be carried using the whole pretreated slurry in the hydrolysis. The dry matter content applied in the pretreatment step had a high impact on the heat duty of the whole process. It should be increased to the greatest extent possible, however, the sugar loss in the pretreatment caused by the increased dry matter content must be avoided. Beside the dry matter content of the pretreatment the dry matter content of the enzymatic hydrolysis was also important, since at higher dry matter content higher sugar concentrations might be obtained, which was inevitable to achieve high ethanol titre in the subsequent fermentation. If the enzymatic hydrolysis and ethanol fermentation were carried out in a simultaneous way (simultaneous saccharification and fermentation), at higher dry matter content higher ethanol titre might be obtained. Increasing the ethanol concentration decreased the heat demand of the distillation.

Based on the results of Aspen sensitivity analysis, experiments of increased dry matter concentration were designed and performed in the case of corn fibre, however, the yields deceased significantly, which did not support the Aspen model results. Meanwhile cooperation was started with the University of Florida, and they could achieve high ethanol yields using sugarcane bagasse, and their results were used in our Aspen models. A techno-economic analysis was conducted for a simplified lignocellulosic ethanol production process developed and proven by the University of Florida at laboratory, pilot, and demonstration scales. Data obtained from all three scales of development were used with Aspen Plus to create models for an experimentally-proven base-case and 5 hypothetical scenarios. The model input parameters that differed among the hypothetical scenarios were the time of liquefaction and simultaneous saccharification and fermentation, the enzyme loading, the enzymatic conversion, the solids loading, and the overall process yield. The minimum ethanol selling price (MESP) varied between 50.38 and 62.72 US cents/L. The feedstock and the capital cost were the main contributors to the production cost, comprising between 23-28% and 40-49% of the MESP, respectively. A sensitivity analysis showed that overall ethanol yield had the greatest effect on the MESP. These findings suggested that future efforts to increase the economic feasibility of a cellulosic ethanol process should focus on optimization for highest ethanol yield.

Cellulase enzymes contribute a significant share of the total costs as well as greenhouse gas emissions of lignocellulosic ethanol production today. A potential future alternative to purchasing enzymes from an off-site manufacturer is to integrate enzyme and ethanol production, using microorganisms and part of the lignocellulosic material as feedstock for enzymes. Together with our Swedish partners at Lund University, Sweden, we modelled and compared two integrated process designs for ethanol production from logging residues of spruce and off-site production of enzymes. Greenhouse gas emissions and primary energy balances were studied in a life cycle assessment, and cost performance in a techno-economic analysis. Greenhouse gas emissions per MJ of ethanol were approximately 50 % lower in the integrated cases than in the off-site case, though the difference between them is reduced with alternative assumptions regarding enzyme dosage and the environmental impact of the purchased enzymes. The comparison of primary energy balances did not show any significant difference between the off-site and the integrated cases. An integrated process design could significantly reduce greenhouse gas emissions from lignocellulose-based ethanol production, and the cost of an integrated process design is comparable to purchasing enzymes produced off-site. This study investigated the circumstances, under which an integrated process could be an environmentally and economically viable alternative for lignocellulosic ethanol production.

Biocarbon can be potentially utilized as a high quality fuel in small-scale heating applications, as charcoal, powder, briquettes or pellets. In this study, the main objectives were to assess the energy efficiency of the whole value chain for utilization of carbonized wood for small-scale biocarbon pellet based stoves and to evaluate the overall heat production cost of the whole value chain by a techno-economic approach. The carbonization temperature did not affect the stove thermal efficiency significantly. However, at higher carbonization temperatures higher biocarbon pellet production cost and higher overall heat production cost were obtained when standalone pellet production was considered. In the case of pellet and district heat coproduction, the pellet production cost was always lower than the corresponding one without district heat production.