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INVESTIGATION OF CORN FIBRE UTILISATION IN BIOREFINERY APPROACH

Thesis book

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INTRODUCTION AND AIMS

Lignocellulosic residues account for the majority of the total biomass present in the world with great potential as annually renewable, low cost resource of carbon-rich raw materials to replace fossil resources for production of chemicals and transportation fuels. The sustainable use of biomass requires integrated manufacturing, which has led to the development of the term biorefinery analogous to oil refinery. The biorefinery concept embraces a wide range of technologies that are able to separate biomass resources into their building blocks (carbohydrates, proteins, oils etc.) and convert those into a wild spectrum of marketable products and energy. In particular, the carbohydrate fraction of the lignocellulosic residues is expected to play the major role to produce bio-based chemicals, since it can be effectively hydrolysed to monosaccharides that can be converted into an array of value-added molecules via fermentations or chemical synthesis.

Corn fibre is an inexpensive by-product of the corn wet-milling process, and contributes to about 8–12% (w/w) of the grain dry matter. Corn fibre is utilised mainly as low-value animal feed or as solid fuel in pelletized form, however, due to its high carbohydrate content, it is a promising raw material for producing value-added products.

The work presented in this thesis aimed the investigation of complex utilisation of corn fibre in biorefinery approach. Bioethanol, biomethane, xylitol and arabinose were considered as possible products, however, particular attention was paid to the investigation of arabinose production. Process model based on literature data was constructed to simulate different process scenarios of a proposed biorefinery and evaluate them in technological point of view. Laboratory experiments were performed to examine novel biotechnological methods aiming the production of arabinose and xylitol from corn fibre. Detailed aims of the presented study are the following:

- Investigate various process configurations of a corn fibre-based biorefinery having the potential to produce bioethanol, biomethane, xylitol, electricity and heat in technological point of view.

- Investigate the possibility of selective arabinose solubilisation from destarched ground corn fibre and soaking in aqueous ammonia pretreated destarched ground corn fibre using commercial enzyme preparations.

- Investigate the production of monosaccharides and oligosaccharides during dilute acid treatments of destarched ground corn fibre and corn fibre to determine the most favourable conditions regarding selective arabinose production.

- Investigate arabinose biopurification and xylitol fermentation on semidefined medium and hemicellulosic hydrolysate derived from corn fibre.

BACKGROUND

Fractionating the biomass into its core constituents is one of the most important steps in a biorefinery, since it allows the effective utilisation of each component. All the biomass components should be valorised through a zero-waste approach and integrated process operation, in which the by-product of a process route serves as a raw material for another. Complex integration of the process steps in terms of their heat demand is also necessary to decrease the overall energy requirement of the biorefinery. An advanced biorefinery plant should aim at running in a self-sustaining way regarding the utilities, like steam and power.

Biorefining is a complex process, which includes several processing steps; for example pretreatments, hydrolyses, fermentations and purification steps. For designing cost-effective configurations of a biorefinery with improved techno-economic and environmental characteristics, it is crucial to understand the entire integrated biorefining process and how one stage of the process can impact the performance of the others. Process modelling provides powerful methods to analyse complex processes such as biorefining, evaluate the interactions between different process units, establish process routes of minimum energy consumption, determine the possible bottlenecks of the processes, hence to identify the directions for further investigation.

In the recent few decades major research effort has been conducted to develop energy-driven lignocellulosic feedstock biorefineries. The main purpose of these biorefineries is to recover the sugars, particularly focusing on glucose derived from cellulose, and subsequently convert them into bioethanol via fermentation. The residual lignin-rich fraction is usually incinerated in a combined heat and power production plant to generate heat and electricity for the biorefinery. Other organic residual streams of the process (e.g. hydrolysis residue derived from cellulose hydrolysis, stillage of the ethanol distillation) are generally subjected to anaerobic digestion to gain biogas or biomethane through an upgrading step.

Nevertheless, in the economically important graminaceous plants such as cereals and non-food crops, beside the cellulose, hemicellulose is also significant component that represents up to 35% of the dry matter. However, less attention has been accorded to hemicelluloses, notably arabinoxylans, which are highly abundant plant polysaccharides convertible into D-xylose and L-arabinose. Although the recent utilisation of these sugars is limited, their future use as platform intermediates is essential to ensure the sustainability of lignocellulosic biorefining and to avoid excessive non-food use of D-glucose. Likewise, less attention has been paid to the development of pentose-specific bioconversion processes despite the fact that the conversion of pentose sugars is not only economically necessary, but advantageous in terms of product diversification of a biorefinery. Hemicellulosic sugars can be easily converted via fermentation technologies into commodity chemicals such as ethanol, xylitol and lactic acid or other bio-based intermediates.

Hemicelluloses are heterogeneous polymers, which contain pentose sugars (D-xylose, L-arabinose), hexose sugars (D-mannose, D-glucose, D-galactose) and sugar acids. The hemicellulose forms an overlying layer through hydrogen bonding with the cellulose, and it is covalently linked with lignin. Hemicelluloses from different sources largely differ in composition. Corn fibre hemicellulose is considered as one of the most complex heteropolysaccharides in nature.

Corn fibre heteroxylan contains homopolimeric backbone chains of 1,4-linked *β*-D-xylopyranose units highly substituted with monomeric sidechains of *α*-L-arabinofuranose and acetic acid linked to *O*-2 and/or *O*-3 positions and *α*-D-glucopyranuronic acid, 4-*O*-methyl-*α*-D-glucopyranuronic acid linked to *O*-2 position. The homoxylan backbone is also substituted by oligomeric sidechains mainly containing *α*-L-arabinofuranose, *β*-D-xylopyranose, *α*-D-galactopyranose and different types of hydroxycinnamic acid linked to *O*-5 position of *α*-L-arabinofuranose moieties. The hemicellulose fraction of corn fibre contains around 48–54% xylose, 33–35% arabinose, 5–11% galactose, and 3–6% glucuronic acid. Hence, corn fibre hemicellulose is a promising raw material for xylitol and arabinose production.

On an industrial scale xylitol is produced through chemical reduction of D-xylose derived from hemicellulosic hydrolysate of birchwood or other xylose-rich materials. As an alternative method, microbial production of xylitol is becoming more attractive. Many studies have been conducted to produce xylitol from the hemicellulose portion of agro-residues like rice straw, corn cob, brewer's spent grain, sugarcane bagasse, corn stover, barley bran and corn fibre by fermentation.

On an industrial scale L-arabinose is produced from gum arabic by acid hydrolysis followed by multiple purification procedures. The relatively high cost of gum arabic and the expensive purification steps required result in the high cost of pure L-arabinose. Therefore, there is biotechnological and commercial interest in the development of new cost-effective methods for producing high purity grade L-arabinose from lignocellulosic residues rich in hemicellulose or pectin, for example from sugar beet pulp, corn hull, wheat bran and corn fibre. Different methods of alternative L-arabinose production have been investigated involving acidic and enzymatic hydrolyses and microbial biopurification.

Acid hydrolysis under mild conditions seems to be an appropriate method to selectively release a significant part of the arabinose from the hemicellulose of lignocellulosic residues. Nevertheless, restricted information is available in the literature about selective arabinose hydrolysis by mild acid treatments, especially in terms of the determination of all hydrolysis products including monomer and oligomer sugars.

The advantages of enzymatic hydrolysis methods are the mild reaction conditions applied, and the pure arabinose solution obtained regarding the solubilised monosaccharides. The drawbacks are, most of the published studies used purified arabinan and arabinoxylan as starting material and purified enzymes (*α*-L-arabinofuranosidase and endo-*α*-1,5-arabinanase) for the hydrolysis, that requires complex and expensive purification processes. Moreover, the recovery of arabinose from the hydrolysates can be challenging, if the starting materials are also soluble in water.

Biopurification of arabinose-rich hydrolysates is an interesting and inexpensive strategy to produce pure arabinose solution through the depletion of other sugars using the adequate microorganisms. It seems to be an effective method of arabinose production with the potential to implement on an industrial scale. Nevertheless, the main drawback of arabinose biopurification is wasting the other sugars convertible into value-added products. Utilisation of the cell mass obtained as by-product of the biopurification is also an issue to be solved.

MATERIALS AND METHODS

Simulation software

Various process configurations of the proposed biorefinery plant were designed and simulated by Aspen Plus v7.3 flow-sheeting software (Aspen Tech Inc, Cambridge, MA, USA). The data for the chemical components were obtained from the built-in databases of Aspen Plus, or from the databank of National Renewable Energy Laboratory (NREL, Golden, CO) on biomass components.

Raw material, enzymes and microorganisms

Corn fibre was donated by Hungrana Starch and Isosugar Manufacturing and Trading Co. Ltd. (Szabadegyháza, Hungary). Xylanase NS22083, Enzyme complex NS22119, Hemicellulase NS22002 and Cellic CTec2 enzyme cocktails were provided by Novozymes A/S (Bagsvaerd, Denmark). *Candida boidinii* NCAIM Y.01308, *Candida parapsilosis* NCAIM Y.01011, *Candida guilliermondii* NCAIM Y.01050, *Hansenula anomala* Y.01499 were purchased from the National Collection of Agricultural and Industrial Microorganisms (Budapest, Hungary).

Enzyme activity and enzymatic hydrolysis

Xylanase activity was assayed using birch wood xylan (Sigma) substrate at 50°C for 5 min. Reducing sugar content of the reaction mixture was measured by using 3,5-dinitrosalicylic acid reagent. Arabinoxylan-arabinofuranohydrolase (AX-AFH) activity was assayed at 50°C for 1 h using water-insoluble wheat arabinoxylan (Megazyme, Bray, Ireland) substrate. The supernatant was analysed to determine arabinose concentration by high-performance liquid chromatography. Enzymatic hydrolysis of destarched ground corn fibre (DGCF) and soaking in aqueous ammonia (SAA) pretreated DGCF were carried out at 3% (w/w) dry matter content using Hemicellulase NS22002 (0.02 g enzyme preparation/g dry matter) for 4 days at 50°C in a rotary shaker (175 rpm).

Sulphuric acid treatments

DGCF and corn fibre were suspended in appropriately diluted sulphuric acid solution at 3% (w/w) and 10 % (w/w) dry matter content, respectively. Treatments were carried out in water bath at 90°C or in an autoclave at 120°C and 140°C without agitation. The reaction times (5–75 min) and the sulphuric acid concentrations (0.25–5% (w/w)) were set according to experimental designs.

Biopurification

Biopurifications were carried out at 30°C in rotary shaker (220 rpm) for 3 or 4 days in 100-mL Erlenmeyer flasks containing 20 mL semidefined medium (10g/L yeast extract, 15g/L KH_2PO_4, 1g/L $MgSO_4 \times 7H_2O$, 3g/L $(NH_4)_2HPO_4$, 15g/L arabinose, 7.5g/L xylose and 7.5g/L galactose) or glucose- and arabinose-rich hydrolysate of corn fibre at pH 6.

Xylitol fermentation

Xylitol fermentations were performed on semidefined medium (10g/L yeast extract, 15g/L KH_2PO_4, 1g/L $MgSO_4 \times 7H_2O$, 3g/L $(NH_4)_2HPO_4$, and 30g/L or 70g/L xylose) or xylose-rich hydrolysate (clarified by activated carbon) of corn fibre at 30°C in rotary shaker in 100-mL Erlenmeyer flasks for 4 days at pH 6 using different volume of medium (25–65 mL) and rotation speed (125, 220 rpm).

RESULTS AND DISCUSSION

Process simulation of a corn fibre-based biorefinery

Two base cases of the proposed biorefinery (base case A and B) were modelled and within those base cases different process configurations were investigated. In the base case A, bioethanol, biomethane and in some cases district heat was produced and the scenarios were compared in terms of energy efficiency. Scenarios containing incineration of the hydrolysis residue had higher energy efficiency than the corresponding scenarios, in which the hydrolysis residue was subjected to anaerobic digestion. Incineration of the sludge significantly increased the energy efficiency of the biorefinery, because it enabled to obtain more biomethane. Implementation of flue gas condensation to produce district heat resulted in an additional increase in energy efficiency. The highest energy efficiency (73%) was achieved in the scenario containing flue gas condensation and incineration of both the hydrolysis residue and the sludge. The heat demand of the different process steps of the biorefinery was also investigated. Fractionation and ethanol distillation were found to be the main heat consuming parts of the proposed process. The products of the base case B were bioethanol, biomethane and crystalline xylitol, and the comparison of the scenarios was based on the mass flows of the products instead of the energy efficiency of the process, since xylitol was produced as a high value chemical and not as energy carrier. Division of the hemicellulose fraction between anaerobic digestion and xylitol fermentation was necessary to produce bioethanol, biomethane and xylitol simultaneously. When the half of the hemicellulose fraction was used for xylitol fermentation, the proposed biorefinery produced 4208 tonnes xylitol, 5599 tonnes biomethane and 15089 tonnes ethanol from 95000 tonnes of dry corn fibre, annually. The amounts of the produced biomethane and xylitol were varied by changing the rate of division of the hemicellulose fraction.

Enzymatic hydrolysis of corn fibre

The pH dependence of xylanase and AX-AFH activities of four commercial, multicomponent enzyme preparations (Xylanase NS22083, Enzyme complex NS22119, Hemicellulase NS22002 and Cellic CTec2) were determined within the range from 3 to 10 in order to investigate the possibility of selective arabinose release from complex polysaccharides. Hemicellulase NS22002 had the highest AX-AFH activity and low xylanase activities compared with the other enzyme preparations, and at the pH optimum of its AX-AFH activity (pH 4) it had relatively low xylanase activity, hence this enzyme preparation was selected to investigate selective arabinose solubilisation from destarched ground corn fibre. Soaking in aqueous ammonia pretreatment was found to be necessary to make the hemicellulose structure accessible for the hemicellulolytic enzymes. During the enzymatic hydrolyses at pH 4 and 6 using Hemicellulase NS22002, high amounts of hemicellulosic oligomers, considerable amount of monomer arabinose and negligible amounts of other monomer sugars were solubilised. Enzymatic hydrolysis at pH 6 resulted in the solubilisation of more than 80% of the hemicellulose fraction and only 13% of the cellulose content within 2 days. Therefore, enzymatic hydrolysis of corn fibre using Hemicellulase NS22002 is a promising method to hydrolyse the hemicellulose fraction under mild reaction conditions, but it is not suitable for selective arabinose release.

Sulphuric acid treatments of corn fibre

Arabinose moieties are more sensitive against acid catalysed hydrolysis compared to the xylose units building up the hemicellulose backbone. Hence, in order to selectively release arabinose acid hydrolysis of destarched ground corn fibre and corn fibre was investigated at different temperatures, acid concentrations and reaction times according to experimental

designs. Temperatures of 140°C and 120°C were found to be extremely high in terms of the selectivity of arabinose release, however, high arabinose yields were achieved. Acidic hydrolysis of destarched ground corn fibre at 5% (w/w) sulphuric acid concentration, 90°C and 5 min reaction time resulted in a total arabinose yield of 82.3% with sufficient selectivity, hence an arabinose-rich liquid fraction was produced. During the investigations of acidic treatments of non-ground corn fibre at 90°C, the previous destarching step was omitted. The most favourable hydrolysis condition was determined by desirability function optimisation. At 1.1% (w/w) sulphuric acid concentration and 51 min reaction time, a total arabinose yield of 75.9% was achieved and the starch fraction was completely solubilised. Acidic hydrolysis of corn fibre under these conditions was referred to as first-hydrolysis in the two-step acidic fractionation process. After the first hydrolysis, considerable amount of oligosaccharides was obtained in the supernatant, thus an oligomer hydrolysis step (120°C, 1.1% (w/w) sulphuric acid, 30 min) was required to recover the sugars in monomeric form, which enabled to produce a glucose- and arabinose-rich supernatant. The solid residue of the first hydrolysis was utilised in a second acidic hydrolysis (120°C, 1.1% (w/w) sulphuric acid, 30 min,10% (w/w) dry matter), which resulted in a xylose-rich supernatant and a cellulose-rich solid fraction.

Arabinose biopurification and xylitol fermentation on semidefined media

The capability of four yeast strains (*C. boidinii, C. guilliermondii, C. parapsilosis* and *H. anomala*) for arabinose biopurification was tested on semidefined medium containing xylose, arabinose and galactose under aerobic conditions. *C. guilliermondii, C. parapsilosis* and *H. anomala* utilised xylose, galactose and arabinose simultaneously, while *C. boidinii* did not consume the arabinose, even if the other carbon sources had been depleted. Biopurification of semidefined media using *C. boidinii* resulted in an arabinose solution with an arabinose purity of 97%.

Xylitol fermentations on semidefined media containing xylose as carbon source were performed by using *C. boidinii* in order to determine the most favourable conditions in terms of xylitol yield. The effects of oxygen transfer rate, initial cell density, initial xylose concentration and cofactor (methanol) addition were investigated in shake flask experiments. A xylitol yield of 58% of theoretical was achieved by using 5 g/L initial cell concentration, 30 g/L initial xylose concentration at an oxygen transfer rate of 2.8 mmol/(L×h). The maximum xylitol concentration was obtained in one day, resulting in a xylitol volumetric productivity of 0.73 g/(L×h).

Integrated process of arabinose biopurification on the glucose- and arabinose-rich hydrolysate and xylitol fermentation on the xylose-rich hydrolysate

As *C. boidinii* was found to be suitable for both arabinose biopurification and xylitol fermentation, it seems to be reasonable to utilise the cell mass produced in the biopurification step as an inoculum in the xylitol fermentation. This kind of integration of the xylitol fermentation and the arabinose biopurification enables the utilization of the by-product cell mass of biopurification and results in a more effective carbon utilization, as the cell propagation of xylitol fermentation does not require additional carbon source or it does not consume xylose convertible into xylitol in the fermentation step. After three days of biopurification of the glucose- and arabinose-rich hydrolysate, the broth contained 9.2 g/L arabinose and 1 g/L galactose, hence the purity of arabinose was 90% of total sugars. Xylitol fermentation on the detoxified xylose-rich hydrolysate, using the cell mass produced in the arabinose biopurification step, resulted in 10.4 g/L xylitol in three days, which corresponds to a xylitol volumetric productivity of 0.14 g/(L×h).

NOVEL SCIENTIFIC FINDINGS

1. Energy efficiency of the corn fibre-based biorefinery producing bioethanol and biomethane can be significantly increased by implementing sludge incineration and district heat production (Paper I).

2. The main heat consuming process steps of the biorefinery producing bioethanol and biomethane from corn fibre are fractionation of the raw material and ethanol distillation (Paper I).

3. Soaking in aqueous ammonia pretreatment of destarched ground corn fibre is an appropriate method to facilitate selective hemicellulose hydrolysis using Hemicellulase NS22002 (Novozymes) enzyme preparation at pH 6 (Paper II).

4. Acid hydrolysis of destarched ground corn fibre under mild conditions results in an arabinose-rich liquid fraction, in which arabinose presents mainly in monomeric form, while the other hemicellulosic sugars are released mainly as oligomers (Paper III).

5. Dilute acid hydrolysis of corn fibre results in a supernatant, from which glucose- and arabinose-rich liquid fraction can be obtained, and a solid fraction, which is appropriate to produce xylose-rich supernatant (Paper III, Paper IV).

6. *C. boidinii* NCAIM Y.01308 is suitable to produce pure arabinose solution regarding total sugars from the glucose- and arabinose-rich hydrolysate of corn fibre through aerobic biopurification. *C. boidinii* NCAIM Y.01308 is also suitable for xylitol production on the activated carbon-treated, xylose-rich hydrolysate of corn fibre at an oxygen transfer rate of 2.8 mmol/(L×h) (Paper IV).

CONCLUSION, POTENTIAL APPLICATIONS

Based on the results of this study, an integrated biorefinery process was proposed that is based on a two-step acidic fractionation of corn fibre and the diverse action of *C. boidinii* yeast (Figure 1). The two-step acidic fractionation of corn fibre results in a glucose- and arabinose-rich hydrolysate, a xylose-rich hydrolysate and a cellulose-rich solid fraction. The glucose- and arabinose-rich hydrolysate can be utilised in arabinose biopurification after pH adjustment. The xylose-rich hydrolysate can be utilised in xylitol fermentation after detoxification by activated carbon and pH adjustment. The utilisation of the cellulose-rich solid fraction, the investigation of purification techniques and recovery processes of arabinose and xylitol, the increase of the achievable arabinose and xylitol concentrations and the scaling-up of the process are the main issues of further development of the biorefinery.

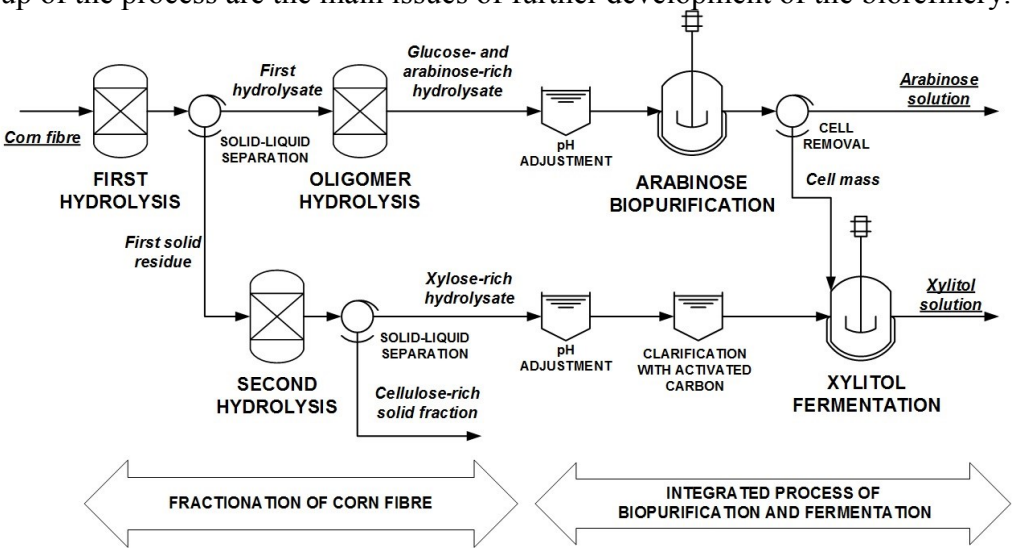

Figure 1: Process scheme of the proposed biorefinery process of corn fibre
Process steps and material streams are indicated in capital letters and in italics, respectively.

PUBLICATIONS

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