INVESTIGATION OF CORN FIBRE UTILISATION IN BIOREFINERY APPROACH

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Investigation of corn fibre utilisation in biorefinery approach

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Department of Applied Biotechnology and Food Science
Édesapámnak †
In memory of my father
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Szeretnék köszönetet mondani minden barátomnak, akik mellettem álltak és bíztattak.

Különös hálával tartozom testvéreimnek és édesanyámnak szüntelen és önzetlen támogatásukért. Édesanyám, köszönöm szépen a rengeteg türelmet, törödést és legfőképp szeretetet, amivel minden nehézségen átsegített!
ABSTRACT

Shifting the dependence of our society from petroleum-based to renewable biomass-based resources is considered to be crucial to the development of a sustainable industry. Biorefinery is defined as the sustainable processing of biomass into a wild spectrum of bio-based products (food, feed, chemicals and/or materials) and bioenergy (biofuels, power and/or heat). Lignocellulosic residues account for the majority of the total biomass present in the world and have a great potential as low cost raw materials of biorefinery processes.

The present PhD study has focused on the investigation of value-added utilisation of corn fibre in biorefinery approach by performing process simulation and experimental work. Corn fibre is an agro-industrial by-product, and currently it is utilised as a component of low value animal feed. However, due to its high carbohydrate content, it has a great potential to be converted into high value chemicals and biofuels.

Process simulation of two base cases of a corn fibre-based biorefinery (base case A producing bioethanol, biomethane and district heat, and base case B producing bioethanol, biomethane and xylitol) was performed. Within the two base cases different process configurations were examined in technological point of view. Within base case A, the highest energy efficiency (73%) was achieved in the scenario containing flue gas condensation and incineration of both the hydrolysis residue and the sludge, and fractionation and ethanol distillation were found to be the main heat consuming parts of the process. In base case B, division of the hemicellulose fraction between anaerobic digestion and xylitol fermentation was essential, and 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.

Through the determination of the pH profiles of xylanase and arabinofuranohydrolase activities of four commercial enzyme preparation, Hemicellulase NS22002 was chosen to investigate selective arabinose solubilisation from destarched ground corn fibre. Soaking in aqueous ammonia pretreatment was found to be necessary before the enzymatic treatment to make the hemicellulose structure accessible for Hemicellulase NS22002. 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, however, it is not suitable for selective arabinose release.

In order to selectively release arabinose acid hydrolysis of destarched ground corn fibre and corn fibre was investigated at different temperatures (90°C–140°C), acid concentrations (0.25%–5% (w/w)) and reaction times (5–75 min) according to experimental designs. 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 a selectivity referred to as satisfactory. Acidic hydrolysis of corn fibre
at 1.1% (w/w) sulphuric acid concentration and 51 min reaction time (first hydrolysis) resulted in a total arabinose yield of 75.9% and completely solubilisation of the starch fraction, however, a subsequent oligomer hydrolysis step was required to recover the sugars in monomeric form, which resulted in 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), which resulted in a xylose-rich supernatant and a cellulose-rich solid fraction.

*Candida boidinii* NCAIM Y.01308 was found to be appropriate for arabinose biopurification under aerobic condition and for xylitol fermentation under microaerobic condition (2.8 mmol/(L×h) oxygen transfer rate) during shake flask experiments on semidefined fermentation media. 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, 6.1 g/L arabinose, 4.1 g/L xylose (+galactose) and 2.7 g/L ethanol in three days.

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.
LIST OF PUBLICATIONS

This thesis is based on the following scientific papers, which will be referred by their roman numerals throughout the thesis. The papers are enclosed at the end of the thesis.


IV. **Csaba Fehér**, Zita Gazsó, Boglárka Gál, Anett Kontra, Zsolt Barta, Kati Réczey. Integrated process of arabinose biopurification and xylitol fermentation based on the diverse action of *Candida boidinii*. *Chemical and Biochemical Engineering Quarterly* (accepted, 05. 03. 2015.) IF: 0.911
Other related articles:


Zoltán Mareczky, Anikó Fehér, **Csaba Fehér**, Zsolt Barta, Katalin Réczey. Effects of pH and aeration conditions on xylitol production by Candida and Hansenula yeasts. *Periodica Polytechnica Chemical Engineering.* (accepted, 30. 06. 2015.) IF: 0.296

Other articles:

István Wagner, Zsombor Kristóf Nagy, Panna Vass, **Csaba Fehér**, Zsolt Barta, Tamás Vigh, Péter Lajos Sóti, Anna Helga Harasztos, Hajnalka Pataki, Geert Verreck, Ivo Van Assche, György Marosi. Stable Formulation of Protein-type Drug in Electrospun Polymeric Fiber Followed by Tableting and Scaling-Up Experiments. *Polymers for Advanced Technologies* (accepted, 06. 05. 2015.) IF: 1.964

Oral presentations:


**Csaba Fehér**, Zita Gazsó, Zsolt Barta, Kati Réczy. Arabinose, xylitol and ethanol production from corn fibre in a biorefinery process based on the diverse action of *Candida boidinii*. 10th International Conference on Renewable Resources and Biorefineries. Valladolid, Spain, June 4–6, 2014.

Poster presentations:


Zoltán Mareczky, **Csaba Fehér**, Hassan Hanan, Máté Kuna, Zsolt Barta, Kati Réczy. The influence of glucose and xylose concentrations and aeration conditions on xylitol production by *Candida* yeasts. *The II Iberoamerican Congress on Biorefineries*. Jaén, Spain, April 10–12, 2013.


Conference proceedings:


# ABBREVIATIONS AND SYMBOLS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AU</td>
<td>arabinoxylan-arabinofuranohydrolase unit</td>
</tr>
<tr>
<td>AWWT</td>
<td>aerobic waste water treatment</td>
</tr>
<tr>
<td>AX-AFH</td>
<td>arabinoxylan-arabinofuranohydrolase</td>
</tr>
<tr>
<td>CE</td>
<td>carbohydrate esterase</td>
</tr>
<tr>
<td>CHP</td>
<td>combined heat and power</td>
</tr>
<tr>
<td>COD</td>
<td>chemical oxygen demand</td>
</tr>
<tr>
<td>DEA</td>
<td>diethanolamine</td>
</tr>
<tr>
<td>DGCF</td>
<td>destarched ground corn fibre</td>
</tr>
<tr>
<td>FP</td>
<td>filterpress</td>
</tr>
<tr>
<td>GH</td>
<td>glycoside hydrolase</td>
</tr>
<tr>
<td>mYA</td>
<td>yield of monomer arabinose</td>
</tr>
<tr>
<td>mYOHS</td>
<td>yield of monomer other hemicellulosic sugars</td>
</tr>
<tr>
<td>m[OHS/A]</td>
<td>ratio of monomer other hemicellulosic sugars (g) and monomer arabinose (g)</td>
</tr>
<tr>
<td>NAD</td>
<td>nicotinamide adenine dinucleotide (oxidised form)</td>
</tr>
<tr>
<td>NADPH</td>
<td>nicotinamide adenine dinucleotide phosphate (reduced form)</td>
</tr>
<tr>
<td>OHS</td>
<td>other hemicellulosic sugars</td>
</tr>
<tr>
<td>OHS/A</td>
<td>ratio of other hemicellulosic sugars (g) and arabinose (g)</td>
</tr>
<tr>
<td>OTR</td>
<td>oxygen transfer rate</td>
</tr>
<tr>
<td>pNPA</td>
<td>( p )-nitrophenyl-( \alpha )-L-arabinofuranoside</td>
</tr>
<tr>
<td>SAA</td>
<td>soaking in aqueous ammonia</td>
</tr>
<tr>
<td>tYA</td>
<td>yield of total arabinose</td>
</tr>
<tr>
<td>tYOHS</td>
<td>yield of total other hemicellulosic sugars</td>
</tr>
<tr>
<td>t[OHS/A]</td>
<td>ratio of total other hemicellulosic sugars (g) and total arabinose (g)</td>
</tr>
<tr>
<td>WIS</td>
<td>water-insoluble solid</td>
</tr>
<tr>
<td>XDH</td>
<td>xylitol dehydrogenase</td>
</tr>
<tr>
<td>XK</td>
<td>xylulokinase</td>
</tr>
<tr>
<td>XU</td>
<td>xylanase unit</td>
</tr>
<tr>
<td>XR</td>
<td>xylose reductase</td>
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1. INTRODUCTION

A great fraction of the energy carriers and material products used today come from non-renewable fossil resources. The intensive consumption of fossilised carbon combined with the diminishing reserves results in the increasing price of fossil derivatives and causes environmental and political concerns. There is clear scientific evidence that emissions of greenhouse gases arising from fossil fuel combustion and land-use change resulting from human activities contribute to the climate change. Currently the primary source of energy for the transport sector and for the production of chemicals is oil, however, the feasibility of oil exploitation is predicted to decrease in the near future (Cherubini, 2010; Nikolau et al., 2008).

Shifting the dependence of our society from petroleum-based to renewable biomass-based resources is considered to be crucial to the development of a sustainable industry, energy independence, and to the effective management of greenhouse gas emissions (Mabee et al., 2005; Ragauskas et al., 2006). Renewable sources for the production of energy and transportation fuels have been extensively investigated by the scientific community in the last decades. However, compared to the bioenergy studies, less attention has been paid to the possibility of replacing existing petrochemicals with chemicals produced from renewable materials. While the production of electricity and heat can rely on a wide spectrum of options (wind, sun, water, nuclear fission and fusion), biomass is very likely the only viable alternative to replace fossil resources for production of chemicals and transportation fuels, since biomass is the only carbon-rich material available on Earth besides fossilised carbons (Cherubini and Strømman, 2011; FitzPatrick et al., 2010).

Biomass can be described as material produced by the growth of microorganisms, plants and animals. Plant biomass is produced via photosynthesis, in which the atmospheric carbon dioxide and water are converted into sugars by using light energy. Plants use these sugars to synthesize complex materials, which are considered to be the most abundant and widely available source of biomass on Earth. The vast majority of the plant biomass resource is lignocellulose, that is composed of three major constituents called cellulose, hemicellulose and lignin (Kumar et al., 2008; Zhang, 2008).

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, which are able to separate biomass resources (wood, grass, crop residues etc.) into their building blocks (carbohydrates, proteins, oils etc.) and convert those into a wide spectrum of marketable products and energy (Cherubini and Strømman, 2011; Kamm and Kamm, 2007). The compositional variety of biomass enables the biorefinery to produce more classes of products than petroleum refinery does, however, larger range of processing technologies is needed, from which the most are still at a pre-commercial stage. Complex utilisation of biomass raw materials using zero-waste approach enables the biorefinery to produce biofuels, biopolymers, resins, food components, animal feed, fertilizers and chemicals (Clark et al., 2012).
In particular, the carbohydrate fraction of lignocellulosic biomass is expected to play the major role to produce bio-based chemicals, since it can be effectively hydrolysed to monosaccharides, which can then be converted into an array of value-added molecules via fermentations or chemical synthesis (Cherubini, 2010). Lignocellulosic residues have the greatest potential to be used as renewable sources to produce value-added materials and chemicals due to their low commercial value and abundant availability. Moreover, biorefining of lignocellulosic residues does not compete with food production and contributes to efficient waste management (Cherubini and Ulgiati, 2010; Kumar et al., 2008; Sánchez, 2009).

The work of this thesis aims the investigation of complex utilisation of the agro-industrial by-product corn fibre in biorefinery approach. Bioethanol, biomethane, xylitol and arabinose are considered as possible products, however, particular attention was paid to the investigation of arabinose production.
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2. BACKGROUND

2.1. BIOREFINERY CONCEPT

Biorefinery is defined by the IEA Bioenergy Task 42 (International Energy Agency, 2009) as the sustainable processing of biomass into a wild spectrum of bio-based products (food, feed, chemicals and/or materials) and bioenergy (biofuels, power and/or heat). Biorefinery is a facility (or a cluster of facilities) that integrates biomass conversion processes and equipment to produce transportation biofuels, power, chemicals and materials from biomass. In this way, biorefining can provide a sustainable approach to produce valuable products, improve biomass processing economics as well as environmental footprint. The term of biorefinery is derived both from the biomass raw material and from the bioconversion processes often applied during the biomass processing (Clark et al., 2012; Kamm and Kamm, 2004).

Biorefinery involves multi-step processing which integrates different biomass conversion technologies such as extraction, biochemical and thermochemical processes. The first steps are pretreatments to make the biomass more accessible for the further processing steps, pre-extraction of valuable products and/or fractionation of the biomass into its core constituent. In the following steps the biomass components are subjected to a combination of biochemical and thermal treatments which include internal recycling of energy and residual streams (FitzPatrick et al., 2010; Smith, 2007). A generalised scheme of the biorefinery concept is shown in Figure 1. The biochemical and thermochemical methods complement each other, resulting in many advantages in terms of products specificity, process flexibility and efficiency (Clark et al., 2012). In the recent years different classifications of biorefineries have been proposed which considered different items.

According to Clark et al. (2012), biorefinery systems can be divided into three types referred to as Phase I, Phase II and Phase III biorefinery. Phase I biorefinery involves integrated facilities with limited process capability to convert a single feedstock into a single major product. An example is a biodiesel plant, in which the oil derived from crushing and extraction of rapeseed or sunflower is transformed to biodiesel using methanol and catalyst. A Phase II biorefinery has the capability to produce a range of bio-products from a single feedstock with flexible process routes to enable adaption to the product demand. An example is a biorefinery utilizing cereal grains (e.g. wheat) to generate multiple products, such as polymers, amino acids, polyols and biofuels. Phase III biorefinery is the most advanced, as it can utilise different types of raw materials to produce wide spectrum of valuable products by integrating a combination of biological and chemical technologies.

Regarding the feedstock utilised in the Phase III biorefinery, it can be subdivided into four main groups: lignocellulosic feedstock biorefinery, whole-crop biorefinery, green biorefinery and marine biorefinery (Diep et al., 2012; Kamm and Kamm, 2007). The lignocellulosic feedstock biorefinery uses nature-dry biomass such as wheat straw, corn stover, wood, paper waste etc. The main component of these raw materials is the lignocellulose which is composed of three main constituents: cellulose, hemicelullose and
lignin. The fractionation of lignocellulosic biomass into its core constituents is considered to be crucial to develop lignocellulose-based biorefinery. Within the lignocellulosic biorefinery different conceptions can be distinguished depending on the process applied and intermediates obtained. Hence, there is thermochemical/syngas platform biorefinery, biochemical/sugar platform biorefinery and, two-platform biorefinery, which combines both processing routes (Carvalheiro et al., 2008). The raw materials for the whole-crop biorefinery are cereals such as rye, wheat, triticale and maize. This concept utilises the entire crop including the stalk, leaves and grain, however, the stalk and leaves can also be processed in a lignocellulosic feedstock biorefinery. The green biorefinery uses nature-wet biomasses, such as green grass, alfalfa, clover and immature cereals, while the marine biorefinery utilises macro- and microalgae (Diep et al., 2012; Kamm and Kamm, 2007). However, the classifications described above oversimplify the highly complex biorefinery concept and provide little information on the specific characteristic.

A more systematic classification approach for biorefinery systems was invented by Cherubini et al. (2009), which was advanced during the IEA Bioenergy Task 42 (2009). This classification is based on four main features, which are identified as platforms, products, feedstocks and processes. The most important terms and features in terms of biorefining will be discussed in the following section.

Figure 1: Scheme of the biorefinery concept (Smith, 2007)
2.1.1. Main features of the biorefinery concept

Feedstocks

The term feedstock means the raw materials used in the biorefinery. Renewable carbon-rich raw materials can be originated from four different sectors, namely: agriculture (dedicated crops and residues), forestry (wood, logging residues), industries (process residues and leftovers) and households (municipal solid waste, waste water), and aquaculture (algae, seaweeds). Another distinction can be made resulting in two subgroups: dedicated feedstocks (e.g. sugarcane, wheat, sweet sorghum, rapeseed) and residues (e.g. crop residues, urban waste, residues of the food industry, sawmill residues) (Cherubini, 2010).

Processes

Several technological processes are applied in biorefinery systems to produce marketable products, which can be divided into four main groups. Mechanical/physical treatments (e.g. pressing, milling, extraction) are performed to reduce the size of the feedstock and to separate different components, but it does not change the chemical structure of the biomass. Biochemical treatments (e.g. fermentation, enzymatic conversion, anaerobic digestion) use microorganisms and enzymes, and usually occur at mild reaction conditions. In chemical processes (e.g. hydrolysis, transesterification, hydrogenation, oxidation) the chemical structure of the substance of biomass changes by reacting with other chemicals. During thermochemical processes (e.g. gasification, pyrolysis, hydrothermal treatments, combustion) the feedstock is exposed to extreme conditions involving high temperature and/or pressure with or without chemical catalyst (Cherubini, 2010; Clark et al., 2012).

Platforms and building block chemicals

By analogy with the current fossil-based chemical industry, a successful bio-based chemical industry will probably build upon the platform chemical approach. In this conception small number of chemical building blocks are produced at first, which are subsequently converted to large number of final products (Cherubini and Strømman, 2011; Nikolau et al., 2008). In 2004, twelve building block chemicals having potential use in the production of bio-based chemicals and materials were identified by the US Department of Energy, which molecules can be produced from sugars via biological or chemical conversions. A common feature of these bio-based building blocks is that their production routes have been known already, however, in most of the cases these routes are not economically viable yet. Hence these bio-based building block chemicals provide promising targets for additional research (Nikolau et al., 2008). The twelve sugar-based building blocks are 1,4-diacids (succinic, fumaric and malic), 2,5-furan dicarboxylic acid, 3-hydroxy propionic acid, aspartic acid, glucaric acid, glutamic acid, itaconic acid, levulinic acid, 3-hydroxybutyrolactone, glycerol, sorbitol, and xylitol/arabinitol (Werpy et al., 2004).
As an example, the possible chemical derivatives of xylitol are shown in Figure 2. Similar molecules can be obtained from the arabinitol. Xylitol and arabinitol are derived from the hydrogenation of the corresponding sugars, xylose and arabinose. The most promising derivatives are xylaric acid, ethylene glycol and propylene glycol. Xylaric acid has a great potential in the production of new, bio-based polymers with specific properties, while the glycols can be used as antifreeze agent or component of unsaturated polyester resins. One of the main challenges to obtain xylitol and arabinitol as building block chemicals is the production of pure feed streams of the platform sugars (Werpy et al., 2004).

![Figure 2: Potential derivatives of xylitol](image)

The term of platform molecules is defined as the key intermediates between the raw material and building block chemicals or final products. By this means the platform molecules of the above listed building blocks are sugars (e.g. glucose, xylose, arabinose). However, most of these molecules can be obtained also as a final marketable product. The platform molecules are considered to be the most important feature in the biorefinery concept (Clark et al., 2012). The most important platforms are the following: biogas (mixture of mainly methane and carbon dioxide) from anaerobic digestion, syngas (mixture of carbon monoxid and hydrogen) from gasification, pyrolysis liquid (multicomponent mixture of different size molecules), C6 sugars (e.g. glucose, mannose, galactose, fructose) form hydrolysis of sucrose, starch, cellulose and hemicellulose, C5 sugars (e.g. xylose, arabinose) from hydrolysis of hemicellulose and pectin, lignin (phenylpropane derivatives) from lignocellulose processing, oil (triglycerides) from oilseed crops and algae processing, organic juice (containing different chemicals) from extraction and pressing of wet biomass (Carvalheiro et al., 2008; Cherubini, 2010; Clark et al., 2012).
Products

In regard to the final application of the products of the biorefinery, two main groups can be distinguished, namely energy products and material products. Energy products are produced, because of their energy content to provide power and heat or transportation fuel. In contrast, the material products are obtained for their special chemical and physical properties (Cherubini et al., 2009). However, some products (e.g. bioethanol) can be used either as fuel or as chemical. The most important energy products of biorefinery systems are: gaseous biofuels (biogas, syngas, hydrogen, biomethane), solid biofuels (pellets, lignin, charcoal), and liquid biofuels for the transportation sector (bioethanol, biodiesel, biobutanol etc.). The chemical and material products achievable through biorefining are the following: chemicals (organic acids, alcohols, sugar derivatives etc.), polymers and resins (starch-based plastics, phenol resins etc.), biomaterials (wood panels, paper etc.) food and animal feed and fertilizers (Cherubini, 2010). In terms of the products the biorefinery systems can be broadly grouped into energy-driven and product-driven biorefineries. The main goal of the energy-driven biorefineries is the production of one or more energy carriers from biomass, and the residues of the process are valorised to bio-based materials to maximise the economic profitability. In comparison, product-driven biorefineries aim to generate one or more bio-based products from biomass, while the residual streams are used for the production of bioenergy for internal or external use to maximise the economic profitability, similar to that highlighted before (Clark et al., 2012).

Based on these main features, the biorefinery systems can be labelled by quoting the involved platforms, marketable products, feedstocks and if necessary the applied processes. For example, there is an integrated process in which glucose, xylose and lignin are recovered from lignocellulosic residues, glucose and xylose are converted into ethanol and xylitol, respectively, while the lignin is burnt to produce heat and electricity. This process can be referred to as three-platform (C6 and C5 sugars, lignin) biorefinery for bioethanol, xylitol, heat and electricity from lignocellulosic residues. This kind of classification approach provides the most sufficient information about a certain biorefinery process, which particularly facilitates the efficient discussion about biorefinery systems (Cherubini et al., 2009).

2.1.2. Main principles to improve biorefinery systems

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. However, the industrial implementation of economically and technically feasible biomass fractionation technologies still has many obstacles (FitzPatrick et al., 2010).

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 process (Gullón et al., 2010). An advanced biorefinery plant should aim at running in a self-sustaining way regarding the utilities, like steam and power. The energy requirements of
the different biomass conversion processes should be internally supplied by in-site production of heat and electricity from the process residues through direct incineration or combustion of biogas produced by anaerobic digestion of the residues (Cherubini, 2010; Octave and Thomas, 2009).

In order to establish a sustainable future for the production of biofuels and bio-chemicals, the integration of green chemistry into the biorefinery concept is mandatory. Green chemistry can be considered as a set of principles for the manufacture and application of products to eliminate the use and generation of substances hazardous to human health and environment. Development and implementation of low environmental impact technologies into the biorefinery are also of importance (Clark et al., 2012).

2.1.3. Biorefinery development by process simulation

Biorefining is a complex process which includes several processing steps; for example pretreatments, hydrolyses, fermentations and purification steps. Many trade-offs are required in the commercial scale design of these process steps owing to their interdependency, which are often overlooked, when each section is analysed independently. For designing cost-effective configurations of a commercial-scale 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 (Geraili et al., 2014). 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 (Geraili et al., 2014; Pham and El-Halwagi, 2012). Therefore, the trade-offs should be incorporated in the process by developing detailed, fully integrated models of biorefinery plants. However, most of the articles recently published focus on the techno-economic analysis and optimization of a specific production pathway only, such as that of ethanol/biodiesel/mixed alcohols (Pham and El-Halwagi, 2012).

Aspen Plus (AspenTechnology, Inc., Cambridge, MA) is commercial, flow-sheeting simulation software, which is widely used to analyse the mass and energy balances in chemical engineering processes. Aspen Plus can be used to develop equilibrium process models to predict the highest conversion or thermal efficiency that can be possibly obtained by a given system. Aspen Plus has abundant library models for different unit operations such as reactions, separations and heat exchange, however, it is also possible to develop new models by the users. Another advantage of Aspen Plus is that it has a large database for the properties of chemicals. Moreover several components playing key role in a biorefinery such as biomass, cellulose, xylan and lignin are also available (Wang et al., 2015). Aspen Plus has been successfully applied to optimise design and operating variables of unit operations in different biorefinery processes such as sugarcane biorefinery (Moncada et al., 2013), microalgae feedstock biorefinery (Gong and You, 2015), olive stone based biorefinery (Hernández et al., 2014) and kraft pulp-mill-based biorefinery (Fornell et al., 2013) with the aim of analysing and improving the overall
efficiency and economics. Aspen Plus based process models can be integrated with economic models and life cycle models to assess the process economics and environmental impacts. The mass and energy balances through the biorefinery system can be calculated by Aspen Plus and the simulation results can be used as the inputs of the software of Aspen Process Economic Analysyer (AspenTechnology, Inc., Cambridge, MA) to estimate the sizes and the costs of the process equipment and to supply data for the life cycle assessment calculations (Wang et al., 2015).

2.2. UTILISATION OF LIGNOCELLULOSIC RESIDUES

Lignocellulosic residues from wood, grass, agricultural, forestry and municipal solid wastes account for the majority of the total biomass present in the word and have a great potential as annually renewable, low cost resource of carbon-rich raw materials (Kumar et al., 2008; Sánchez, 2009). Agro-residues consist of many and various residues from agriculture and food industry, including materials like bagasse, oilseed cakes, wheat straw, corn stover, corn milling by-products and brewer’s residues (Singh Nee Nigam and Pandey, 2009). Such residual streams are only partially valorised at different value-added levels (spread on land, animal feed, composting), whereas the largest fractions are disposed as wastes, with negative impacts on the sustainability of the food processing industry (Fava et al., 2013).

Crop residues encompass all agricultural residues such as straw, stem, stalk, leaves, husk, shell, peel, lint, seed, pulp etc. which come from cereals (rice, wheat, corn, sorghum, barley, millet), cotton, ground-nut, jute, legumes (bean, soya) coffee, cacao, olive, tea, fruits (banana, mango, coco, cashew) and palm oil (Singh Nee Nigam and Pandey, 2009). Agricultural lignocellulosic residues are quite abundant: around 2.9×10^3 million tons from cereal crops and 1.6×10^2 million tons from pulse crops, 1.4×10 million tons from oil seed crops and 5.4×10^2 million tons from plantation crops are produced annually worldwide. Apart from the aforementioned lignocellulosic residues, approximately 6.0×10^2 million tons of harvestable palm oil biomass is being produced worldwide annually. However, only 10% of it is used as finished products such as palm oil and palm kernel oil. The remaining 90% (empty fruit bunches, fibres, fronds, trunks, kernels, palm oil mill effluent) is discarded as waste (Kumar et al., 2008).

Agro-residues are of a wide variety, however, they composed of the same main constituents: cellulose, hemicellulose and lignin. Therefore, they have a huge potential to be used for the production of fuels, chemicals, animal feed and food components in an appropriate biorefinery process. The main advantages of the biorefining of agro-residues for biofuel and bio-products is that, it does not compete with food production and is considered to be advantageous from the environmental point of view, as it contributes to waste management (Cherubini and Ulgiati, 2010; Doherty et al., 2011). The composition of lignocellulosic residues derived from different agricultural sources is listed in Table 1.
Table 1: Composition of agro-residues

<table>
<thead>
<tr>
<th>Lignocellulosic residue</th>
<th>Composition (percentage of dry matter)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cellulose</td>
<td>Hemicellulose</td>
</tr>
<tr>
<td>Barley husk</td>
<td>21</td>
<td>34</td>
</tr>
<tr>
<td>Barley straw</td>
<td>41</td>
<td>27</td>
</tr>
<tr>
<td>Corn cob</td>
<td>33</td>
<td>34</td>
</tr>
<tr>
<td>Corn stover</td>
<td>30</td>
<td>26</td>
</tr>
<tr>
<td>Corn fibre</td>
<td>14</td>
<td>39</td>
</tr>
<tr>
<td>Cotton stalk</td>
<td>40</td>
<td>17</td>
</tr>
<tr>
<td>Rice straw</td>
<td>36</td>
<td>24</td>
</tr>
<tr>
<td>Rice husk</td>
<td>42</td>
<td>18</td>
</tr>
<tr>
<td>Sugarcane bagasse</td>
<td>38</td>
<td>32</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>35</td>
<td>22</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>34</td>
<td>22</td>
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</table>

2.2.1. Structure of the lignocellulose

The composition of lignocelluloses shows wide variety depending on the type and origin of the biomass. In general it consists of 25–50% cellulose, 20–35% hemicellulose and 10–25% lignin and contains other components in smaller quantity such as proteins, oils and minerals (Menon and Rao, 2012; Van Dyk and Pletschke, 2012). The schematic structure of the lignocellulose matrix is shown in Figure 3.

Cellulose is a linear polymer which is composed of D-glucose subunits linked by $\beta$-1,4 glycosidic bonds forming the dimer celllobiose. These form long chains (or elemental fibrils) which are linked together by hydrogen bonds and van der Waals forces. Major part of the cellulose is present in crystalline form and a small amount of non-organized cellulose chains forms amorphous regions. Cellulose is found to be embedded in the matrix of hemicellulose and lignin (Sánchez, 2009).

Lignin is composed of three major phenolic components, namely $p$-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. Lignin is synthesized by polymerization of these components and their ratio varies between different plants, wood tissues and cell wall layers. Lignin is a complex hydrophobic, cross-linked aromatic polymer and it is present in the cellular wall to give structural support, impermeability and resistance against microbial attack and oxidative stress (Menon and Rao, 2012; Sánchez, 2009).
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<th>Hemicellulose</th>
<th>Lignin</th>
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<tr>
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</tr>
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<tr>
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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 (Beg et al., 2001). Hemicelluloses from different sources largely differ in composition. Softwood hemicelluloses contain mostly glucomannans, whereas hemicelluloses of hardwood and graminaceous plants contain mostly xylans. Xylans are composed of a backbone containing xylose units and side chains consisted of mainly arabinose, xylose, glucuronic acid, acetic acid and phenolic acids (Koukiekolo et al., 2005; Van Dyk and Pletschke, 2012). In terms of the main constituents, xylans can be categorised as homoxylan, arabinoxylan, glucuronoxylan and glucurono-arabinoxylan. The frequency and composition of branches essentially depend on the source of xylan (Saha, 2003). Corn fibre hemicellulose is considered as one of the most complex heteropolysaccharides in nature, and its composition is presented in detail below.

2.2.2. Corn fibre hemicellulose

Corn fibre heteroxylan is referred to as glucurono-arabinoxylan, hence the main constituents are xylose, arabinose and glucuronic acid. It 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 (Saulnier et al., 1995b, 1993; Wang et al., 2008). The hemicellulose fraction of corn fibre contains around 48–54% xylose, 33–35% arabinose, 5–11% galactose and 3–6% glucuronic acid (Saha, 2003). The highly branched heteroxylans, which are cross-linked by di-, tri-, and tetraferulic bridges, constitute a network in which the cellulose microfibrils may be imbedded. Structural wall proteins might be cross-linked together by isodityrosine bridges and with the feruloylated heteroxylans, thus contributing to create an insoluble network (Allerdings et al., 2006; Appeldoorn et al., 2010; Saha, 2003). In consequence, corn fibre hemicellulose is highly recalcitrant for enzymatic hydrolysis (Appeldoorn et al., 2013), however, it can be easily solubilised at relatively mild conditions by mineral acids (Grohmann and Bothast, 1997; Noureddini and Byun, 2010). A schematic structure of the heteroxylan and cell wall of corn fibre are shown in Figure 4.

Figure 4: Schematic structure of the heteroxylan and cell wall of corn fibre (adapted from (Saha, 2003))
2.2.3. Biofuels production from lignocellulose

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 hexoses, and subsequently convert them into bioethanol via fermentation. The residual lignin-rich fraction is usually incinerated in 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 (Barta et al., 2013). Therefore, short description about the basics of bioethanol fermentation and biogas production is provided below.

Bioethanol production

Lignocellulosic raw materials can provide environmental, economic, and strategic benefits for the production of bioethanol (Viikari et al., 2012). Bioethanol produced from lignocellulose is commonly called as second-generation bioethanol. The basic process steps in second-generation bioethanol production are: (1) pretreatment to render the cellulose and hemicellulose more accessible to the subsequent steps; (2) enzymatic hydrolysis to convert polysaccharides into monomer sugars; (3) fermentation of the sugars to ethanol using adequate microorganisms; (4) separating and concentrating the obtained ethanol by distillation and dehydration (Sánchez and Cardona, 2008).

The pretreatment generally involves a mechanical step to reduce the particle size of the biomass and a chemical treatment (dilute acid, alkaline, steam explosion etc.) to make the biomass more digestible in the enzymatic hydrolysis. Enzymatic hydrolysis of pretreated lignocellulosic materials involves enzymatic reactions that convert cellulose into glucose and hemicellulose into pentoses (xylose and arabinose) and hexoses (glucose, galactose, and mannose). In the cellulose hydrolysis at least three major groups of cellulases are involved, namely endoglucanase, exoglucanase, and β-glucosidase (Cheng and Timilsina, 2011). The fermentation of hexose sugars are particularly performed by *Saccharomyces cerevisiae* yeast due to its good fermentative capacity, high tolerance to ethanol and other inhibitory compounds, however, there are other microorganisms such as the bacteria *Zymomonas mobilis* under investigations. Pentose fermenting microorganisms, such as *Escherichia coli* and *Pichia stipitis*, can be used to convert xylose and arabinose to ethanol, which is usually carried out separately from the hexose fermentation. However, simultaneous fermentation of hexoses and pentoses to ethanol can be performed by genetically modified microorganisms (Balat, 2011).

To improve the ethanol yield and the process efficiency, different configurations have been invented beside the basic scheme of separated hydrolysis and fermentation (SHF), such as simultaneous saccharification and fermentation (SSF), prehydrolysis and simultaneous saccharification and fermentation (PSSF), simultaneous saccharification and co-fermentation (of pentose sugars) (SSCF) and consolidated bioprocessing (CBP), which comprises saccharification, fermentation and cellulase enzyme production (Sarkar et al.,
Biogas production by anaerobic digestion

Anaerobic digestion is a biological process in which the biomass is converted into biogas. Anaerobic digestion of lignocellulosic residues consists of a complex series of metabolic interactions involving different anaerobic microorganisms in an oxygen-free environment. This process can be divided into four main steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Divya et al., 2015; Monlau et al., 2013). Each stage requires the activity of its own specific group of microorganisms. Hydrolysis is the conversion of polysaccharides into soluble sugar monomers (e.g. glucose, mannose, xylose, arabinose), acidogenesis is the transformation of the sugar monomers into volatile fatty acids. During acetogenesis, volatile fatty acids are converted into acetate, carbon dioxide and hydrogen. Homoacetogenesis is of particular interest as it produces acetate from the mixture carbon dioxide and hydrogen. Finally, methanogenesis is the conversion of acetate, carbon dioxide and hydrogen to methane by archaeabacteria. Hence, the final product of anaerobic digestion of lignocelluloses is biogas, which mainly consists of methane (55–75%) and carbon dioxide (25–45%). Anaerobic digestion is strongly affected by the following environmental factors: temperature, pH and toxic compounds. Regarding the temperature applied, anaerobic digestion can be divided into psychrophilic (10–20°C), mesophilic (20–40°C), or thermophilic (50–60°C) processes (Monlau et al., 2013; Parawira, 2012).

The advantage of the biogas process is the option to use different organic residues to produce energy, such as electrical power and heat, in relatively easy-to-manage and small industrial units. Alternatively, the gas can be compressed after purification using biogas upgrading techniques (e.g. water scrubbing, amine absorption, pressure swing adsorption) and then fed to the gas grid or used as fuel in combustion engines. Its greatest advantage is the environmentally friendly aspect of the technology, which includes the potential for complete recycling of minerals, nutrients (e.g. phosphate) and fibre material (for humification) by returning them to the fields through the utilisation of the residue of anaerobic digestion as a bio-fertilizer (Antoni et al., 2007; Yang et al., 2014).

2.2.4. Hemicellulose-specific biorefining

Major research efforts over the last few decades have been focused on cellulose extraction and deconstruction. Nevertheless, in the economically important graminaceous plants such as cereals and non-food crops, hemicellulose is also significant component, representing 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, partly due to the fact that high quality D-xylose and L-arabinose have not been produced yet as commodity chemicals, their future use as platform intermediates is essential to ensure the sustainability of biorefining lignocellulosic biomass 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 into commodity chemicals such as ethanol, xylitol, 2,3-butanediol and lactic acid or other bio-based intermediates via fermentation technologies (Dumont et al., 2012; Saha, 2003).

2.3. HEMICELLULOSE RECOVERY AND HYDROLYSIS

2.3.1. Chemical methods

The recovery of the hemicellulose fraction is an essential step during the fractionation of lignocellulosic biomass. During hydrolysis, hemicelluloses are hydrolysed into their constituents by complex series of chemical reactions that disrupt molecular interactions within hemicelluloses and with other cell wall components (Kapu and Trajano, 2014). Several effective methods to hydrolyse and solubilise hemicellulose components have been investigated so far, including dilute-acid pretreatment, liquid hot water extraction, steam explosion-based fractionation and alkaline extraction (Huang et al., 2008).

Dilute sulphuric acid treatment is one of the most promising approaches, because of its relatively low operating cost and high achievable hemicellulose yield, which could exceed 90% of the theoretical maximum. Dilute sulphuric acid treatment (0.5–1% (w/w) acid) at moderate reaction temperature (140–190°C) usually results in the hydrolysis of the hemicellulose polymer to soluble monomer sugars (Huang et al., 2008). Two categories of dilute acid treatments can be distinguished: high temperature (>160°C) continuous-flow for low solid loading (5–10% (w/w)) and low temperature (<160°C) batch process for high solid loading (10–40% (w/w)) (Saha, 2003). During dilute acid hydrolysis of lignocellulosic residues, parameters such as temperature, time, acid concentration and solid-to-liquid ratio play critical role in obtaining optimum sugar recovery and low concentrations of inhibitors. The establishment of these parameters is of fundamental importance to define optimal conditions of hydrolysis in view of the following microbial conversions (da Silva and Chandel, 2012). Dilute sulphuric acid treatment of destarched corn fibre at 1% (w/w) acid concentration, 120°C, 8% (w/w) dry matter content, 2 h residence time results in almost complete hemicellulose solubilisation in the form of monomer sugars (Kálmán and Réczey, 2007).

Water extraction at high temperatures (200–230°C) can effectively recover the hemicellulose content from woody and herbaceous materials avoiding significant degradation of sugars. The advantages of the hot water treatments over the acid catalysed methods are the reduced corrosion of the equipment and the limited degradation of xylose resulting in lower inhibitor formation, however, in many cases the hemicellulose is solubilised in the form of sugar oligomers, which requires post-hydrolysis steps to obtain monosaccharides (Huang et al., 2008; Nabarlatz et al., 2007). Hot water treatment of destarched corn fibre at 160°C, 20 min residence time dissolves 75% of the xylan content, however, the obtained xylan oligomers show high recalcitrance against enzymatic decomposition (Dien et al., 2006).
Steam explosion is widely considered as an effective pretreatment for hemicellulose hydrolysis. During this process, the biomass is pretreated by pressurised steam followed by rapid relieving of pressure, which breaks down the complex structure of the lignocellulose. As a result, the hemicellulose is easily hydrolysed. By steam explosion of woody biomass, optimal solubilisation and degradation of hemicellulose can generally be achieved by either high temperature with short residence time (270°C, 1 min) or relatively low temperature with longer reaction time (109°C, 10 min) (Duff and Murray, 1996).

The aim of the alkali treatments (sodium hydroxide and potassium hydroxide treatments, ammonia steeping) of lignocellulosic materials is to disrupt the cell wall by dissolving hemicellulose and/or lignin, by hydrolysing uronic and acetic acid esters and by swelling the cellulose. Therefore, alkali treatments result in increased biodegradability of the cell wall due to cleavage of the bonds between lignin and cellulose, while the hemicellulose fraction is solubilised in the form of polysaccharides and oligosaccharides (Gáspár et al., 2007; Menon and Rao, 2012). During alkali extraction of poplar hemicellulose by using an extruder-type twin-screw reactor, 90% of the initial pentosans can be recovered (N’Diaye et al., 1996). Alkali treatment (1% (w/w) sodium hydroxide or potassium hydroxide solutions) of destarched corn fibre (10% (w/w) dry matter) at 120°C, 2 h residence time results in the solubilisation of 80% of the hemicellulose fraction (Gáspár et al., 2007).

2.3.2. Enzymatic hydrolysis

Complete biodegradation of hemicellulose requires a mixture of different enzymatic functionalities including endo-xylanase (endo-1,4-β-xylanase, E.C.3.2.1.8), β-xylosidase (xylan-1,4-β-xylosidase, E.C.3.2.1.37), α-glucuronidase (α-glucosiduronase, E.C.3.2.1.139), α-arabinofuranosidase (α-L-arabinofuranosidase, E.C.3.2.1.55), acetylxylan esterase (E.C.3.1.1.72) and ferulic/coumaric acid esterases (EC 3.1.1.73) (Dodd and Cann, 2009; Juturu and Wu, 2013, 2012). The sites of the action of the above mentioned hemicellulose degrading enzymes are indicated in a schematic representation of glucurono-arabinoxylans in Figure 5.

Endo-xylanases and β-xylosidases (collectively xylanases) are the two key enzymes responsible for the hydrolysis of the xylan backbone. Endo-xylanases cleave the β-1,4 glycosidic linkage between xylose residues in the backbone of xylans. Endo-xylanases have been classified into glycoside hydrolase (GH) families 5, 7, 8, 10, 11, and 43 on the basis of their amino acid sequences, structural folds, and catalysis mechanisms (Cantarel et al., 2009). Endo-xylanases (even those classified into the same GH family) display quite different pH optima, thermostabilities and also substrate specificities, which is of great importance in terms of the degradation mechanism of hemicelluloses (Beg et al., 2001). Xylan side chain decorations can be recognized by xylanases, and the degree of substitution in xylan influences the products of the hydrolysis. For example, GH 10 enzymes are able to hydrolyze xylose linkages closer to the side chain residues than GH 11, hence GH 10 enzymes release shorter products than GH 11 enzymes when incubated with arabino-glucuronoxylan substrates (Biely et al., 1997). Besides, α-glucuronidases can only release methyl glucuronic acid from a terminal xylose unit present at the
non-reducing end. Thus the products of a GH 10 enzyme acting on glucuronoxylan can be a direct substrate for α-glucuronidase, whereas α-glucuronidase is unable to hydrolyze GH 11 products (Dodd and Cann, 2009). The β-xylosidases release xylose monomers from the non-reducing end of xylo-oligosaccharides produced by endo-xylanases. β-xylosidases are grouped into five GH families: GH 3, 39, 43, 52, and 54 (Shallom and Shoham, 2003).

α-arabinofuranosidases are mainly exo-acting enzymes releasing arabinose units through the cleavage of the α-1,2, α-1,3 and α-1,5 bonds which link L-arabinosyl sidechain decorations to the main chain of arabinoxylans, arabinogalactans and arabinans (Dumon et al., 2012). Those acting specifically on arabinoxylans are usually referred to as arabinoxylan arabinofuranohydrolases (Pitson et al., 1996). Enzymes displaying arabinofuranosidase activity present in GH families 3, 43, 51, 54 and 62. The action of α-arabinofuranosidases is particularly important, since the L-arabinosyl side chains can both hinder the action of xylanases and act as substrate specificity determinants (Beaugrand et al., 2004; Vardakou et al., 2005). α-arabinofuranosidases can act in synergy with endo-xylanases, since endo-xylanases usually hydrolyse more effectively the xylan chains from which arabinose moieties are removed, while most of the α-arabinofuranosidases are more active on soluble arabinose-substituted xylo-oligosaccharides obtained after xylanase treatment (Dumon et al., 2012). The activity of different α-arabinofuranosidases is strongly influenced by the type of the glycosidic bond (α-1,2, α-1,3 or α-1,5), the location of the arabinose unit (connect to mono- or disubstituted xylose subunit), the frequency of the arabinose moieties and the presence of other substituents (Lagaert et al., 2010; Numan and Bhosle, 2006; Saha, 2000).

α-glucuronidases cleave the α-1,2-glycosidic bond of the 4-O-methyl-D-glucuronic acid side group when it is located in the non-reducing end of xylo-oligomers, and are found exclusively in GH family 67. An additional limitation to the release of methyl glucuronic

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**Figure 5: Enzymes required for decomposition of glucurono-arabinoxylan**

(adapted from (Dodd and Cann, 2009))
acid by α-glucuronidases is the extent of acetylation of the xylose chain in proximity to
the methyl glucuronic acid substituent. (Dodd and Cann, 2009; Shallom and Shoham, 2003)

The carbohydrate esterases (CE) hydrolyse the ester linkages between xylose units of the
xylan and acetic acid (acetyl xylan esterase) or between arabinose side chain residues and
phenolic acids, such as ferulic acid (ferulic acid esterase) and p-coumaric acid (p-
coumaric acid esterase) (Saha, 2003). The synergistic action between acetyl xylan esterase
and endoxylanase results in the efficient degradation of acetylated xylans (Sharma and
Kumar, 2013). The release of acetic acid by acetyl xylan esterase increases the
accessibility of the xylan backbone for endoxylanase attack. The endoxylanase creates
shorter acetylated polymers, that are preferred substrates for esterase activity (Beg et al.,
2001). Acetyl xylan esterase activity has been described for members of CE families 1–7,
12 and 16, whereas ferulic and coumaric acid esterases belong to the CE family 1 (Li et
al., 2008).

2.4. XYLITOL PRODUCTION

Xylitol is a naturally occurring five-carbon sugar alcohol that has a lower caloric content
(2.4 cal/g) than that of sucrose (4 cal/g) but with almost the same sweetness level
(Granström et al., 2007a), and its metabolism is partially independent of insulin (Natah
and Hussien, 1997). Therefore xylitol has been used as a sweetener in low energy and
diabetics’ foods as well as an excipient or sweetener in syrups, tonics and vitamin
formulations (Aranda-Barradas et al., 2010). Xylitol has a potential use in the
pharmaceutical and odontological industries due to its health promoting properties, such
as its anticariogenicity, tooth remineralisation, prevention of otitis and other upper
respiratory infections (da Silva and Chandel, 2012; Silva, 2009).

On an industrial scale xylitol is produced through chemical reduction of D-xylose derived
from hemicellulosic hydrolysate of birchwood or other xylose-rich materials. The overall
xylitol yield is approximately 50–60% from the total xylan content of the wood
hemicellulose (Nigam and Singh, 1995). The moderate yield as well as the difficult
purification and separation steps required to remove other by-products from the xylose
and xylitol make the process relatively expensive (Nigam and Singh, 1995). Currently the
annual production is estimated to lie between 20000 and 40000 tonnes annually with a
market value of 40–80 M€, however, the market is expected to extend in the near future
(Granström et al., 2007b; Mohamad et al., 2014).

As an alternative method, microbial production of xylitol is becoming more attractive,
since the downstream processing is expected to be cheaper and, unlike the chemical route,
mild reaction conditions are required (Winkelhausen and Kuzmanova, 1998). Xylitol can
be produced by some bacteria and filamentous fungi, but the most efficient producers are
yeasts, especially species of genus Candida such as C. guilliermondii, C. pelliculosa, C.
parapsilosis, C. tropicalis and C. boidinii (Vandeska et al., 1996).
The key enzymes for assimilation of D-xylose in yeasts are the xylose reductase (XR), which catalyses the reduction of D-xylose to xylitol requiring NADPH as the main cofactor, and the NAD-dependent xylitol dehydrogenase (XDH) catalysing the xylitol conversion to xylulose (Figure 6) (Aranda-Barradas et al., 2010). Xylulose is phosphorylated to xylulose-5-phosphate by xylulokinase (XK). Xylulose-5-phosphate enters the pentose phosphate pathway in which the necessary NADPH for XR activity is regenerated (Figure 6) (Granström et al., 2007a). The NAD cofactor for XDH is mostly supplied by the respiratory chain in the mitochondria (Lighthelm et al., 1988). Xylitol accumulation and secretion under microaerobic conditions are the result of a deficient NAD regeneration that leads to a diminished XDH activity. Higher aeration normally results in larger XDH activity, hence less xylitol and more xylulose accumulates during the xylose fermentation (Aranda-Barradas et al., 2010; Vandeska et al., 1995a). Formation of by-products during the fermentation can also contribute to maintain the intracellular redox balance. Production of ethanol and glycerol regenerates NAD for xylitol oxidation by XDH, whereas acetate production regenerates NADPH for D-xylose reduction by XR (Figure 6) (Granström et al., 2007a).

Many studies have been conducted to produce xylitol from the hemicellulose portion of agro-residues like rice straw (Mayerhoff et al., 1997), corn cob (Tada et al., 2012), brewer’s spent grain (Mussatto et al., 2005), sugarcane bagasse (Rodrigues et al., 2006), corn stover (Maciel de Mancilha and Karim, 2003), barley bran (Cruz et al., 2000) and corn fibre (Rao et al., 2006) using microbial processes. Microbial production of xylitol

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**Figure 6**: Metabolic and cofactor regeneration pathways of xylose metabolism in yeasts (Granström et al., 2007a)

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from hemicellulose hydrolysate is influenced by several factors including the type of the strain, the fermentation conditions (pH, dissolved oxygen concentration, initial cell concentration, temperature) employed in the process and the composition of the fermentation medium (initial xylose concentration, concentrations of other sugars and the ratios of these sugars, concentrations of inhibitor compounds and nutrients) (Parajó et al., 1998; Winkelhausen and Kuzmanova, 1998).

2.4.1. Xylitol fermentation by Candida boidinii

Vandeska et al. (1995a) investigated the effect of initial xylose concentration on xylitol production of C. boidinii NRRL Y-17213 in shake flask experiments using xylose medium. The xylose concentration was varied from 20 g/L to 200 g/L and it was found that 150 g/L initial xylose concentration is the most favourable resulting in a xylitol yield of 0.47 g/g xylose consumed (after 14 days). Xylose concentration of 200 g/L resulted in a strong decrease in xylitol production. In contrast, Vongsuvanlert and Tani (1989) reported that increasing xylose concentration up to 150 g/L resulted in lower xylitol production than that of 100 g/L of xylose, using C. boidinii no. 2201. Accordingly, high initial xylose concentration increases the xylitol production until a certain value, above which it has strong negative effect. This might be due to the osmotic stress on the cells of C. boidinii.

The improvement of xylitol yield by increasing the initial cell density of C. boidinii NRRL Y-17213 was also reported by Vandeska et al. (1995a). When an initial cell concentration of 5.1 g/L was used instead of 1.3 g/L, the xylitol yield (g/g xylose consumed) and the specific xylitol production rate (g/(g xylose consumed×h)) were doubled during shake flask experiments on xylose medium.

The effect of the oxygen availability on the xylitol production of C. boidinii NRRL Y-17213 in 2-L bench top fermentor using xylose (130 g/L) as carbon source was investigated under different oxygen transfer rates (OTR) by Vandeska et al. (1995b). The OTR was varied between 10 and 30 mmol/(L×h). The highest xylitol yield, 0.48 g/g xylose consumed, was reached at an OTR of 14 mmol/(L×h) in 12 days.

Methanol addition is considered to be favourable for polyols production using methylotrophic yeasts, since the oxidation of methanol results in formation of NADH, which is needed for the reduction of sugars (Suryadi et al., 2000). Vongsuvanlert and Tani (1989) reported that xylitol production using C. boidinii no. 2201 significantly increased by adding methanol. A xylitol yield of 0.48 g/g xylose consumed was obtained in a medium containing 10% (w/v) xylose and 2% (v/v) methanol after 4 days of shake flask fermentation.

2.4.2. Xylitol fermentation on corn fibre hydrolysate

Leathers and Dien (2000) developed a two-stage, sequential fermentation process for xylitol and arabinitol production from neutralised and deionised corn fibre hydrolysate using P. guilliermondii. Corn fibre was hydrolysed by dilute sulphuric acid treatment (4.5 mL acid solution/g solid, 1% (v/v) H$_2$SO$_4$, 121°C for 1 h), neutralised by adding calcium
hydroxide and deionised using a mixed-bed resin. This strategy resulted in a xylitol yield of 0.27 g xylitol/g initial xylose within 4 days.

Rao et al. (2006) investigated xylitol production from corn fibre hydrolysate (1% (v/v) H₂SO₄ at a ratio of 1 g of biomass to 5 mL of acid solution, 121°C for 1 h), which was neutralised, treated with activated charcoal and ion exchange resin. C. tropicalis cells were adapted by sub-culturing in hydrolysate containing medium for 20 cycles. This method resulted in a xylitol yield of 0.58 g/g xylose utilised within 2 days.

Buhner and Agblevor (2004) investigated different detoxification methods to produce xylitol from concentrated corn fibre hydrolysate (obtained from dilute sulphuric acid hydrolysis at 121°C using different reaction times and acid concentrations) by using C. tropicalis. The highest xylitol yield, 0.4 g/g xylose utilised, was obtained within 4 days in the case of the highest concentrations (three times of the original hydrolysate) that had been partially neutralised by adding calcium hydroxide and treated with activated charcoal prior to the fermentation.

### 2.5. L-ARABINOSE PRODUCTION

L-arabinose, a five-carbon sugar, is widely found in the nature as a component of hemicellulose and pectin polymers. Traditionally, it is used for the production of flavours in the food industry. It can be used as a natural and non-caloric sweetener, its taste is similar to sucrose, however with half sweetness (Loeza-Corte et al., 2007). Moreover, physiological experiments have revealed that L-arabinose inhibits the sucrose activity of intestinal mucosa. Addition of L-arabinose in combination with sucrose results in suppressed blood glucose and insulin responses in humans (Krog-mikkelsen et al., 2011). Hence, L-arabinose is a promising additive for functional foods. Different possible applications of L-arabinose have been also reported, for example for analytical purposes and for bacteriological diagnostics (Otto and Pickett, 1976), as a starting material in the synthesis of non-ionic surfactants (Bouquillon, 2011), as intermediate for antivirus drug synthesis (Du et al., 1999), as a precursor to the drug intermediate (R)-3,4-dihydroxybutyric acid, to carnitine and agrichemicals (Zhang et al., 2012) and as a base material in the production of vitamin B₂ (Aguedo et al., 2013).

On an industrial scale it 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. In 2010, China produced about 500 tons of L-arabinose at the cost of more than 70 USD per kg (Cheng et al., 2011).

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 (Kim et al., 2012; Spagnuolo et al., 1999), corn hull (Kurakake et al., 2011), wheat bran (Aguedo et al., 2013) and corn fibre (Shibanuma et al., 1999). Different methods of alternative L-arabinose production have been investigated involving acidic and enzymatic hydrolysates.
2.5.1. Dilute acid hydrolysis

Dilute acid hydrolysis of destarched corn fibre was investigated by Shibanuma et al. (1999) for the purpose of studying arabinose production. The concentrations of oxalic acid, hydrochloric acid and sulphuric acid were varied between 0.01–2 N, 0.01–0.1 N and 0.05–0.5 N, respectively at 100°C using 10% (w/w) dry matter content, and the reaction time was changed from 0.5 to 6 h at the selected acid concentrations. Arabinose was liberated rapidly at the beginning of hydrolyses and then slowed when the yield reached 42–54% of theoretical. Conversely, xylose liberation was relatively slow but linearly increased to more than 66% of theoretical. The most favourable condition in terms of selective arabinose liberation was 0.3 N oxalic acid concentration and 1 h reaction time, resulting in around 55% arabinose yield and 15% xylose yield based on theoretical, however, the authors did not conclude it in this form. Although significant amount of oligosaccharides were also produced during the acidic treatments, data about the amount of oligomers were not published.

Dilute acid catalysed hydrolysis of enzymatically destarched wheat bran using water bath or microwave irradiation for heating was investigated by Aguedo et al. (2013). Experiments using water as heating medium were carried out at pH 1, 2, 3 adjusted by hydrochloride acid, at 80°C and 100°C using 10% (w/w) dry matter for 2, 6, and 24 h reaction time. The arabinose yield changed according to a saturation curve as a function of the reaction time, and an arabinose yield of 70% of theoretical was reached at 100°C and pH 1 within 6 h. During these conditions significant amount of xylose was also recovered, but the exact amount of the solubilised xylose was not published. Microwave heating was investigated at 4.75% (w/w) dry matter content according to a Box-Behnken experimental design, in which the effect of temperature (130, 140, 150°C), irradiation duration (1, 3, 5 min) and pH of the medium (1, 2, 3) on the arabinose yield was examined. Microwave heating for 4–5 min at 150°C and pH 1 appeared as a fast and highly efficient method to recover more than 90% of the arabinose content of destarched wheat bran. The experimental design gave an adequate model to describe the release of xylose and arabinose. According to the proposed model a range of conditions could be selected to minimise xylose release and hydrolyse around 50% of the total arabinose, yielding a high purity arabinose fraction, whereas when an arabinose yield of 80–90% was achieved the xylose yield was more than 80% of theoretical. Sugar oligomers might have been produced along the sugar monomers, however, the oligosaccharides were not analysed in this study.

These results implied that the $\alpha$-1→2/3 bonds connecting arabinose moieties to the xylan backbone are more sensitive to the effects of pH and temperature than the $\beta$-1→4 bonds of the xylan, thus 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.

2.5.2. Enzymatic hydrolysis

Lim et al. (2011) investigated arabinose production from purified debranched arabinan and sugar beet arabinan using thermostable α-L-arabinofuranosidase and endo-α-1,5-arabinanase of *Caldicellulosiruptor saccharolyticus* simultaneously. The enzymes were produced by recombinant *Escherichia coli*, and after cell disruption the enzymes were purified through a multistep process involving chromatography and dialysis. The effects of the dosage and ratio of the enzymes, the temperature, the pH and the substrate concentration on the arabinose yield and productivity were examined. In the case of sugar beet arabinan the most favourable conditions were the following: pH 6.0, 75°C, 20 g/L sugar beet arabinan, 3 U/mL endo-1,5-α-L-arabinanase and 24 U/mL α-L-arabinofuranosidase. Under these conditions, 16 g/L arabinose was obtained after 2 h, resulting in a volumetric productivity of 8 g/(L×h). (One unit (U) of endo-1,5-α-L-arabinanase activity was defined as the amount of enzyme required to liberate 1 µmol arabinose per min at 75°C and pH 6.5 from debranched arabinan. One unit of α-L-arabinofuranosidase activity was defined as the amount of enzyme required to liberate 1 µmol of *p*-nitrophenol per min at 80°C and pH 5.5 from *p*-nitrophenyl-α-L-arabinofuranoside.) Based on these results, Kim et al. (2012) developed a continuous process of arabinose hydrolysis from sugar beet arabinan by immobilised enzymes in a packed-bed bioreactor that resulted in a productivity of 9.9 g/(L×h).

Kurakake et al. (2011) investigated arabinose production from purified corn hull arabinoxylan using α-L-arabinofuranosidase of *Arthobacter aurescens* MK5. The cells were grown in a liquid medium containing corn hull arabinoxylan in which the arabinose/xylose ratio was 0.6. The suspension of the separated and washed cells of *Arthobacter aurescens* MK5 was used in the determination of enzyme activities and the hydrolysis of corn hull arabinoxylan. The cell suspension had relatively high arabinoxylan hydrolase activity, while its α-L-arabinofuranosidase and β-xylosidase activities were low. Enzymatic hydrolysis of the soluble corn hull arabinoxylan was performed at pH 7, 40°C for 43 h using 1 U/mL arabinoxylan hydrolyse activity at different substrate concentrations (2%, 4.5% and 16% (w/w)). (One unit was defined as the amount of the cell suspension that could produce 1 µmol of reducing sugar (glucose base) in 1 min from corn hull arabinoxylan.) The arabinose yields achieved were 45%, 44% and 16% of theoretical at 2%, 4.5% and 16% (w/w) substrate concentrations, respectively. During the hydrolysis only arabinose was released.

The advantages of these methods are the mild reaction conditions applied, and the pure arabinose solution obtained regarding the solubilised monosaccharides. The drawbacks are that the production of the starting materials investigated (purified arabinan and arabinoxylan) and the purification of enzymes (α-L-arabinofuranosidase and endo-α-1,5-arabinanase) require complex and expensive processes. Moreover, the purification and recovery of arabinose from the hydrolysates can be challenging, as the starting materials were also soluble in water.
2.5.3. Biopurification

Hydrolysis of the whole hemicellulose content of lignocelluloses results in a mixture of xylose, arabinose and other sugars, from which the arabinose can be separated by chromatographic methods. However, on an industrial scale it might be difficult and expensive. 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) using the adequate microorganisms (Cheng et al., 2011; Park et al., 2001).

Cheng et al. (2011) performed yeast-mediated arabinose biopurification on xylose mother liquor using *Pichia anomala* Y161, which strain was selected by screening of 306 strains of yeasts. Xylose mother liquor is an acid hydrolysate by-product derived from the preparation of xylose from corncob or sugarcane bagasse. It generally contains 350–400 g/L xylose, 150–180 g/L arabinose and 150–180 g/L glucose and galactose. Biopurification experiments were carried out with a mixture of yeast extract containing fermentation medium and xylose mother liquor under aerobic conditions in shake flasks. In order to determine the optimal conditions of the arabinose biopurification response surface methodology was employed. Three parameters, namely the fermentation time (50, 60, 70, 80, 90 h), temperature (30, 31, 32, 33, 34°C) and concentration of xylose mother liquor in the medium (15, 20, 25, 30, 35% (v/v)) were investigated according to central composite experimental design in terms of the purity of arabinose solution obtained. Under the optimised condition of biopurification (32.5°C, 75 h and 21% (v/v) xylose mother liquor) an arabinose purity of 86% (of total sugars) was achieved. Biopurification under the optimised condition was accomplished in a 3-L fermentor. After cell removal, the fermentation broth subjected to consecutive process steps of activated carbon treatment, ion-exchange chromatography, concentration and crystallization, resulting in pure arabinose crystals (99%) with a recovery of 69% of theoretical.

Biopurification of arabinose-rich residual streams seems to be an effective method 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.

2.5.4. Combined process of enzymatic hydrolysis and biopurification

Park et al. (2001) developed a method to produce arabinose from purified corn fibre arabinoxylan by enzymatic hydrolysis followed by arabinose biopurification. Commercially available enzyme preparation (Cellulase C-0901) derived from *Penicillium funiculosum* was used for the enzymatic hydrolysis of the purified arabinoxylan containing 28% (w/w) arabinose and 33% (w/w) xylose. The purified arabinoxylan was obtained from alkali treatment of corn fibre, however, the conditions of extraction and purification were not published. Enzymatic hydrolysis was performed at 40°C, 3.5 pH and 45.5 g/L substrate concentration using an enzyme dosage corresponding to 5940 units β-xylanase, 9 units β-xylosidase and 21 units α-L-arabinofuranosidase in a 5-L jar.
fermenter. (One unit of the enzyme activity was defined as the amount of enzyme which released 1 μmol xylose from soluble 4-O-methyl-D-glucurono-D-xylan or p-nitrophenyl from the corresponding p-nitrophenyl-glucosides per min.) At the end of the hydrolysis (72 h) the resultant supernatant contained xylose, arabinose and small amount of other mono- and oligosaccharides. The arabinose and xylose concentrations were 9.7 g/L and 8.5 g/L, respectively. _Williopsis saturnus_ var. _saturmus_ yeast was cultured in the hydrolysate aerobically at 30°C, 4.5 pH and 96 h residence time. After 72 h of biopurification almost all of the xylose was consumed without any loss of arabinose, however, the concentrations of other components were not reported. The solution obtained after biopurification was decolorized with activated carbon, deionised with cation- and anion-exchange resins, concentrated under reduced pressure and then subjected to crystallization. Finally, 57% of the arabinose present in the initial arabinoxylan was obtained as crude crystals. However, in order to get pure crystals further purification was performed by recrystallization 3 times using ethanol-water mixture, which gave 61% (w/w) yield based on the crude crystalline arabinose.

Although this is a promising method to produce crystalline arabinose, the difficulties of the production of purified arabinoxylan, which was used as raw material, and the significant arabinose loss during the downstream processes might cause a strict barrier in terms of industrial implementation.

### 2.6. CORN FIBRE

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 (Grohmann and Bothast, 1997; Noureddini and Byun, 2010). Wet-milling of corn involves steeping the grain in water and sulphur dioxide to increase the moisture content and to soften the grain structure that facilitates separation of the components: starch, gluten, fibre and germ. The major marketable products of wet-milling are the starch and the oil derived from germ. Co-products of wet-milling include corn fibre, corn gluten and steeping liquor (Leathers, 2003; Nghiem et al., 2011). In 2008, 25.6×10^6 tonnes of corn was wet-milled, thus about 2.43×10^6 tonnes of corn fibre was produced (Rose et al., 2010).

Corn fibre consists of thick-walled cells originated from the aleurone layer, testa and pericarp, cell wall material from the endosperm and significant amount of residual starch. The walls of the various cell types are mainly composed of hemicellulose and cellulose, however, they also contain phenolic acids, proteins, lipids and low amount of lignin (Akin and Rigsby, 2007; Akin, 2008; Saulnier et al., 1995a). Focusing on the polysaccharide content, corn fibre contains around 20% starch, 35% hemicellulose and 15% cellulose (dry matter). However, the exact chemical composition depends on the origin of the corn, the milling technology applied and it also varies year by year (Leathers et al., 2006; Saha and Bothast, 1999).

Corn fibre is utilised mainly as low-value animal feed or as solid fuel in pelletized form, however, it is a promising raw material for producing value-added products (Gáspár et al., 2005). The main fields of research concerning corn fibre utilization are the production of
bioethanol (Rasmussen et al., 2010; Shrestha et al., 2010) and food additives like cellulosic fibre gel (Xu et al., 2010), corn fibre gum (Yadav et al., 2012), xylo-oligosaccharide prebiotics (Samala et al., 2012), corn fibre oil (Kálmán et al., 2006), ferulic acid (Buranov and Mazza, 2009) and vanillin (Lesage-Meessen et al., 2002). Moreover, as it was detailed in the previous sections, corn fibre could be a raw material for xylitol and arabinose production.
3. AIMS OF THE THESIS

General aim of my PhD study is to investigate the possibilities of the value-added utilisation of corn fibre in biorefinery approach, hence provide some novel knowledge to the development of biorefinery processes for lignocellulosic residues. Two ways of gathering new information was performed: constructing a process model based on literature data to simulate different process scenarios of a proposed biorefinery and evaluate them in technological point of view, and performing laboratory experiments to examine novel methods leading to the improvement of biorefining of 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.

The work was carried out at the Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics. The simulation software used in the modelling investigation was available at the Department of Chemical and Environmental Process Engineering, Budapest University of Technology and Economics.
4. MATERIALS AND METHODS

4.1. PROCESS SIMULATION

4.1.1. 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), which is capable to solve mass and energy balances, and to calculate the thermodynamic properties of all the streams involved in the process. The accuracy of the target values adjusted by design specifications was ± 0.1%. 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 (Wooley and Putsche, 1996). Data not directly available were estimated from data on similar components (Wingren et al., 2003).

4.1.2. Process description

Corn fibre, the raw material of the proposed biorefinery, is assumed to contain 21.3% starch, 15% cellulose, 22% xylan, 10.9% arabinan, 2.2% hemicellulosic glucan, 2% acetate, 14% protein, 5% lipids, 3% lignin and 1% inorganic compounds related to the dry matter. These data are based on the publication of Kálmán et al. (2006). An additional component referred to as others is defined to close the mass balance, however, this component is not involved in any reaction during the process. The dry matter content of the raw material is assumed to be 40% (w/w). The simulated biorefinery plant processes 95000 tonne dry corn fibre annually.

4.2. EXPERIMENTAL INVESTIGATION

4.2.1. Raw material, enzymes and microorganisms

Corn fibre derived from the corn wet-milling process of Hungrana Starch and Isosugar Manufacturing and Trading Co. Ltd. (Szabadegyháza, Hungary) was kindly donated by the manufacturer and it was used as raw material in this study. Corn fibre was received at a dry matter content of 30–40% (w/w), hence it was air-dried, and then it was stored at room temperature.

Xylanase NS22083, Enzyme complex NS22119, Hemicellulase NS22002 and Cellic CTec2 enzyme cocktails, which are dedicated to hydrolysis of lignocellulosic materials, were generously provided by Novozymes A/S (Bagsvaerd, Denmark). The main characteristics of these enzyme preparations are summarized in Table 2. Thermostable α-amylase enzyme preparation was donated by Hungrana Starch and Isosugar Manufacturing and Trading Co. Ltd.
Table 2: Main characteristics of the enzyme preparations

<table>
<thead>
<tr>
<th>Enzyme complex NS22119</th>
<th>Main activities</th>
<th>Source</th>
<th>Optimal pH regarding the main activities</th>
<th>Optimal temperature regarding the main activities (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>polygalacturonase, mannanase, β-glucanase</td>
<td><em>Aspergillus aculeatus</em></td>
<td>4.5-6.0</td>
<td>25-55</td>
</tr>
<tr>
<td>Xylanase NS22083</td>
<td>endo-xylanase</td>
<td>n.a.</td>
<td>4.5-6.0</td>
<td>35-55</td>
</tr>
<tr>
<td>Hemicellulase NS22002</td>
<td>β-glucanase, xylanase</td>
<td><em>Humicola insolens</em></td>
<td>5.0-6.5</td>
<td>40-60</td>
</tr>
<tr>
<td>Cellic CTec2</td>
<td>cellulase</td>
<td>n.a.</td>
<td>5.0-5.5</td>
<td>45-50</td>
</tr>
</tbody>
</table>

Information on Xylanase NS22083, Enzyme complex NS22119, Hemicellulase NS22002 and Cellic CTec2 is derived from the product sheets of Novozymes A/S (Bagsvaerd, Denmark). n.a. – not available.

*Candida boidinii* NCAIM Y.01308, *Candida parapsilosis* NCAIM Y.01011, *Candida guilliermondii* (Pichia guilliermondii) NCAIM Y.01050, *Hansenula anomala* (Pichia anomala) Y.01499 were purchased from the National Collection of Agricultural and Industrial Microorganisms (Budapest, Hungary).

4.2.2. Enzyme activity assays

Xylanase activity

Xylanase activity of the enzyme cocktails from Novozymes was investigated as a function of pH within the range 3 to 10. The xylanase activity was assayed in a reaction mixture (1.5 mL) containing 0.1 mL of appropriately diluted enzyme solution and 1.4 mL of 1% (w/w) birch wood xylan (Sigma) solution, at least in duplicate. The xylan solution was prepared with 0.1 mol/L sodium acetate buffer (pH=3, 4, 5) or 0.1 mol/L phosphate buffer (pH=6, 7, 8, 9, 10). The enzyme preparations were diluted with distilled water. After incubation at 50°C for 5 min with continuous agitation, the reaction was stopped by adding 3 mL of 3,5-dinitrosalicylic acid reagent and the reducing sugar content of the reaction mixture was measured. Xylanase activity was expressed in xylanase unit/g enzyme preparation. One xylanase unit (XU) was defined as the amount of released reducing sugar in xylose equivalent (μmol) per minute under the assay conditions.

Arabinoxylan-arabinofuranohydrolase activity

Arabinoxylan-arabinofuranohydrolase (AX-AFH) activity of the enzyme preparations from Novozymes was determined within the range from 3 to 10. The AX-AFH activity was assayed in a reaction mixture (2 mL) containing 1 mL of appropriately diluted enzyme solution and 1 mL of 2% (w/w) water-insoluble wheat arabinoxylan (Megazyme, Bray, Ireland) suspension, at least in duplicate. The enzyme solution and the arabinoxylan suspension were prepared with 0.1 mol/L sodium acetate buffer (pH=3, 4, 5) or 0.1 mol/L phosphate buffer (pH=6, 7, 8, 9, 10). After incubation at 50°C for 1 h with continuous agitation, the reaction was stopped by adding 2 mL of 1 mol/L disodium carbonate solution, and the supernatant was separated by filtration (0.45 μm). The supernatant was
analysed to determine arabinose concentration. AX-AFH activity was expressed in AX-AFH unit/g enzyme preparation. One AX-AFH unit (AU) was defined as the amount of released arabinose (µmol) per min under the assay conditions.

4.2.3. Pretreatments

Destarching

Ground (particle size less than 1 mm) corn fibre was suspended in sodium acetate buffer (pH=4.8, 100 mmol/L) at 3% (w/w) dry matter content, and treated by thermostable α-amylase (5 g/kg dry matter) in 1 L closed glass-flasks at 90°C for 3 h with continuous agitation (250 rpm) in a water bath. The solid fraction was separated by vacuum filtration through a 150 µm pore sized nylon filter and washed with distilled water at 80°C to completely remove the soluble substances. The volume of distilled water used in the washing step was three times that of the liquid volume of the corn fibre suspension. Destarched ground corn fibre (DGCF) was dried at 40°C, and stored at room temperature.

Soaking in aqueous ammonia

Soaking in aqueous ammonia (SAA) treatment of DGCF was performed using the method of Nghiem et al. with minor modifications (Nghiem et al., 2011). DGCF was treated at 10% (w/w) dry matter content in closed glass-flasks using 15% (w/w) ammonia solution for 6 h at 55°C in rotary shaker (175 rpm). The solid fraction was separated by vacuum filtration through nylon filter (150 µm), washed with distilled water (80°C) until neutral pH, and immediately processed in enzymatic hydrolysis experiments.

4.2.4. Enzymatic hydrolysis

Enzymatic hydrolysis of DGCF and SAA-pretreated DGCF were carried out at 3% (w/w) dry matter content and at three different pH values (3, 4, 6) with Hemicellulase NS22002 (0.02 g enzyme preparation/g dry matter) for 4 days. The suspensions were prepared with 0.1 mol/L sodium acetate buffer (pH=3, 4) or 0.1 mol/L phosphate buffer (pH=6) supplemented with a small amount of Thimerosal (Sigma-Aldrich, St. Louis, MO, USA) to avoid microbial infection. The suspensions were incubated at 50°C in a rotary shaker (175 rpm). Homogenous samples were taken daily and the supernatants were separated by centrifugation (10 min, 1000×g). The supernatants were analysed to determine monosaccharide and total sugars content.

4.2.5. Acidic treatments

Sulphuric acid treatment

DGCF (50 g total weight) and corn fibre (100 g total weight) were suspended in appropriately diluted sulphuric acid solution at 3% (w/w) and 10 % (w/w) dry matter content in 100-mL and 250-mL closed glass-flasks, 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 and the sulphuric acid concentrations were set according to the experimental design. The warm-up period was 15 min at 140°C and 10 min at 90°C and 120°C. After treatments at 90°C the flasks were cooled in cold water for 1 min and then the solid residues were separated by vacuum filtration through a nylon filter (150 μm). At 120°C and 140°C the autoclave was cooled to 100°C in 19 and 37 min, respectively. After that the flasks were cooled in cold water for 1 min and then the solid residues were separated by vacuum filtration through a nylon filter (150 μm). The supernatants were analysed to determine monosaccharide and total sugars content.

Two-step acidic fractionation

The fractionation process of corn fibre includes two sequential hydrolyses catalysed by sulphuric acid. The first acidic hydrolysis was carried out in 1000-mL closed glass-flasks containing 800 g corn fibre suspensions at 90°C for 51 min (plus 15 min warm-up period) without agitation in water bath. The corn fibre suspensions contained 10% (w/w) dry matter and 1.1% (w/w) sulphuric acid. Subsequently, the flasks were cooled in cold-water for 1 min and the solid fractions were separated by vacuum filtration through nylon filter (150 μm). The supernatants were collected and stored at -10°C (first hydrolysate). The solid fractions were washed with distilled water until neutral pH, collected and dried at 40°C (first solid residue). The first hydrolysate was treated at 120°C for 60 min to decompose oligosaccharides. The solution obtained is referred to as glucose- and arabinose-rich hydrolysate. The first solid residue was used in the second acidic hydrolysis step, which is performed in 500-mL closed glass flasks containing 300 g suspensions at 120°C for 30 min in autoclave. The suspensions of the first solid residue contained 10% (w/w) dry matter and 1.1% (w/w) sulphuric acid. After the flasks were removed from the autoclave, they were cooled in cold-water for 1 min, and the solid fractions were separated by vacuum filtration through nylon filter (150 μm). The supernatants were collected and stored at -10°C, and called as xylose-rich hydrolysate. The solid fractions were washed with distilled water until neutral pH, collected and dried at 40°C (cellulose-rich solid fraction).

4.2.6. Yeast cultivation

Yeast strains (*Candida boidinii* NCAIM Y.01308, *Candida parapsilosis* NCAIM Y.01011, *Candida guilliermondii* NCAIM Y.01050, *Hansenula anomala* Y.01499) were maintained on agar slants containing 1% (w/w) glucose, 1% (w/w) peptone, 0.3% (w/w) yeast extract and 2% (w/w) agar at room temperature. The medium used for inoculum preparation (pH=6) contained 10 g/L yeast extract, 15 g/L KH2PO4, 1 g/L MgSO4·7H2O, 3 g/L (NH4)2HPO4 and 30 g/L xylose (Walther et al., 2001). The solutions of the xylose and the other components were sterilised separately at 120°C for 15 min in autoclave. Cells were cultivated in 750-mL cotton-plugged Erlenmeyer flasks containing 150 mL inoculum medium at 220 rpm rotation speed in a rotary shaker at 30°C for 72 h, subsequently recovered by centrifugation (1000×g, 5 min), washed with sterile distilled water and the adequate amount of cell mass was directly resuspended in the xylitol fermentation medium. Biopurification experiments were inoculated by addition of 2 mL inoculum medium.
4.2.7. Biopurification

Biopurifications were carried out at 30°C in rotary shaker (220 rpm) for 3 or 4 days in 100-mL cotton-plugged Erlenmeyer flasks containing 20 mL semidefined medium or glucose- and arabinose-rich hydrolysate of corn fibre, and monitored through daily sampling. Semidefined biopurification medium (pH=6) contained 10 g/L yeast extract, 15 g/L KH2PO4, 1 g/L MgSO4×7H2O, 3 g/L (NH4)2HPO4, and 30 g/L or 70 g/L xylose (Walther et al., 2001). The solution of the xylose and that of the other components were sterilised separately at 120°C for 15 min in autoclave. The glucose- and arabinose-rich hydrolysate was sterilised using the same conditions. Before sterilization, the pH of the glucose- and arabinose-rich hydrolysate (pH=1) was adjusted to 6 by addition of calcium hydroxide. The precipitated gypsum was removed by filtration with folding filter.

4.2.8. Xylitol fermentation

Xylitol fermentations were performed on semidefined medium or xylose-rich hydrolysate of corn fibre at 30°C in rotary shaker for four days, and monitored through daily sampling. Semidefined fermentation medium (pH=6) contained 10 g/L yeast extract, 15 g/L KH2PO4, 1 g/L MgSO4×7H2O, 3 g/L (NH4)2HPO4, and 30 g/L or 70 g/L xylose (Walther et al., 2001). The solution of the xylose and that of the other components were sterilised separately at 120°C for 15 min in autoclave. The xylose-rich hydrolysate was sterilised under the same conditions. Before sterilization, pH adjustment and clarification of the xylose-rich hydrolysate were performed. The pH of xylose-rich hydrolysate (pH=1) was adjusted to 6 by adding calcium hydroxide. The precipitated gypsum was removed by filtration with folding filter. After pH adjustment, the xylose-rich hydrolysate was clarified by activated carbon (0.05 g/100 g hydrolysate) at room temperature for 30 min with continuous agitation, subsequently the activated carbon was removed by filtration with folding filter. Activated carbon (Norit DX ULTRA 8005.3) was kindly donated from Cabot Norit Activated Carbon (Amersfoort, The Netherlands). Fermentations were carried out in 100-mL Erlenmeyer flasks closed with cotton plugs.

4.2.9. Compositional analysis

Carbohydrates, acetate and acid-insoluble solid content was determined from solid samples using the method of National Renewable Energy Laboratory with minor modifications (Sluiter et al., 2012). Half a gram of dry matter was mixed with 2.5 mL of 72% (w/w) sulphuric acid and the mixture was kept at room temperature for 2 h. Then, 75 mL of distilled water were added and the suspension was treated at 120°C in the autoclave for 1 h. The acid-insoluble fraction was separated by filtration through a G4 glass filter, washed with hot distilled water, dried at 105°C and measured gravimetrically. The supernatant was analysed to determine its monosaccharide and acetate content. Starch content of the corn fibre was determined using α-amylase. Ground, air-dried corn fibre was suspended in sodium acetate buffer (pH=4.8, 100 mmol/L) at 3% (w/w) dry matter content, and then treated by thermostable α-amylase (5 g/kg dry matter) in 1-L closed glass-flasks at 90°C for 3 h with continuous agitation (250 rpm) in a water bath. The supernatant was separated by vacuum filtration through nylon filter (150 μm), mixed with
8% (w/w) sulphuric acid at a volume ratio of 1:1 and treated at 120°C in autoclave for 15 min to decompose oligosaccharides. Then it was analysed for glucose. The cellulose content was calculated as the difference of the total glucan and starch content.

4.2.10. Analytical methods

Reducing sugar

Reducing sugar content of the reaction mixture of xylanase activity assay was measured colorimetrically using dinitrosalicylic acid reagent according to Miller’s method (Miller, 1959). After stopping the enzymatic reaction by adding 3 mL of 3,5-dinitrosalicylic acid reagent, the reaction mixture was boiled for 5 min, then cooled to room temperature and mixed with 16 mL of distilled water. The adsorption value was measured at a wavelength of 550 nm. Reducing sugar content was determined in xylose equivalent from a calibration curve prepared using pure xylose solution.

Monosaccharides, alcohols and organic acids

Concentration of glucose, xylose, arabinose, xylitol, ethanol, methanol and acetic acid was determined by high-performance liquid chromatography (HPLC) using BioRad (Hercules, CA, USA) Aminex HPX-87H (300 × 7.8 mm) column at 65°C. The eluent was 5 mmol/L sulphuric acid at a flow rate of 0.5 mL/min. Determination of galactose was performed using Phenomenex (Torrance, CA, USA) Rezex RPM-Monosaccharide Pb+2 (300 × 7.8 mm) column at 80°C. The eluent was ultra-pure (milli-Q) water at a flow rate of 0.5 mL/min. In both cases, the sample volume was 40 μL and the components were detected and quantified by refractive index. Xylose and galactose appeared as one peak in the chromatogram measured by Aminex HPX-87H column, hence in samples derived from the acidic treatments xylose and galactose were determined as one component. This component was referred to as other hemicellulosic sugars (OHS) (Paper II and III) or xylose (+galactose) (Paper IV).

Total sugars

Total sugars include monomer sugars and sugar oligomers solubilised. To determine the total sugar content the samples were mixed with 8% (w/w) sulphuric acid at a volume ratio of 1:1, and treated at 120°C in autoclave for 15 min. Hence, the sugar oligomers were hydrolysed into monomers, which were analysed by HPLC.

Cell concentration

Cell concentration in the inoculum and fermentation samples was calculated from the optical density of the sample using a calibration curve based on the relationship of optical density and cell dry weight. Cell dry weight was determined gravimetrically after separation a certain volume of inoculum broth (48 hour) by centrifuge (1000×g, 5 min), washing it with distilled water and drying the cells at 105°C. Optical density was
determined by spectrophotometer (Ultrospec III, Pharmacia LKB, Uppsala, Sweden) at a wavelength of 600 nm.

Total phenols

Total phenolics content was estimated using Folin-Ciocalteu reagent according to the method described by Guo et al. (2013).

Oxygen transfer rate

Gas-liquid mass transfer coefficients ($K_{La}$) of oxygen from the headspace of the flask to the media of xylitol fermentation (semidefined medium and xylose-rich hydrolysate) were determined by using a non-fermentative gassing-out method (Roseiro et al., 1991). A 100-mL Erlenmeyer flask was equipped with an optical oxygen sensor (VisiFerm DO 120, HAMILTON Bonaduz AG, Switzerland) to measure dissolved oxygen concentration ($C$). After gassing out of the fermentation media with nitrogen, the increase of the dissolved oxygen concentration due to the shaking of the flask was measured until constant level of the dissolved oxygen concentration ($C^*$). The measurements were performed at 30°C by using different levels of medium volume (35, 50 and 65 mL) and rotation speed (125 and 220 rpm). The values of the expression $-\ln(1-(C/C^*))$ was plotted as a function of time. The slope of the fitted linear curve gave the value of $K_{La}$ (1/h) (Roseiro et al., 1991). Maximum oxygen transfer rate (OTR) was calculated by multiplying $K_{La}$ (1/h) and $C^*$(mmol/L).

4.2.11. Calculations and statistical analysis

Yields

Sugar yields of acidic and enzymatic hydrolysis were expressed as percentage of theoretical based on the composition of the starting material used in the experiment. Yield of monosaccharides and yield of total sugars – including monomers and oligomers solubilised – were distinguished. The yields of sugars obtained in oligomer form were calculated as the difference between total and monomer sugars.

Xylitol yield was considered to evaluate the fermentation experiments. Xylitol yield was calculated from the highest xylitol concentration obtained during the fermentation and expressed as percentage of theoretical. Theoretical xylitol yield was calculated from the initial xylose concentration by assuming a complete (stoichiometric) conversion. Xylitol volumetric productivity was also calculated at the time of the maximum xylitol concentration.

OHS/A value

To evaluate the selectivity of acidic and enzymatic hydrolysis in terms of the selective arabinose release the ratio of OHS (g) and arabinose (g) (OHS/A) appearing in the supernatant was defined. In the most suitable case much more arabinose is released than
other sugars resulting in an OHS/A value close to 0, however, in undesirable case all the hemicellulosic sugars are solubilised resulting in an OHS/A value of approximately 1.9, which comes from the carbohydrate composition of the starting materials used in these investigations.

Experimental design and optimisation of sulphuric acid treatment

Sulphuric acid treatments were carried out according to a full factorial orthogonal design \( (3^2) \) in quadruplicate at the centre point to determine the effects of the independent variables (sulphuric acid concentration and reaction time), the interactions between the variables, and to reduce the number of experiments. The sulphuric acid concentrations and reaction times were set according to the following: 0.25, 0.5, 0.75 % (w/w) or 1, 3, 5 % (w/w) and 5, 10, 15 min in the case of DGCF, and 0.25, 0.75, 1.25 % (w/w) and 25, 50, 75 min in the case of corn fibre. The monomer arabinose yield \( (mY_A) \), monomer OHS yield \( (mY_{OHS}) \), total arabinose yield \( (tY_A) \), total OHS yield \( (tY_{OHS}) \), OHS/A for monosaccharide \( (m[OHS/A]) \) and OHS/A for total sugars \( (t[OHS/A]) \) were chosen as response variables in the experimental design. Statistica\textsuperscript{TM} v.11 (Statsoft\textsuperscript{®}, Tulsa, USA) software was used to fit a second-order polynomial model for the measured data, and to enable the analysis of variance. The quadratic model was expressed as: \( Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \), where \( Y \) represents the response variable, \( b_0 \) is the intercept, \( b_1 \) and \( b_2 \) are the linear coefficients, \( b_{11} \) and \( b_{22} \) are the quadratic terms and \( X_1 \) and \( X_2 \) represent the independent variables studied. The independent variables were expressed in original physical values. Where possible, the model was simplified by eliminating statistically insignificant terms. The goodness of the reduced model was checked by the value of lack of fit and its statistical significance was evaluated by F-test at 5% significance level. The statistical significance of the effect of variables was checked by Pareto chart and half normal probability plot. In order to determine the optimum condition in terms of \( t[OHS/A] \) and \( tY_A \) simultaneously, a desirability function approach was applied. The desirability function \( D \) involves transformation of each estimated response variable \( Y_i \) to a desirability value \( d_i \), where \( 0 \leq d_i \leq 1 \), and \( i=1,2,\ldots,k \) corresponded to the number of estimated response variables. The individual desirabilities are then combined using the geometric mean: \( D = (d_1 \times d_2 \times \ldots \times d_k)^{1/k} \). This single value of \( D \) gives the overall assessment of the desirability of the combined response levels (Derringer, 1980). In our study \( d_1 \) and \( d_2 \) functions were set to change linearly by changing \( t[OHS/A] \) and \( tY_A \) respectively. The desired interval for the response variables were the following: \( 1.2 \geq [OHS/A] \geq 0 \) and \( 50 \leq tY_A \leq 100 \), where \( [OHS/A] = 0 \) and \( tY_A = 100 \) corresponded to \( d_1 \) and \( d_2 \) values of 1, and \( [OHS/A] = 1.2 \) and \( tY_A = 50 \) corresponded to \( d_1 \) and \( d_2 \) values of 0.
5. RESULTS AND DISCUSSIONS OF PROCESS SIMULATION

5.1. MODEL DEVELOPMENT OF THE BIOREFINERY PROCESS

Two base cases of the biorefinery – A and B showed in Figure 7 and Figure 8, respectively – were modelled and investigated. In the base case A, bioethanol, biomethane and in some scenarios district heat were produced as products. In the base case B, beyond bioethanol and biomethane, crystalline xylitol was also obtained as a value-added product. In both base cases the steam requisite of the process was covered by on-site steam generation and considerable amount of electricity was produced. Part of the excess electricity could be also realized as a product, however, calculation to determine the electricity consumption of the process was not carried out. Scenarios in the base case A were compared in terms of energy efficiency, which was defined as the energy output divided by the energy input of the process. Raw material was considered as input, while the products (bioethanol, biomethane, district heat) and the electricity produced in the process step of combined heat and power production (CHP) were considered as output. Determination of the energy of the raw material, biomethane and bioethanol was based on higher heating values. In the base case B, the comparison of the scenarios was based on the mass flows of the products instead of their energy efficiencies, since xylitol is not an energy carrier.

Figure 7: Process scheme of base case A

_Dashed lines and texts in italics indicate the different configurations of scenarios investigated. FP – filterpress_
Fractionation

The fractionation process consists of two minor steps. The first step of fractionation is separation of the starch content, which is carried out by hot water treatment at 120°C. After hot water treatment the solubilised starch (starch fraction) is removed from the solids in filterpress (Figure 7 and Figure 8). The second step of fractionation is weak acid treatment using 1% (w/w) sulphuric acid at 120°C to hydrolyse the hemicellulosic polymers into monomeric sugars. The solubilised hemicellulosic sugars (hemicellulose fraction) are separated from the solid residue (cellulose fraction) in filterpress only in the base case B (Figure 8), while in the base case A, they are recovered with the distillation residue (Figure 7). In both base cases, the WIS (water-insoluble solid) contents before the hot water and the weak acid treatments are set to 30% (w/w) and 22% (w/w), respectively. All of the filterpress units built in the model are operated with the same settings which are the followings. The solid retention of the filterpress is assumed to be 0.99, and the dry matter content of the solid stream is 40% (w/w). If it was necessary, washing liquid (water) is used to move 90% of the soluble component into the supernatant. The washing curve is based on a washing model developed for the recovery of lignin from pulp residue (Grahs, 1975). The reactions and the conversion factors used in the model are based on the experimental data published by Kálmán et al. (2006).

Figure 8: Process scheme of base case B

*Dashed lines indicate the streams which have different mass flows in the scenarios investigated. FP – filterpress*
Enzymatic hydrolysis and ethanol fermentation

The starch fraction is liquefied at 90°C by α-amylase (0.025 g/g starch) and then saccharified by glucoamylase (0.055 g/g starch). The cellulose fraction containing 10% (w/w) WIS is directed to cellulose hydrolysis, which is carried out by cellulase enzyme complex (5 filter-paper unit/g dry matter) at 50°C (Kálmán et al., 2006). The cellulose fraction is not separated from the solubilised hemicellulosic sugars in the base case A, hence in that case this fraction is referred to as lignocellulose fraction (Figure 7). Residual solid of the cellulose hydrolysis (hydrolysis residue) is separated from the supernatant in filterpress after the hydrolysis (Figure 7, Figure 8). The sugar-rich liquors derived from the starch and cellulose hydrolysis are mixed and then subjected to yeast cultivation and ethanol fermentation. Ordinary baker’s yeast is used to convert 95% of the glucose into ethanol at 35°C in the fermentation step, while pentose sugars are not consumed. Data implemented in this step of the process are based on the results of Kálmán et al. (2006) also.

Distillation and dehydration

Distillation and molecular sieve adsorption are used to produce pure (>99.8% (w/w)) ethanol (Figure 9). The model of distillation and dehydration is based on the model developed by Sassner et al. (2008).

![Figure 9: Scheme of process step of distillation and dehydration](image)

*Dashed lines represent vapour streams*

The distillation step consists of two stripper columns (25 trays) operating in parallel to separate the ethanol from the fermented broth, and a rectification column (35 trays) to concentrate the ethanol to 94% (w/w). The columns operate at different pressures to be thermally coupled in order to reduce the energy demand. The feed is preheated in two steps, first by process streams and then by primary steam, before being divided between the two strippers. The reboiler of the first stripper is heated using primary steam (144°C, 4 bar) generated in the process step of CHP. Overhead vapour from the first stripper (3 bar)
is used as heating medium in the reboiler of the second stripper before being fed to the rectifier. The overhead vapour from the second stripper (1.25 bar) is used as heating medium in the reboiler of the rectifier, together with some primary steam, if it is necessary. The ethanol recovery is set to 99.5% in all of the columns. The remaining water in the overhead vapour of the rectifier (0.35 bar) is removed in the dehydration columns, which are then regenerated with pure ethanol steam. The regenerate is returned to the rectifier.

Anaerobic digestion

The feed streams of the anaerobic digestion are the stillage derived from the distillation and, in some scenarios in the base case A, the separated hydrolysis residue (Figure 7). In the base case B, mother liquor derived from the xylitol crystallization and part of the hemicellulose fraction are also directed to anaerobic digestion (Figure 8). Anaerobic digestion is performed under mesophilic conditions (37°C). Degradation factors of the different components are based on literature data (Barta et al., 2010). The yields of the methane and the anaerobic sludge are assumed to be 0.35 Nm$^3$/kg COD (chemical oxygen demand) removed and 0.03 kg sludge dry matter/kg COD fed, respectively. The biogas is assumed to consist of approximately 50% (w/w) methane, 46% (w/w) carbon dioxide and 4% (w/w) water (Barta et al., 2010).

Biogas upgrading

The removal of carbon dioxide is performed in the gas upgrading step, which is based on amine absorption-desorption (Figure 10). The model of the biogas upgrading step is based on the model developed by Ljunggren and Zacchi (2010).

![Figure 10: Scheme of process step of biogas upgrading](image)

Grey lines represent gas streams. DEA – diethanolamine

The upgrading step consists of an absorption column, in which the carbon dioxide reacts with the amine solution, and a desorption column to regenerate the carbon dioxide-rich
is used as heating medium in the reboiler of the second stripper before being fed to the rectifier. The overhead vapour from the second stripper (1.25 bar) is used as heating medium in the reboiler of the rectifier, together with some primary steam, if it is necessary. The ethanol recovery is set to 99.5% in all of the columns. The remaining water in the overhead vapour of the rectifier (0.35 bar) is removed in the dehydration columns, which are then regenerated with pure ethanol steam. The regenerate is returned to the rectifier.

Anaerobic digestion

The feed streams of the anaerobic digestion are the stillage derived from the distillation and, in some scenarios in the base case A, the separated hydrolysis residue (Figure 7). In the base case B, mother liquor derived from the xylitol crystallization and part of the hemicellulose fraction are also directed to anaerobic digestion (Figure 8). Anaerobic digestion is performed under mesophilic conditions (37°C). Degradation factors of the different components are based on literature data (Barta et al., 2010). The yields of the methane and the anaerobic sludge are assumed to be 0.35 Nm$^3$/kg COD (chemical oxygen demand) removed and 0.03 kg sludge dry matter/kg COD fed, respectively. The biogas is assumed to consist of approximately 50% (w/w) methane, 46% (w/w) carbon dioxide and 4% (w/w) water (Barta et al., 2010).

Biogas upgrading

The removal of carbon dioxide is performed in the gas upgrading step, which is based on amine absorption-desorption (Figure 10). The model of the biogas upgrading step is based on the model developed by Ljunggren and Zacchi (2010).

The upgrading step consists of an absorption column, in which the carbon dioxide reacts with the amine solution, and a desorption column to regenerate the carbon dioxide-rich amine solution. Losses of amine and water are made up, and the lean amine solution is pumped back to the absorption tower. The amine used is diethanolamine (DEA). The DEA load is 2.5 mol amine/mole carbon dioxide, and the DEA concentration is 15% (w/w). The boilup ratio in the reboiler is set to achieve 90% regeneration of the amine solution (Ljunggren and Zacchi, 2010).

Aerobic waste water treatment

The whole effluent of the anaerobic digestion is subjected to the aerobic waste water treatment (AWWT) to remove the remaining organic materials (Figure 7 and Figure 8). The organic material is assumed to be removed entirely, so the degradation factors of all organic compounds are unity. The aerobic sludge is presumed to form at a yield of 0.5 kg sludge dry matter/kg COD (Wei et al., 2003). The sludge is separated from the liquid fraction in filterpress.

Combined heat and power production

Superheated steam (91 bar, 470°C) is generated in a steam boiler (Figure 11) by burning part of the raw biogas and in some scenarios also the hydrolysis residue, the sludge and the separated cell mass from the xylitol production (Figure 7 and Figure 8). The generated steam is allowed to expand to 4 bar through a high-pressure turbine to produce electricity and primary steam (144°C, 4 bar) to cover the steam requirement of the whole plant (Figure 11). The isentropic and the mechanical efficiency of the turbine are presumed to be 90% and 97%, respectively. The amount of the biogas incinerated in the burner is set to produce enough primary steam to satisfy the heat demand of the biorefinery. The return condensate from the heating system of the process is used as feed water for the superheated steam generation (Figure 11). The flue gas leaving the boiler is used to preheat the compressed air flow to 220°C before combustion. The temperature of the flue gas after the air heater is 150°C. In the flue gas condenser the temperature is reduced to 50°C to recover energy by condensation of the steam part of flue gas. The recovered heat is used during the production of district heat in some scenarios of the base case A, and in the evaporation of the fermented broth derived from the xylitol production in the base case B. The temperature of the return water from the district-heating system is raised from 45°C to 90°C by passing the stream through the flue gas condenser and by condensation some of the primary steam withdrawn from the high-pressure turbine (Figure 11). The model of combined heat and power production is based on the model constructed by Sassner and Zacchi (2008).
Xylitol fermentation and recovery

The xylitol fermentation and recovery steps are implemented only in the base case B, in which the hydrolysed hemicellulosic sugars are separated from the solid fraction, before the ethanol fermentation (Figure 8). The hemicellulose fraction is utilised to produce xylitol from xylose and arabinose in two fermentation steps operating sequential. In the first step the whole xylose content is consumed by a *Candida* yeast strain to produce xylitol and cell mass. The conversion factor of the xylose to xylitol reaction is set to 0.67 based on literature data (Walther et al., 2001). After the first fermentation the cell mass is separated from the broth in filterpress to prevent xylitol consumption, which may occur after the depletion of the other carbon sources (Walther et al., 2001). In the second fermentation step, the xylitol formation from arabinose is carried out by a genetically engineered *Escherichia coli* strain, with the conversion factor of 0.71. This strain is assumed to use added glycerol in order to maintain the redox balance in the cells, and to form cell mass (Sakakibara et al., 2009). The genetically modified *Escherichia coli* cell mass is separated from the xylitol-rich broth in filterpress. The downstream steps are clarification with activated charcoal, evaporation and crystallization (Figure 12). The xylitol-rich broth is treated with charcoal at a concentration of 15 g/L to remove the impurities, however, 5% of the sugars and sugar alcohols are also adsorbed to the charcoal surface. Separation of the solid and liquid fractions is performed in filterpress. To concentrate the liquid fraction until a xylitol concentration of 637 g/L, before the crystallization, vacuum evaporation (0.1 bar) is used. Xylitol is crystallized from the
purified and concentrated broth in one step, the crystallization yield is set to 47% (Misra et al., 2011). The purity of the recovered crystals is assumed to be more than 99%.

![Diagram](Figure 12: Scheme of process step of xylitol recovery)  
*FP – filterpress*

### 5.2. INVESTIGATION OF DIFFERENT PROCESS CONFIGURATIONS

#### 5.2.1. Investigation of scenarios in base case A

Within the base case A, six different configurations (Table 3) of the process were investigated and compared with each other in terms of their energy efficiency.

<table>
<thead>
<tr>
<th>Scenarios</th>
<th>Processing of the hydrolysis residue</th>
<th>Processing of the sludge</th>
<th>Utilizing of the heat of flue gas condensation</th>
<th>Ratio of the hemicellulose fraction to produce xylitol</th>
<th>Ratio of the hemicellulose fraction to produce biogas</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>CHP</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A2</td>
<td>CHP</td>
<td>CHP</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A3</td>
<td>CHP</td>
<td>CHP</td>
<td>District heating</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A4</td>
<td>AD</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A5</td>
<td>AD</td>
<td>CHP</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A6</td>
<td>AD</td>
<td>CHP</td>
<td>District heating</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B1</td>
<td>CHP</td>
<td>CHP</td>
<td>Evaporation</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>B3</td>
<td>CHP</td>
<td>CHP</td>
<td>Evaporation</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*Base cases A and B are shown in Figure 7 and Figure 8, respectively. AD: anaerobic digestion, CHP: combined heat and power.*

Two options of the utilization of the hydrolysis residue were investigated: anaerobic digestion to produce biogas or incineration in the process step of CHP to generate steam and electricity. Scenarios containing incineration of the hydrolysis residue (A1, A2, A3) had higher energy efficiency than the corresponding scenarios (A4, A5, A6) in which the hydrolysis residue was subjected to anaerobic digestion, while other settings were equal (Figure 13). However, the differences were small in those cases in which the sludge derived from the AWWT was burnt in the process step of CHP (A2 vs A5, Figure 13).
Part of the hydrolysis residue remained undigested after anaerobic digestion, so it was eliminated during AWWW. In this step the organic matter converted into cell mass and part of that into carbon dioxide and water. Thus, energy from the incineration of the hydrolysis residue is more than the energy from the incineration of the biogas and sludge, which were produced in anaerobic digestion and AWWW from the hydrolysis residue.

Figure 13: Energy efficiencies of the investigated scenarios of base case A. Summary of the scenarios is given in Table 3. HHV – higher heating value.

Incineration of the sludge had significant effect on the energy efficiency of the biorefinery. The sludge replaced some biogas required in the process step of CHP, thus more biomethane appeared as product. Energy efficiency increased from 59% to 66% and from 53% to 64% in the cases of incineration (A1, A2) and anaerobic digestion (A4, A5) of the hydrolysis residue, respectively, by implementing sludge incineration (Figure 13). Therefore, processing of the sludge increased the energy efficiency to a larger extent, when the hydrolysis residue was subjected to anaerobic digestion. The reason is that in the case of anaerobic digestion of the hydrolysis residue (A5) more sludge was produced during the AWWW – from the organic matter remained from the hydrolysis residue after anaerobic digestion – compared to the case in which the hydrolysis residue was combusted (A2).

When the streams containing relatively high amount of water (for example: hydrolysis residue, sludge) are incinerated, the flue gas leaving the burner contains considerable amount of steam, which can be condensed in flue gas condenser to regain energy. This energy can be used during the production of hot water for district heating system. However, to produce district heat it was also necessary to consume some primary steam (Figure 11). Production of district heat resulted in considerable increase in energy
efficiency (A2 vs. A3 and A5 vs. A6, Figure 13). In the scenarios containing production of district heat, more biogas had to be incinerated, hence less biomethane was produced. However, the increased steam production resulted in more electricity. The positive effect of production of district heat on the energy efficiency is greater in the scenario, where hydrolysis residue is digested anaerobically (A5 vs. A6, Figure 13) instead of incineration (A2 vs. A3, Figure 13). The highest energy efficiency (73%) was achieved in the scenario containing flue gas condensation and incineration of both the hydrolysis residue and the sludge (A3, Figure 13). In this scenario 10346 tonnes of biomethane and 15610 tonnes of ethanol were produced from 95000 tonnes of dry corn fibre annually.

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 biorefinery. The ethanol distillation required 41–46% of the whole heat consumption of the biorefinery (Table 4) in spite of the heat integration implemented by thermally coupled columns (Figure 9), which is due to the low ethanol concentration (3 g/L) in the fermented broth. However, the applied temperature of the fractionation process was relatively low (120°C), the heat requisite of the fractionation process was almost the same (36–41% of the whole heat consumption) compared to that of the distillation (Table 4). Thus, decreasing the heat demand of fractionation and distillation is important to increase the energy efficiency of the biorefinery.

### Table 4: Heat demand of process steps

<table>
<thead>
<tr>
<th>Scenarios</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
<th>A6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractionation (%)</td>
<td>38</td>
<td>38</td>
<td>41</td>
<td>41</td>
<td>36</td>
<td>40</td>
</tr>
<tr>
<td>Distillation (%)</td>
<td>42</td>
<td>42</td>
<td>46</td>
<td>46</td>
<td>41</td>
<td>45</td>
</tr>
<tr>
<td>Other (%)</td>
<td>20</td>
<td>20</td>
<td>13</td>
<td>13</td>
<td>23</td>
<td>15</td>
</tr>
</tbody>
</table>

*Other includes all process steps except the fractionation and distillation. Heat demand of the process steps are expressed as percentage of the heat demand of the whole biorefinery.*

#### 5.2.2. Investigation of scenarios in base case B

In the base case B, the process was extended to be able to produce xylitol as an additional, value-added product of the biorefinery. In these scenarios the hemicellulose fraction was separated in filterpress and then part of that is directed to xylitol fermentation (Figure 8). A preliminary study proved that the hemicellulose fraction had to be shared between the xylitol fermentation and the anaerobic digestion to cover the heat demand of the whole process. Using 80% of the hemicellulose fraction for xylitol fermentation and 20% of that in anaerobic digestion, the heat requisite of the process can be satisfied by combustion of the raw biogas produced. Hence, in that scenario (B1) bioethanol and xylitol were obtained as marketable products (Table 5). The achieved yield of ethanol production was 77% of theoretical, as 15089 tonnes of ethanol was obtained annually (Table 5) and
theoretically, 19587 tonnes/year ethanol can be produced from the starch and cellulose inputs of the process. The theoretical amount of the xylitol produced from the xylan and arabinan content of the corn fibre is 36030 tonnes/year. The proposed process produced 6733 tonnes/year crystalline xylitol (Table 5), which corresponds to 19% of the theoretical. Theoretically, 35568 tonnes/year of xylose and arabinose can be released from the processed raw material, from which 22540 tonnes/year was obtained in the inlet stream of the xylitol fermentation (Table 5). It corresponds to 63% of the theoretical, which is mostly due to the xylose and arabinose loss during the solid-liquid separations of the fractionation process. During the two-step, sequential fermentation 15496 tonnes/year xylitol was produced, from which 65% derived from xylose (Table 5). Forty-three percent of the fermented xylitol was obtained in the form of pure crystals, which is due to the low yield of crystallization and xylitol loss during filtrations and activated charcoal treatment. When the half of the hemicellulose fraction was used for xylitol fermentation instead of 80%, the proposed biorefinery can simultaneously produce bioethanol, biomethane and xylitol. In that scenario (B3) 4208 tonnes xylitol, 5599 tonnes biomethane and 15089 tonnes ethanol were produced from 95000 tonnes of dry corn fibre annually (Table 5). (The scenario is referred to as B3 to be in accordance with the nomenclature of paper I.)The methane obtained from the mother liquor were 37% and 20% of total methane produced during anaerobic digestion in the scenarios utilizing 80% and 50% of hemicellulose fraction to xylitol fermentation, respectively (Table 5), which verified the significant role of mother liquor in biogas production.

Table 5: Process details of the scenarios investigated in base case B

<table>
<thead>
<tr>
<th>Scenarios</th>
<th>B1</th>
<th>B3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part of the hemicellulose fraction used for xylitol</td>
<td>0.8</td>
<td>0.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component flows of xylitol fermentation (tonne/year)</th>
<th>B1</th>
<th>B3</th>
</tr>
</thead>
<tbody>
<tr>
<td>xylose input</td>
<td>14875</td>
<td>9297</td>
</tr>
<tr>
<td>arabinose input</td>
<td>7665</td>
<td>4790</td>
</tr>
<tr>
<td>xylitol derived from xylose</td>
<td>10115</td>
<td>6322</td>
</tr>
<tr>
<td>xylitol derived from arabinose</td>
<td>5379</td>
<td>3362</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biogas streams (tonne/year)</th>
<th>B1</th>
<th>B3</th>
</tr>
</thead>
<tbody>
<tr>
<td>methane obtained from mother liquor</td>
<td>3851</td>
<td>2407</td>
</tr>
<tr>
<td>total methane produced</td>
<td>10487</td>
<td>12245</td>
</tr>
<tr>
<td>methane incinerated in CHP plant</td>
<td>10487</td>
<td>6603</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Products (tonne/year)</th>
<th>B1</th>
<th>B3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ethanol</td>
<td>15089</td>
<td>15089</td>
</tr>
<tr>
<td>methane</td>
<td>-</td>
<td>5599</td>
</tr>
<tr>
<td>xylitol</td>
<td>6733</td>
<td>4208</td>
</tr>
</tbody>
</table>

Summary of the scenarios is given in Table 3. CHP: combined heat and power.

Therefore, division of the hemicellulose fraction between anaerobic digestion and xylitol fermentation allows producing of bioethanol, biomethane and xylitol simultaneously, and by varying the rate of the division the amount of biomethane and xylitol could be adjusted to market conditions.
6. RESULTS AND DISCUSSIONS OF THE EXPERIMENTAL WORK

The raw material used in this study was corn fibre derived from the wet-milling process of Hungrana Starch and Isosugar Manufacturing and Trading Co. Ltd. Carbohydrate, acetate and acid-insoluble solid content of corn fibre was determined and the results are listed in Table 6.

Table 6: Carbohydrate, acetate and acid-insoluble solid content of corn fibre

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage of dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>13 (0.6)</td>
</tr>
<tr>
<td>Cellulose</td>
<td>17 (0.8)</td>
</tr>
<tr>
<td>Xylan</td>
<td>19 (0.7)</td>
</tr>
<tr>
<td>Arabinan</td>
<td>12 (0.5)</td>
</tr>
<tr>
<td>Galactan</td>
<td>4 (0.1)</td>
</tr>
<tr>
<td>Acetate</td>
<td>3 (0.4)</td>
</tr>
<tr>
<td>Acid-insoluble solid</td>
<td>8 (0.5)</td>
</tr>
</tbody>
</table>

Standard deviations are indicated in parenthesis.

6.1. INVESTIGATION OF SELECTIVE ARABINOSE RELEASE FROM CORN FIBRE

Two different methods, enzymatic hydrolysis and acidic treatment, were investigated to selectively release arabinose from the hemicellulose matrix of corn fibre. The aim of this part of the study was the selective solubilisation of arabinose moieties from corn fibre, an insoluble substrate, to obtain a liquid fraction rich in arabinose and a solid fraction containing the main part of other carbohydrates. Therefore, a simple solid-liquid separation could result in the separation of arabinose from other sugars present in the corn fibre hemicellulose. Yields of solubilised monosaccharides and total sugars (including monomers and oligomers) were distinguished during the investigations. Targets for the yield of arabinose solubilised and the selectivity of hydrolysis were defined as the following: total arabinose yield unacceptable below 50%, satisfactory between 50% and 70%, and good above 70%. The t[OHS/A] unacceptable above 1.2, satisfactory between 1.2 and 0.4 and good below 0.4.

6.1.1. Enzymatic hydrolysis using commercial enzyme preparations

Four commercial enzyme preparations – dedicated for lignocellulose hydrolysis – from Novozymes were investigated in terms of selective release of arabinose. Beside their main activities (Table 2) these enzyme preparations could contain several types of side activity, such as α-L-arabinofuranosidase. During the determination of arabinoxylan-arabinofuranohydrolase (AX-AFH) activity of a multi-component enzyme cocktail by using complex polysaccharide (e.g. arabinoxylan) as a substrate, the activity depends on
both the $\alpha$-L-arabinofuranosidase activity and the other activities present in the mixture. By this means the AX-AFH activity characterizes the capability of the enzyme preparations to release arabinose from complex polysaccharides. In many studies the enzyme preparations for arabinose liberation were characterised by their $\alpha$-L-arabinofuranosidase activity determined on $p$-nitrophenyl-$\alpha$-L-arabinofuranoside ($p$NPA) model substrate, however, the activity on $p$NPA does not guarantee the ability to liberate arabinose from natural substrates (van den Broek et al., 2005; Van Laere et al., 1997). Hence, investigation of AX-AFH activity on water-insoluble wheat arabinoxylan was chosen in this study.

Multi-component enzyme preparations contain different enzyme activities, which can have different pH and temperature optima. This implies the possibility of performing selective hydrolysis reactions by shifting the pH without using purified enzymes. The pH dependence of xylanase and AX-AFH activities of the selected enzyme mixtures were determined within the range from 3 to 10 to investigate the possibility of selective arabinose release during the hydrolysis of corn fibre.

Determination of xylanase and AX-AFH activities

Figure 14 shows the relative xylanase and AX-AFH activities of Xylanase NS22083, Enzyme complex NS22119, Cellic CTec2 and Hemicellulase NS22002 enzyme preparations as a function of pH. Relative activity is expressed as percentage of the highest activity value obtained for a given enzyme preparation and type of activity (Table 7).

Xylanase NS22083 had maximum xylanase activity at pH 5. In AX-AFH activity two maxima were observed, one at pH 5 (acidic maximum), which is considered as the absolute maximum, and another at pH 8 (alkaline maximum) with 78% relative activity. The highest AX-AFH and xylanase activities were obtained at the same pH, furthermore at pH 8 – at the alkaline maximum of AX-AFH activity – the relative xylanase activity was 46%. These results imply that Xylanase NS22083 is not applicable to selectively release arabinose from an arabinoxylan containing substrate.

The optimum pH of xylanase activity of Enzyme complex NS22119 was 6. Regarding AX-AFH activity it also had an acidic maximum at pH 4 with 100% relative activity and an alkaline maximum at pH 9 with a relative activity of 48%. At the pH values of acidic and alkaline maximum of AX-AFH activity, 70% and 32% relative xylanase activities were obtained, respectively. Hence, enzyme complex NS22119 is considered to be inappropriate for selective release of arabinose also.

The existence of two maximum values of AX-AFH activity of Xylanase NS22083 and Enzyme complex NS22119 implies the presence of two different types of $\alpha$-L-arabinofuranosidase in these enzyme mixtures, however, further investigation is required to confirm this hypothesis.
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Determination of xylanase and AX-AFH activities

Figure 14 shows the relative xylanase and AX-AFH activities of Xylanase NS22083 (A), Enzyme complex NS22119 (B), Cellic CTec2 (C) and Hemicellulase NS22002 (D) as a function of pH. Relative activity is expressed as percentage of the highest activity value obtained for a given enzyme preparation and type of activity (Table 7).

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The existence of two maximum values of AX-AFH activity of Xylanase NS22083 and Enzyme complex NS22119 implies the presence of two different types of $\alpha$-L-arabinofuranosidase in these enzyme mixtures, however, further investigation is required to confirm this hypothesis.

Xylanase activity of Hemicellulase NS22002 as a function of pH had a broad optimum. The optimum pH was 7, however, it kept around 90% of its maximum activity within the pH range from 5 to 8. At pH 4 and 3 significant decrease occurred resulting in 9% and 1% relative xylanase activities, respectively. However, regarding AX-AFH activity, pH 4 was found to be the optimum, and at pH 3 it decreased only by 29%. More than 50% relative AX-AFH activity of Hemicellulase NS22002 was retained over the whole pH range investigated. The considerable difference between the relative activities of xylanase and AX-AFH at pH 4 and 3 implies the possibility to selectively release arabinose by using Hemicellulase NS22002 from arabinoxylan-containing raw materials.
Comparing the maximum absolute values of xylanase activity of the four commercial enzyme preparations Xylanase NS22083 had the largest one followed by Cellic CTec2 (Table 7). The xylanase activity of Xylanase NS22083 is 10-fold and 35-fold of the Hemicellulase NS22002 and Enzyme complex NS22119, respectively (Table 7). Low absolute values of AX-AFH activity of the commercial enzyme preparations were obtained (Table 7), which is due to the fact that AX-AFH is side activity, and it was measured on water-insoluble substrate. The highest value was observed in Hemicellulase NS22002 (Table 7).

Table 7: Measured highest activities of xylanase and arabinoxylan-arabinofuranohydrolase (AX-AFH)

<table>
<thead>
<tr>
<th>Enzyme preparation</th>
<th>AX-AFH activity (AU/g enzyme preparation)</th>
<th>Xylanase activity (XU/g enzyme preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme complex NS22119</td>
<td>1.66 (0.12)</td>
<td>538 (40)</td>
</tr>
<tr>
<td>Xylanase NS22083</td>
<td>6.66 (0.06)</td>
<td>18862 (794)</td>
</tr>
<tr>
<td>Hemicellulase NS22002</td>
<td>7.30 (0.29)</td>
<td>1893 (54)</td>
</tr>
<tr>
<td>Cellic CTec2</td>
<td>3.28 (0.43)</td>
<td>14803 (121)</td>
</tr>
</tbody>
</table>

AU – arabinoxylan-arabinofuranohydrolase unit, XU – xylanase unit. Standard deviations are calculated from triplicates, and are indicated in parenthesis.

Considering that Hemicellulase NS22002 had the highest AX-AFH and low xylanase activities compared with the other enzyme preparations (Table 7), and at the pH optimum of its AX-AFH activity it had relatively low xylanase activity (Figure 14), this enzyme preparation was selected to investigate selective arabinose solubilisation from DGCF and SAA-pretreated DGCF.

Enzymatic hydrolysis

Enzymatic hydrolyses of DGCF using Hemicellulase NS22002 was performed at pH 4. Hydrolyses of SAA-pretreated DGCF were carried out at pH 3 and 4, and as a control experiment at pH 6, where AX-AFH activity is minimal and the xylanase activity is close to the maximum (Figure 14).

In the case of DGCF the amount of recovered monosaccharides in the supernatant was negligible, as the yields of monomer glucose, arabinose and OHS were less than 5% (Table 8). Regarding total sugars an arabinose yield of 17%, glucose and OHS yields of 8% were obtained (Table 8). The low yields of total sugars imply that the lignocellulose structure of DGCF is recalcitrant for the efficient enzymatic hydrolysis with Hemicellulase NS22002 at pH 4.

To make the hemicellulose fraction of DGCF fibre more accessible for enzymatic digestion, SAA pretreatment was performed. Following the SAA pretreatment, the monomer and total arabinose yields were significantly increased (Table 8). The monomer arabinose yield continuously increased during enzymatic hydrolysis up to 17% (Figure 15). The monomer glucose yield also continuously increased to 9% (Table 8), while the
amount of released monomer OHS was negligible. The yields of total arabinose and total OHS reached their maximums within 1 day (Figure 15) and were 63% and 57%, respectively (Table 8). The yield of total glucose increased continuously to 16% (Table 8). The high yields of total arabinose and OHS and the low yields of monomer arabinose and OHS indicate that the hemicellulose fraction was solubilised mainly in the form of oligosaccharides. The fast liberation and accumulation of oligosaccharides can be explained by high endo-xylanase and low β-xylosidase activities of Hemicellulase NS22002 at pH 4. This combination of endo-xylanase and β-xylosidase activities could also be the reason for the relatively low xylanase activity determined by the measurement of reducing sugars at pH 3 and 4 (Figure 14). The presence of glucose monomers and oligomers in the supernatant of enzymatic hydrolysis indicates that Hemicellulase NS22002 contains cellulase and β-glucosidase enzymes with considerable activity at pH 4.

Table 8: Yields of monomer and total sugars and ratios of OHS to arabinose at the end of the hydrolysis of DGCF and SAA-pretreated DGCF using Hemicellulase NS22002

<table>
<thead>
<tr>
<th></th>
<th>Hydrolysis at pH 4</th>
<th>Hydrolysis at pH 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DGCF</td>
<td>SAA-DGCF</td>
</tr>
<tr>
<td></td>
<td>monomer total</td>
<td>monomer total</td>
</tr>
<tr>
<td>Y_arabinose (% of theoretical)</td>
<td>4.2 (0.2)</td>
<td>16.5 (0.9)</td>
</tr>
<tr>
<td>Y_OHS (% of theoretical)</td>
<td>0.3 (0.0)</td>
<td>8.2 (0.5)</td>
</tr>
<tr>
<td>Y_glucose (% of theoretical)</td>
<td>2.5 (0.2)</td>
<td>8.4 (0.3)</td>
</tr>
<tr>
<td>OHS/arabinose (g/g)</td>
<td>0.12 (0.01)</td>
<td>0.93 (0.01)</td>
</tr>
</tbody>
</table>

OHS – other hemicellulosic sugars, DGCF – destarched ground corn fibre; SAA – soaking in aqueous ammonia, Y_arabinose – yield of arabinose; Y_OHS – yield of other hemicellulosic sugars (xylose and galactose); Y_glucose – yield of glucose. The hydrolysis was performed for 4 days. Standard deviations are calculated from triplicates, and are indicated in parenthesis.

Regarding the monomer sugars, the selectivity of hydrolyses of DGCF and SAA-pretreated DGCF at pH 4 were found to be good (Table 8). Regarding the total sugars appearing in the supernatant, the hydrolysis selectivity was satisfactory in the case of DGCF and unacceptable in the case of SAA-pretreated DGCF (Table 8).

Monomer glucose, arabinose and OHS were not detected in the supernatant, when enzymatic hydrolysis of SAA-pretreated DGCF was performed at pH 3 with Hemicellulase NS22002. The yields of total arabinose and total OHS were 14% and 12%, respectively, and the yield of total glucose was 3% at the end of hydrolysis (data not shown). The results show that without formation of the appropriate amount of solubilised oligosaccharides the liberation of monomer arabinose is retarded.

Enzymatic hydrolysis of SAA-pretreated DGCF was performed also at pH 6 with Hemicellulase NS22002. Higher yields of monosaccharides and total sugars were achieved than at pH 3 and 4. The yields of monomer sugars increased until the end of hydrolysis (Figure 15), where 36% monomer arabinose yield and 12% monomer glucose yield were obtained (Table 8). This yield of monomer arabinose is comparable with the highest yield achieved on purified corn hull arabinoxylan (45%) by Kurakake et al.
The yield of monomer OHS was only 6% (Table 8), whereas the xylanase activity of Hemicellulase NS22002 at pH 6 is close to the maximum (Figure 14). This could be due to the highly substituted structure of the oligosaccharides derived from corn fibre hemicellulose, which can make the oligosaccharides recalcitrant against enzymatic decomposition. Xylo-oligomers derived from corn fibre that resist the hydrolytic enzymes currently available were published by Appeldoorn et al. (2010, 2013) and Hespell et al. (1997).

![Image of Figure 15](image)

**Figure 15**: Enzymatic hydrolysis of SAA-pretreated DGCF using Hemicellulase NS22002 at pH 4 (A) and pH 6 (B).

DGCF – destarched ground corn fibre, SAA – soaking in aqueous ammonia, OHS – other hemicellulosic sugars (xylose and galactose). Standard deviations are calculated from triplicates.

The yields of total arabinose and OHS reached their maximums within 2 days, in contrast with the yield of total glucose, which increased until the end of hydrolysis (Figure 15). The yields of total arabinose and OHS were 88% and 83%, respectively (Table 8). The yield of total glucose increased to 20% (Table 8). Although the AX-AFH activity of Hemicellulase NS22002 – determined on water-insoluble wheat arabinoxylan – at pH 6
was only half of that at pH 4, at the end of hydrolysis of SAA-pretreated DGCF approximately two times more monomeric arabinose was released at pH 6 than at pH 4 (Figure 15). The yield of total arabinose was much higher at pH 6 than at pH 4 (Figure 15), which means that much more arabinose-containing oligosaccharides were liberated. The higher amount of oligosaccharides can contribute to obtain higher yield of monomer arabinose. The monomer and total yield of glucose were only 6% and 13%, respectively (Figure 15) in two days of hydrolysis, indicating that the major part of cellulose remained intact in the solid fraction.

At the end of hydrolysis at pH 6 the OHS/arabinose value regarding solubilised monosaccharides was 0.34 (Table 8), which is considered to be good hydrolysis selectivity. The OHS/arabinose value regarding total sugars present in the supernatant was 1.78 (Table 8), which is considered to be unacceptable.

Therefore, SAA pretreatment has been found to be an appropriate method to make the structure of DGCF accessible to the hemicellulose-degrading enzymes, as a significant part of the hemicellulose fraction was solubilised during hydrolysis with Hemicellulase NS22002 at pH 4 and 6. Whereas, there is not any pH value allowing the selective solubilisation of arabinose from arabinoxylan-containing insoluble materials using the multi-component enzyme preparations investigated in this study.

6.1.2. Sulphuric acid treatment under mild conditions

Acidic treatments were carried out by using sulphuric acid, and relatively short reaction times was chosen, as it has been found that the liberation of arabinose is much faster than that of xylose in the early period of dilute acid hydrolysis of corn fibre (Noureddini and Byun, 2010; Shibanuma et al., 1999). Acidic hydrolysis of DGCF were performed at 140°C, 120°C and 90°C at different acid concentrations and reaction times, according to experimental design. Investigation of acidic hydrolysis of corn fibre was carried out at 90°C.

Acid hydrolysis of DGCF at 140°C and 120°C

Fast dilute sulphuric acid treatments of DGCF were carried out at 140°C and 120°C according to experimental design, in which sulphuric acid concentration and reaction time varied from 0.15 to 0.75% (w/w) and from 5 to 15 min. The major part of the arabinose was solubilised immediately at both 140°C and 120°C, however, a great amount of OHS also appeared in the supernatant in all cases. During the hydrolysis of 140°C, at an acid concentration of 0.15% (w/w) and a reaction time of 5 min, the total OHS yield was more than 90% and all of the arabinose was solubilised. At the most severe reaction condition (0.75% (w/w) acid concentration, 15 min reaction time) both OHS and arabinose were completely recovered in the supernatant as monosaccharides. Therefore, in the applied range of acid concentration and reaction time, 140°C was found to be extremely high in terms of the selective arabinose release. However, it is a good choice to completely remove the hemicellulose fraction within a short time and with low acid consumption. At 120°C, the total arabinose yield and total OHS yield changed between 74% and 93% and
between 37% and 84%, respectively. Most of the OHS/A value regarding total sugars were in the range considered to be unacceptable. Although high arabinose yields were achieved, the treatments at 120°C did not meet our targets due to the great amount of released OHS.

Acid hydrolysis of DGCF at 90°C

During the acidic hydrolysis of DGCF at 90°C, the sulphuric acid concentration and reaction time were changed from 0.15 to 0.75% (w/w) and from 5 to 15 min, respectively (Table 9). The sugar yields and the OHS/A values obtained are listed in Table 9.

Table 9: Reaction conditions, sugar yields and OHS/A values of DGCF hydrolysis at 90°C

<table>
<thead>
<tr>
<th>Reaction conditions</th>
<th>Monomer</th>
<th>Oligomer **</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mY&lt;sub&gt;OH&lt;/sub&gt;</td>
<td>mY&lt;sub&gt;A&lt;/sub&gt;</td>
<td>mY&lt;sub&gt;[OHS/A]&lt;/sub&gt;</td>
</tr>
<tr>
<td>S (w/w %)</td>
<td>T (min)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>0.15</td>
<td>5</td>
<td>0.1</td>
<td>2.7</td>
</tr>
<tr>
<td>0.15</td>
<td>10</td>
<td>0.2</td>
<td>4.2</td>
</tr>
<tr>
<td>0.15</td>
<td>15</td>
<td>0.2</td>
<td>4.9</td>
</tr>
<tr>
<td>0.45</td>
<td>5</td>
<td>0.4</td>
<td>8.8</td>
</tr>
<tr>
<td>0.45</td>
<td>10</td>
<td>0.7*</td>
<td>12.6*</td>
</tr>
<tr>
<td>0.45</td>
<td>15</td>
<td>0.9</td>
<td>16.0</td>
</tr>
<tr>
<td>0.75</td>
<td>5</td>
<td>0.8</td>
<td>15.5</td>
</tr>
<tr>
<td>0.75</td>
<td>10</td>
<td>1.1</td>
<td>19.8</td>
</tr>
<tr>
<td>0.75</td>
<td>15</td>
<td>1.5</td>
<td>24.4</td>
</tr>
</tbody>
</table>

DGCF – destarched ground corn fibre, OHS – other hemicellulosic sugars (xylose and galactose), A – arabinose, m – monomer, o – oligomer, t – total, Y – yield expressed in percentage of the theoretical, S – sulphuric acid concentration, T – reaction time.

*Average values. **Variables related to oligomers were calculated as the difference of total sugars and monomers.

Negligible amount of OHS was recovered in monomer form, while the monomer arabinose yield changed between 3% and 24%. Monomer OHS and arabinose yields increased as a first-order function of both the acid concentration and the reaction time (Figure 16). The mY<sub>[OHS/A]</sub> was between 0.10 and 0.11, which means that approximately nine times more arabinose monomers were obtained in the liquid fraction than OHS monomers, corresponding to good selectivity of hydrolysis. The total OHS yields were low in all cases, even under the most severe conditions (0.75% (w/w) acid concentration and 15 min reaction time) it was only 7%. The total arabinose yield varied between 4% and 33%. The total OHS and arabinose yields changed as a first-order function of the acid concentration and the reaction time (Figure 16). The amount of OHS in oligomers was always larger than the amount of OHS monomers, while for arabinose the opposite was observed. The oligomer yields of arabinose and OHS increased with increasing acid concentration and/or reaction time. The OHS/A values for both the solubilised oligomers and total sugars were lower than the OHS/A of the starting material, which suggests that only the side chains of the hemicellulose structure was attacked at 90°C. According to the statistical evaluation, the linear term of acid concentration has the greatest effect on all the
response variables. The selectivities of the treatments at 90°C were satisfactory, but the achievable total arabinose yields were unacceptable.

Acid hydrolysis of DGCF at 90°C

The acid concentrations were increased from 0.15%, 0.45% and 0.75% (w/w) to 1%, 3% and 5% (w/w), respectively, with the aim of increasing the arabinose yield achievable. The sugar yields and the OHS/A values obtained are listed in Table 10.
Table 10: Reaction conditions, sugar yields and OHS/A values of DGCF hydrolysis by applying increased sulphuric acid concentration at 90°C

<table>
<thead>
<tr>
<th>Reaction conditions</th>
<th>Monomer</th>
<th>Oligomer **</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S (w/w %)</td>
<td>T (min)</td>
<td>mY_{OHS} (%)</td>
<td>mY_A (%)</td>
</tr>
<tr>
<td>------</td>
<td>------</td>
<td>--------</td>
<td>-------</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>1.4</td>
<td>24.7</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>1.9</td>
<td>30.7</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>2.5</td>
<td>37.7</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>4.4</td>
<td>51.9</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>6.2*</td>
<td>60.8*</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>8.4</td>
<td>66.4</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>6.9</td>
<td>61.3</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>10.5</td>
<td>71.0</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>14.4</td>
<td>74.9</td>
</tr>
</tbody>
</table>

DGCF – destarched ground corn fibre, OHS – other hemicellulosic sugars (xylose and galactose), A – arabinose, m – monomer, o – oligomer, t – total, Y – yield expressed in percentage of the theoretical, S – sulphuric acid concentration, T – reaction time.

*Average values. **Variables related to oligomers were calculated as the difference of total sugars and monomers.

The monomer arabinose yield varied from 25% to 75% as a second-order function of the acid concentration and as a first-order function of the reaction time (Figure 17). The monomer OHS yield depended on both the reaction time and the acid concentration linearly (Figure 17). It changed from 1% to 14% resulting in good hydrolysis selectivity. Although the m[OHS/A] increased with increasing acid concentration and/or reaction time, it was satisfactory (less than 0.4) at all points of the experimental design. The total arabinose yield also changed as a second-order function of the acid concentration and as a first-order function of the reaction time, and the total OHS yield also depended linearly on both the reaction time and the acid concentration (Figure 17). The total arabinose and OHS yields varied from 36% to 97% and from 8% to 64%, respectively. The yields of both arabinose and OHS oligomers increased with increasing acid concentration and/or reaction time. The amount of OHS in oligomer form was higher than the amount of monomer OHS in all cases. However, the monomer arabinose content of the hydrolysates was always higher than the amount of arabinose present as oligomers. By increasing the acid concentration and/or the reaction time the OHS proportion in the oligomers was increased. The linear term of acid concentration has the greatest effect on all response variables. As in most of the cases the total arabinose yield and t[OHS/A] were in the satisfactory range, optimisation by D-function approach was carried out. According to the optimization of D-function, the optimum conditions for our targets are 4.6% (w/w) acid concentration and 5 min reaction time. At this point the model predicted a total arabinose yield of 80.5% and a t[OHS/A] value of 0.74. Similar yield and selectivity were obtained experimentally at 5% (w/w) acid concentration and 5 min reaction time, as a total arabinose yield of 82.3% and a t[OHS/A] value of 0.77 were achieved (Table 10). Hence, under the appropriate conditions of acidic hydrolysis, an arabinose-rich liquid fraction can be produced from DGCF.
Table 10: Reaction conditions, sugar yields and OHS/A values of DGCF hydrolysis by applying increased sulphuric acid concentration at 90°C

DGCF – destarched ground corn fibre, OHS – other hemicellulosic sugars (xylose and galactose), A – arabinose, m – monomer, o – oligomer, t – total, Y – yield expressed in percentage of the theoretical, S – sulphuric acid concentration, T – reaction time.

*Average values. **Variables related to oligomers were calculated as the difference of total sugars and monomers.

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<table>
<thead>
<tr>
<th>Reaction conditions</th>
<th>Monomer</th>
<th>Oligomer</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomer Arabinose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligomer OHS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Arabinose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(w/w %) (min) (%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>1 5 1.4 24.7 0.11 6.7 11.5 1.11 8.2 36.1 0.43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 10 1.9 30.7 0.12 8.9 12.9 1.31 10.9 43.6 0.47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 15 2.5 37.7 0.13 11.6 14.1 1.56 14.2 51.9 0.52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 5 4.4 51.9 0.16 17.3 15.7 2.10 21.7 67.6 0.61</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 10 6.2* 60.8* 0.19* 24.4* 17.4* 2.66* 30.6* 78.3* 0.74*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 15 8.4 66.4 0.24 33.2 19.7 3.21 41.7 86.1 0.92</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>5 5 6.9 61.3 0.21 26.5 21.0 2.40 33.3 82.3 0.77</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 10 10.5 71.0 0.28 39.7 21.5 3.51 50.2 92.5 1.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 15 14.4 74.9 0.37 49.6 22.3 4.23 64.0 97.2 1.25</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 17: Response surfaces of monomer arabinose yield, monomer OHS yield, total arabinose yield and total OHS yield during acid hydrolysis of DGCF at 90°C by increased sulphuric acid

The response surfaces represent the reduced models and yields expressed in percentage of the theoretical. DGCF – destarched ground corn fibre, OHS – other hemicellulosic sugars (xylose and galactose).

During the sulphuric acid treatments of DGCF, small amount of glucose (2–5% of the glucan content of DGFC in the cases of hydrolysis at 90°C) was also released. That implies the existence of a non-starch glucan fraction in corn fibre, which can be easily hydrolysed by dilute acid treatments.

Acid hydrolysis of corn fibre at 90°C

Non-ground corn fibre was also tested in dilute acid treatments, since at an industrial scale milling of corn fibre should be omitted due to the high energy demand. In this case destarching process was also omitted, therefore the aim of this experiments was to obtain a supernatant containing glucose – derived from the hydrolysis of starch – and arabinose as the main sugar components.
Table 11: Reaction conditions, sugar yields and OHS/A values of corn fibre hydrolysis at 90°C

<table>
<thead>
<tr>
<th>Reaction conditions</th>
<th>Monomer</th>
<th>Oligomer **</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S (w/w %)</td>
<td>T (min)</td>
<td>mY&lt;sub&gt;OHs&lt;/sub&gt; (%)</td>
</tr>
<tr>
<td>0.25</td>
<td>25</td>
<td>5.4</td>
<td>4.5</td>
</tr>
<tr>
<td>0.25</td>
<td>50</td>
<td>5.6</td>
<td>8.3</td>
</tr>
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<td>75</td>
<td>5.8</td>
<td>11.8</td>
</tr>
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<td>25</td>
<td>6.7</td>
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</tr>
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<td>0.75</td>
<td>50</td>
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<td>42.3*</td>
</tr>
<tr>
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<td>75</td>
<td>9.9</td>
<td>53.2</td>
</tr>
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</tr>
<tr>
<td>1.25</td>
<td>75</td>
<td>17.2</td>
<td>70.3</td>
</tr>
</tbody>
</table>

OHS – other hemicellulosic sugars (xylose and galactose), A – arabinose, m – monomer, o – oligomer, t – total, Y – yield expressed in percentage of the theoretical, S – sulphuric acid concentration, T – reaction time. *Average values. **Variables related to oligomers were calculated as the difference of total sugars and monomers.

In these experiments the acid concentrations were 0.25%, 0.75% and 1.25% (w/w) and the reaction times were 25, 50 and 75 min. The sugar yields and the OHS/A values obtained are listed in Table 11. Monomer and total arabinose yields changed as a second-order function of sulphuric acid concentration, and as a first-order function of reaction time (Figure 18). The monomer and the total arabinose yields increased from 5% to 70% and from 8% to 86%, respectively. Both the monomer and total OHS yields depend in a quadratic way on the acid concentration and linearly on the reaction time (Figure 18). The monomer and total OHS yields increased from 5% to 17% and from 5% to 59%, respectively. The amount of oligomers, including the OHS and the arabinose, increased with increasing acid concentration and/or reaction time, and in parallel the proportion of arabinose in the oligomers decreased. When the acid concentration and the reaction time were higher than 0.75% (w/w) and 25 min, the solubilised OHS were mainly present in oligomers, unlike the solubilised arabinose. In most of the cases, values of m[OHS/A] and t[OHS/A] were in the satisfactory range, and apparently they have a minimum value. According to the evaluation of standardized effects of the independent variables and the interactions, the linear term of acid concentration has the greatest effect on all the response variables except for the t[OHS/A], where the interaction of linear terms of reaction time and acid concentration does. At 1.25% (w/w) sulphuric acid concentration and 75 min reaction time, a monomer arabinose yield of 70% and a monomer OHS yield of 17% were achieved. Shibanuma et al. (1999) achieved 55% arabinose yield and 15% xylose yield at the most favourable condition (0.3 N oxalic acid concentration, 1 h reaction time, 100 °C) found for selective arabinose liberation from destarched corn fibre.

The amount of glucose released in monomer form was negligible during the conditions investigated, it was less than 4% of the glucan content of corn fibre. The total glucose yield was around 35% in the cases of low acid concentrations (0.25% (w/w)) and it was around 45% in the cases of higher acid concentrations (0.75% and 1.25% (w/w)). It
implies that at 0.75% and 1.25% (w/w) acid concentrations almost the whole amount of starch was obtained in the supernatant as oligomers.

According to the optimization of the D-function, the optimum conditions for our targets are 1.1% (w/w) acid concentration and 51 min reaction time. At this point the model predicted a total arabinose yield of 73.6% and a t[OHS/A] value of 0.79. To verify the optimum, corn fibre was treated under these conditions in triplicate. The averaged results were in line with the model prediction, as a total arabinose yield of 75.9% and a t[OHS/A] value of 0.77 were achieved. Both values are inside the prediction interval, which proves the goodness of the model developed. Therefore, acidic hydrolysis of corn fibre under mild conditions is appropriate to produce a liquid fraction rich in glucose oligomers and arabinose.

Figure 18: Response surfaces of monomer arabinose yield, monomer OHS yield, total arabinose yield and total OHS yield during acid hydrolysis of corn fibre at 90°C

The response surfaces represent the reduced models and yields expressed in percentage of the theoretical. OHS – other hemicellulosic sugars (xylose and galactose).
Summarizing, sulphuric acid treatments of DGCF and corn fibre under appropriately mild conditions result in moderate arabinose yields with good hydrolysis selectivity, or high arabinose yields with significant amounts of other sugars solubilised. The other sugars are solubilised mainly in the form of oligosaccharides, while the major part of the arabinose is released in monomeric form.

6.1.3. Two-step acidic fractionation of corn fibre

The fractionation process of corn fibre includes two sequential hydrolyses catalysed by sulphuric acid (Figure 19). Based on the results of the previous section, in the first step corn fibre was treated under mild acidic conditions (90°C, 1.1% (w/w) sulphuric acid, 51 min) to solubilise the starch content and liberate most of the arabinose. After the first hydrolysis, considerable amount of oligosaccharides was obtained in the supernatant, thus an oligomer hydrolysis step was required to recover the sugars in monomeric form (Figure 19). Additional acid was not required to decompose oligomers of the first hydrolysate, when the oligomer hydrolysis step was carried out at 120°C for 1 h. After the first and oligomer hydrolyses, glucose, arabinose and xylose (+galactose) were recovered in the supernatant with the yields of 103%, 74% and 32% of theoretical based on the raw material composition (Table 6), respectively. In this case, glucose yield is calculated from the starch content of corn fibre as the cellulose is assumed to be intact under mild conditions applied in the first hydrolysis. Glucose yield of 103% might be explained by the presence of small amount of glucose in the hemicellulose fraction, which can be easily hydrolysed under mild acidic treatments. The glucose- and arabinose-rich hydrolysate contained 15 g/L glucose, 8.4 g/L xylose (+galactose), 10.5 g/L arabinose and 0.9 g/L acetic acid.

The solid residue of the first step was utilised in the second acidic hydrolysis to produce xylose-rich hydrolysate (Figure 19). Glucose, xylose (+galactose) and arabinose were recovered in the supernatant with the yields of 10%, 88% and 63% of theoretical based on the composition of first solid residue, respectively. The lower yield of arabinose than that
of xylose might be due to arabinose degradation. The low glucose recovery during the second hydrolysis resulted in a cellulose-rich solid fraction (Figure 19) containing 50% cellulose based on dry weight. The xylose-rich liquid fraction contained 3 g/L glucose, 28 g/L xylose (+galactose), 6.6 g/L arabinose and 2.3 g/L acetic acid. The total phenolics content was 5.3 g/L.

6.2. INVESTIGATION OF ARABINOSE BIOPURIFICATION AND XYLITOL FERMENTATION

Through the two-step acidic fractionation of corn fibre a glucose- and arabinose-rich liquid fraction, a xylose-rich liquid fraction and a cellulose-rich solid fraction were produced. The removal of the other sugars (glucose, xylose, galactose) from the glucose- and arabinose-rich liquid fraction is necessary to obtain pure arabinose solution. Therefore, microbial depletion of the undesirable sugars beside arabinose – referred to as arabinose biopurification – was investigated by using yeast strains under aerobic conditions. The xylose-rich hydrolysate is a promising raw material for microbial production of xylitol. Hence, yeast-mediated xylitol fermentation experiments were carried out and the effects of different factors for xylitol yield were investigated. Finally, an integrated process was developed and studied, in which the arabinose biopurification and xylitol fermentation were coupled.

6.2.1. Arabinose biopurification on semidefined medium

The capability of the investigated yeast strains (C. boidinii, C. guilliermondii, C. parapsilosis and H. anomala) for arabinose biopurification was tested on semidefined medium containing sugars typically present in hemicellulosic hydrolysates (xylose, arabinose and galactose) under aerobic conditions.

C. guilliermondii, C. parapsilosis and H. anomala utilised xylose, galactose and arabinose simultaneously to produce cell mass. C. parapsilosis consumed all of the sugars in two days, C. guilliermondii and H. anomala depleted xylose and galactose in two days and arabinose in three days (data not shown). Hence, these strains are found to be inappropriate for arabinose biopurification from hemicellulosic hydrolysate.

C. boidinii metabolised almost all of the xylose in one day (Figure 20). The galactose concentration was continuously decreased, 85% of the initial was consumed in two days and 7% of the initial was remained after four days. C. boidinii did not consume arabinose, even if the other carbon sources were present in negligible quantities (Figure 20). The purity of the arabinose solution was defined as the ratio of arabinose content to total sugar content, in percentage. After four days, the biopurificated medium contained 13 g/L arabinose and 0.4 g/L galactose, which resulted in a purity of 97%. As C. boidinii was found to be appropriate for arabinose biopurification on semidefined media, it was selected for further investigations.
6.2.2. Xylitol fermentation on semidefined medium

Preliminary studies showed that *Candida boidinii* can convert xylose into xylitol by using semidefined fermentation medium, however, the achievable xylitol yield was strongly affected by the aeration condition. In order to simulate different aeration conditions in shake flasks, the fermentations were performed at three levels of filling ratio (0.35, 0.5 and 0.65) each of them at two levels of rotation speed (125 and 220 rpm) using semidefined medium. Filling ratio is defined as the ratio of the medium volume to the flask volume. Oxygen transfer rate of the different settings of filling ratio and rotation speed was determined. The investigation of the effect of OTR to the xylitol yield achievable was performed with 1 g/L initial cell concentration and 30 g/L initial xylose concentration.

The higher filling ratio resulted in the lower OTR, while the higher rotation speed increased the OTR, during shake flask fermentations (Figure 21). At the rotation speed of 220 rpm, the OTR value decreased from 6.6 mmol/(L×h) to 5.2 mmol/(L×h) by increasing the filling ratio from 0.35 to 0.65. At the rotation speed of 125 rpm, the OTR value decreased from 4.2 mmol/(L×h) to 1.9 mmol/(L×h) by increasing the filling ratio from 0.35 to 0.65. The xylitol yield continuously increased by decreasing the OTR value until 2.8 mmol/(L×h), where a xylitol yield of 44% was obtained (Figure 21). That condition of aeration can be referred to as microaerobic condition (Walther et al., 2001), and was set for the subsequent experiments. Further decrease in the OTR resulted in significant decrease in the xylitol yield (Figure 21). The xylitol fermentation performed at 1 g/L initial cell concentration and 30 g/L initial xylose concentration at 2.8 mmol/(L×h) OTR is referred to as base case in the following sections.
Figure 20: Arabinose biopurification on semidefined medium using *Candida boidinii* NCAIM Y.01308. Standard deviations are calculated from duplicates.

### 6.2.2. Xylitol fermentation on semidefined medium

Preliminary studies showed that *Candida boidinii* can convert xylose into xylitol by using semidefined fermentation medium, however, the achievable xylitol yield was strongly affected by the aeration condition. In order to simulate different aeration conditions in shake flasks, the fermentations were performed at three levels of filling ratio (0.35, 0.5 and 0.65) each of them at two levels of rotation speed (125 and 220 rpm) using semidefined medium. Oxygen transfer rate of the different settings of filling ratio and rotation speed was determined. The investigation of the effect of OTR to the xylitol yield achievable was performed with 1 g/L initial cell concentration and 30 g/L initial xylose concentration.

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Figure 21: Xylitol yields achieved on semidefined medium using *Candida boidinii* NCAIM Y.01308 at different aeration conditions. Filling ratios and rotation speeds applied are indicated under the xylitol yields. Standard deviations are calculated from triplicates.

Xylitol fermentations of the same initial conditions (1 g/L initial cell concentration and 30 g/L initial xylose concentration) using *C. boidinii* followed the same trends, which is demonstrated in Figure 22.

Figure 22: Fermentation profile of xylitol production on semidefined medium under microaerobic condition (2.8 mmol/(L×h) OTR) at 1 g/L initial cell concentration and 30 g/L initial xylose concentration by using *Candida boidinii* NCAIM Y.01308 (base case). Standard deviations are calculated from triplicates.
Xylitol concentration keeps increasing as long as xylose is available in the fermentation broth. After xylose is depleted, *C. boidinii* starts to consume xylitol, hence xylitol concentration has a maximum value during the fermentation. Small amount of ethanol is always produced simultaneously with xylitol, however, after xylose depletion the ethanol is also consumed. The cell mass continuously increases, which indicates that xylitol and ethanol are used to form cell mass after xylose depletion.

The effects of initial cell concentration, initial xylose concentration and methanol addition to the xylitol yield were investigated. The effect of high initial xylose concentration for the fermentative capacity of *C. boidinii* was also investigated using 70 g/L initial xylose concentration (Table 12). The high initial xylose concentration resulted in a xylitol yield of 40%, which is slightly lower compared to the xylitol yield of the base case (44%) (Table 12). The xylitol concentration continuously increased until the end of the fermentation resulting in a volumetric productivity of 0.3 g/(L×h), which is similar to that obtained in the base case (0.28 g/(L×h)).

The effect of high cell density on the fermentation process was investigated using 5 g/L initial cell concentration. High initial cell density resulted in significantly higher xylitol yield within shorter fermentation time, compared to the base case, as 58% xylitol yield was achieved in one day (Table 12). The volumetric productivity of the fermentation was 0.73 g/(L×h).

Addition of methanol as a co-substrate did not result in significant increase of xylitol yield, as a xylitol yield of 60% was obtained when 12 g/L methanol was added to the fermentation broth containing 30 g/L initial xylose and 5 g/L initial cell concentrations (Table 12). On the other hand, longer time was needed (2 days) to reach the highest xylitol concentration compared to that of without methanol addition (1 day), which resulted in a volumetric productivity of 0.38 g/(L×h).

Table 12: Fermentation conditions, maximal xylitol yields achieved on semidefined media and times required to achieve the maximal yield

<table>
<thead>
<tr>
<th>OTR (mmol/Lh)</th>
<th>Initial cell concentration (g/L)</th>
<th>Initial xylose concentration (g/L)</th>
<th>Co-substrate</th>
<th>Xylitol yield (% of theoretical)</th>
<th>Time (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.8</td>
<td>1</td>
<td>30</td>
<td>-</td>
<td>44 (4)</td>
<td>2</td>
</tr>
<tr>
<td>2.8</td>
<td>1</td>
<td>70</td>
<td>-</td>
<td>40 (1)</td>
<td>4</td>
</tr>
<tr>
<td>2.8</td>
<td>5</td>
<td>30</td>
<td>-</td>
<td>58 (5)</td>
<td>1</td>
</tr>
<tr>
<td>2.8</td>
<td>5</td>
<td>30</td>
<td>12 g/L MeOH</td>
<td>60 (5)</td>
<td>2</td>
</tr>
</tbody>
</table>

*Standard deviations are calculated from triplicates and indicated in parenthesis.*

According to our investigation of xylitol production on semidefined media using *C. boidinii*, aeration and initial cell density have the greatest effects on xylitol yield. Xylitol production is favoured under microaerobic condition (2.8 mmol/(L×h) OTR) using increased (5 g/L) initial cell concentration.
6.2.3. Integration of arabinose biopurification and xylitol fermentation using corn fibre hydrolysate

The glucose- and arabinose-rich hydrolysate derived from the fractionation process of corn fibre was utilised to produce pure arabinose solution through biopurification using *C. boidinii*. The biopurification medium contained 13.5 g/L glucose, 9.2 g/L arabinose, 5.8 g/L xylose, 2.3 g/L galactose and 0.8 g/L acetic acid after the inoculation, and the initial cell concentration was 0.5 g/L. During the biopurification of glucose- and arabinose-rich hydrolysate of corn fibre, the consumptions of glucose, xylose and galactose occurred simultaneously. Glucose and xylose were totally depleted in one day and two days, respectively (Figure 23). Galactose was not consumed completely, 32% and 57% of the initial amount were removed in one day and four days, respectively (Figure 23). Acetic acid was consumed within one day (data not shown). Most of the cell mass (4.5 g/L) was formed in one day, however, cell concentration increased continuously until 6.5 g/L (Figure 23). Beside the cell formation, considerable amount of ethanol was produced (2 g/L) in one day. However, it was consumed within the next day (Figure 23). The arabinose concentration was constant through the whole biopurification process (Figure 23). After three days of biopurification the medium contained 9.2 g/L arabinose and 1 g/L galactose (Figure 23), that is the purity of arabinose was 90% of total sugars, which exceeds the purity requirement for crystallization of L-arabinose from solution (Cheng et al., 2011).

According to our investigation of xylitol production on semidefined media using *C. boidinii*, aeration and initial cell density have the greatest effects on xylitol yield. Xylitol production is favoured under microaerobic condition (2.8 mmol/(L×h) OTR) using increased (5 g/L) initial cell concentration.

![Figure 23: Arabinose biopurification on glucose- and arabinose-rich hydrolysate of corn fibre using *Candida boidinii* NCAIM Y.01308](image)

*Standard deviations are calculated from triplicates.*

According to the investigation of Cheng et al. (2011), biopurification of xylose mother liquor (350–400 g/L xylose, 150–180 g/L arabinose and 150–180 g/L glucose and galactose) by *P. anomala* under optimized conditions (32.5°C, 75 h and 21% (v/v) xylose mother liquor) results in an arabinose purity of 86% of total sugars. Park et al. (2001)
developed a method to produce arabinose from purified corn fibre arabinoxylan by enzymatic hydrolysis and arabinose biopurification by *Williopsis saturnus* yeast strain. During the biopurification of the enzymatic hydrolysate of corn fibre, the xylose was consumed almost entirely in three days without any loss of arabinose, however data of the concentrations of other components were not reported. Compared to these methods, arabinose biopurification from acidic hydrolysate of corn fibre using *C. boidinii* is a competitive strategy.

The xylose-rich hydrolysate derived from the fractionation of corn fibre was utilised to produce xylitol, however, it contained significant amount of phenolic compounds, that are strong inhibitors of microbial xylitol production (Parajó et al., 1998). In order to remove phenolic substances the xylose-rich hydrolysate was treated with activated carbon before xylitol fermentation. After the activated carbon treatment, the total phenolic content was reduced to 0.3 g/L, while the xylose concentration did not change. The initial composition of the xylitol fermentation medium was the following: 2.2 g/L glucose, 24.1 g/L xylose (+galactose) and 6.1 g/L arabinose, and 2.3 g/L acetic acid. The fermentation was performed under microaerobic condition (2.8 mmol/(L×h) OTR) using 5 g/L initial cell concentration. The *C. boidinii* cell mass used to inoculate the fermentation was harvested from the arabinose biopurification. 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. The fermentation profile of xylitol production in the integrated process is shown in Figure 24. Glucose was present in the medium in small concentration and it was depleted within one day (Figure 24). The arabinose concentration was unaltered through the whole fermentation (Figure 24). The cell concentration was almost constant, less than 0.5 g/L was produced in four days (Figure 24). Xylitol concentration increased until the third day, when it was 10.4 g/L (Figure 24), resulting in a volumetric productivity of 0.14 g/(L×h). This concentration corresponds to a xylitol yield of 43% of theoretical based on the initial concentration of xylose (+galactose). However, according to our previous results, 80% of the component measured as xylose (+galactose) in the xylose-rich hydrolysate was xylose, thus the initial xylose concentration can be assumed to be 19.3 g/L. By using this assumption during the calculation of the concentration of theoretically achievable xylitol, a xylitol yield of 53% was obtained. It is only 5% less, than the xylitol yield of the case performed under the same conditions (microaerobic aeration, 5 g/L initial cell concentration) on semidefined media. During the fermentation of xylose-rich hydrolysate, higher amount of ethanol (3 g/L) was produced in two days, than that obtained under the same conditions on semidefined medium (1 g/L). After the third day of xylitol fermentation on xylose-rich hydrolysate of corn fibre, the fermentation broth contained 10.4 g/L xylitol, 6.1 g/L arabinose, 4.1 g/L xylose (+galactose) and 2.7 g/L ethanol (Figure 24). As the xylitol concentration is approximately the same as the concentration of the residual sugars, further purification steps might be required to enable xylitol crystallization from the broth.
Leathers and Dien (2000) developed a two-stage, sequential fermentation process for xylitol and arabinose production from neutralized and deionized corn fibre hydrolysate using *P. guilliermondii*. This strategy resulted in a xylitol yield of 0.27 g xylitol/g initial xylose within four days, which yield is around half of that achieved in our process. Rao et al. (2006) investigated xylitol production from corn fibre hydrolysate, which was neutralized, treated with activated charcoal and ion exchange resins. *C. tropicalis* cells were adapted by sub-culturing in hydrolysate containing medium for 20 cycles. This method resulted in a xylitol yield of 0.58 g/g xylose utilized within two days. In our process a xylitol yield of 0.54 g/g xylose utilized was obtained within three days, by assuming that the initial xylose was 19.3 g/L and it was completely utilized, however deionization step was not included. Buhner and Agblevor (2004) investigated different detoxification methods to produce xylitol from concentrated corn fibre hydrolysate by using *C. tropicalis*. The highest xylitol yield, 0.4 g/g xylose utilized, was obtained within four days in the case of the highest concentrations (three times of the original hydrolysate) that had been partially neutralized by adding calcium hydroxide and treated with activated charcoal prior to the fermentation. Hence, xylitol production from corn fibre could be more favourable in our integrated process than in those found in the literature.

Summarizing, *C. boidinii* NCAIM Y.01308 was found to be suitable to produce pure arabinose solution from hemicellulosic hydrolysate through biopurification, and to be appropriate for xylitol production using high initial cell concentration. An integrated biorefinery process based on the diverse action of *C. boidinii* was developed, in which cell mass produced in aerobic biopurification was used to perform the xylitol fermentation under microaerobic condition.
The work presented in this thesis focuses on the development of biorefinery processes for value-added utilisation of corn fibre. Two kinds of approaches, i.e. process simulation and experimental investigations were involved in this progress. In the process simulation part, different process configurations of a corn fibre-based biorefinery producing bioethanol, biomethane and xylitol as main products were evaluated in technological point of view. In the experimental part, different biotechnological methods aiming the production of arabinose and xylitol from corn fibre were examined. Finally, an integrated biorefinery process is proposed, which is based on a two-step acidic fractionation of corn fibre and the diverse action of *C. boidinii* yeast.

**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

Biopurification of hemicellulose hydrolysate is an interesting and inexpensive strategy to produce pure arabinose solution through the depletion of other sugars using the adequate microorganisms. 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

During aerobic biopurification, the undesired sugars are utilised mainly to produce cell mass, which occurs as by-product of the process. In the xylitol fermentation high initial cell concentration is required to achieve high xylitol yield and volumetric productivity. 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. Utilization of on-site by-products and integration of different production routes are thought to be crucial to develop a viable biorefinery process. Considering those conceptions and the results of the acidic treatments of corn fibre, an integrated biorefinery process was invented, which is based on the two-step acidic fractionation of corn fibre and the diverse action of *C. boidinii* (Figure 25). The two-step acidic fractionation of corn fibre resulted in a glucose- and arabinose-rich hydrolysate, a xylose-rich hydrolysate and a cellulose-rich solid fraction. The glucose- and arabinose-rich hydrolysate was utilised in arabinose biopurification after pH adjustment. After three days of biopurification the medium contained 9.2 g/L arabinose and 1 g/L galactose, hence the purity of arabinose was 90% of total sugars. The xylose-rich hydrolysate was utilised in xylitol fermentation after detoxification by activated carbon and pH adjustment. After the third day of xylitol fermentation the broth contained 10.4 g/L xylitol, 6.1 g/L arabinose, 4.1 g/L xylose (+galactose) and 2.7 g/L ethanol. As the xylitol concentration was approximately the same as the concentration of the residual sugars, further purification steps might be required to enable xylitol crystallization from the broth. The xylitol volumetric productivity was 0.14 g/(L×h).
Future investigations

Utilisation of the cellulose-rich solid fraction is one of the main issues of the further development of the proposed biorefinery process. Preliminary studies have shown that the cellulose-rich solid fraction can be easily hydrolysed by commercial cellulase enzyme preparations using relatively high solid loading (15 (w/w) dry matter). Hence a glucose-rich liquid fraction can be obtained, which might be utilised to produce bioethanol or other value-added products via fermentation.

Investigation of purification techniques and recovery processes of arabinose and xylitol from the fermented broths are also crucial in terms of the development of a viable process. Connected to this issue, it is important to increase the achievable arabinose and xylitol concentrations.
8. 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. Energy efficiency increases from 59% to 73% in the case of incineration of the hydrolysis residue and from 53% to 71% in the case of anaerobic digestion of the hydrolysis residue, 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. Heat demand of fractionation and distillation are 36–41% and 41–46% of the heat demand of the whole biorefinery, respectively (Paper I).

3. Soaking in aqueous ammonia pretreatment (60°C, 6 hours, 15% (w/w) of ammonia solution, 10% (w/w) dry matter) of destarched ground corn fibre is an appropriate method to facilitate selective hemicellulose hydrolysis using Hemicellulase NS22002 (Novozymes) enzyme preparation at pH 6, as more than 80% of the hemicellulose fraction and only 13% of the cellulose content are solubilised within 2 days of hydrolysis (50°C, 3% (w/w) dry matter, 0.02 g enzyme preparation/g dry matter) (Paper II).

4. Acid hydrolysis of destarched ground corn fibre at appropriately mild conditions (90°C, 5 min, 5% (w/w) sulphuric acid, 3% (w/w) dry matter) 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 acidic hydrolysis of corn fibre (90°C, 1.1% (w/w) sulphuric acid, 51 min, 10% (w/w) dry matter) results in a supernatant, from which glucose- and arabinose-rich liquid fraction can be obtained (120°C, 1.1% (w/w) sulphuric acid, 30 min), and a solid fraction, which is appropriate to produce xylose-rich supernatant (120°C, 1.1% (w/w) sulphuric acid, 30 min, 10% (w/w) dry matter) (Paper III, Paper IV).

6. *C. boidinii* NCAIM Y.01308 is suitable to produce pure arabinose solution from hemicellulosic hydrolysates through aerobic biopurification. Arabinose biopurification of the glucose- and arabinose-rich hydrolysate of corn fibre using *Candida boidinii* results in a liquid fraction containing 9.2 g/L arabinose and 1 g/L galactose, which corresponds to an arabinose purity of 90% based on sugar content (Paper IV).

7. *C. boidinii* NCAIM Y.01308 is suitable for xylitol production at an oxygen transfer rate of 2.8 mmol/(L×h). Xylitol fermentation on the activated carbon-treated, xylose-rich hydrolysate of corn fibre using *Candida boidinii* results in a xylitol volumetric productivity of 0.14 g/(L×h) (Paper IV).
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STATEMENT

I the undersigned, hereby declare, that this PhD thesis was written by me, and I only used the sources listed in the reference list.

NYILATKOZAT

Alulírott Fehér Csaba kijelentem, hogy ezt a doktori értekezést magam készítettem és abban csak a megadott forrásokat használtam fel. Minden olyan részt, amelyet szó szerint, vagy azonos tartalomban, de átfogalmazva más forrásból átvettem, egyértelműen, a forrás megadásával megjelöltem.

Budapest, 2015. augusztus 24. Fehér Csaba
Abstract
Corn fibre, a co-product of corn wet milling, can be a suitable raw material of a biorefinery producing biofuels and value-added chemicals. The simulated process is able to produce bioethanol, biomethane and xylitol synergistically, while it also covers its own heat demand. The proposed plant consists 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. Various scenarios of the biorefinery were investigated and the process configurations were compared in terms of energy efficiency, or mass flows of the products. Incineration of the sludge and production of district heat are found to be effective methods to increase the energy efficiency, on which aerobic sludge yield has a great effect. The solid-liquid separations, which are carried out in filterpress, have a curial role in terms of energy efficiency. Combustion of the solid part of cellulose hydrolysis residue is favourable compared with the anaerobic digestion, except if the dry matter content of the filterpressed solid was set to 30% instead of 40%. The amounts of the produced xylitol and biomethane are variable, which ensures the ability of market adaptation for the biorefinery.

Keywords
Biorefinery · process simulation · corn fibre · xylitol · biofuels

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1 Introduction
Bioethanol is expected to be one of the main renewable alternatives replacing fossil fuels in the transport sector [1]. It is very important considering both the danger of running out of fossil fuels in the next decades and environmental problems, like the acceleration of the global warming [2]. Bioethanol used as a transportation fuel can help in reducing carbon dioxide accumulation by allowing the carbon dioxide content of the fossil fuels to remain in storage [3]. Traditional bioethanol production is based on materials rich in sugar or starch. The ethanol production worldwide was 74 billion litres in 2009 [4], and is expected to reach 100 billion litres in 2015 [3]. To be able to meet the future increasing demands, bioethanol also needs to be produced from lignocellulosic feedstocks. However, the economically viable technologies to produce bioethanol from lignocellulosic biomass are still under development all over the world, and no process concept has been demonstrated on an industrial scale yet. The main barrier can be solved by the utilizations of the by-products and wastes of the bioethanol process. Many investigations were carried out in this topic [5]-[8], which led to the idea of producing bioethanol in biorefinery process [9].

The biorefinery concept describes a large highly-integrated complex incorporating individual processes that synergistically convert biomass feedstocks into fuels, electrical power and/or heat, value-added chemicals, and other materials, with zero waste approach [10, 11]. Depending on raw materials, technological processes, and products obtained, the following biorefinery platforms can be distinguished: sugar (biochemical), syngas (thermochemical), biogas, or carbon-rich chains platforms [11]. However, they can incorporate other processes from other platforms: e.g. the distillation stillage can be digested anaerobically, or the lignin can be transformed thermochemically in a sugar platform biorefinery. In these cases, the process is called two platform biorefinery. Economic viability of the biorefinery is highly sensitive to raw material cost, operating scale, and energy consumption of the applied process steps. High energy efficiency is of great importance regarding economic feasibility [1]. Increased productivity and efficiency can be achieved through operations that decrease overall energy demand of the biorefini-
supply its own steam requisite. In the base case A, bioethanol, as a base requirement for both base cases that the process can – were modelled and investigated in this study. It is defined
finery plant – A and B showed in Figs. 1 and 2, respectively
dry metric ton corn fibre annually. Two base cases of the biore-
ance, other component is defined, which is not involved in any
on the publication of Kálmán et al. [14]. To close the mass bal-
ifications is
cess. The accuracy of the target values adjusted by design spec-
transportation properties of all the streams involved in the pro-
ese. The potential to achieve this goal: i; increasing the dry matter (DM) content during the ethanol fermentation to decrease the energy demand of the distillation step [5] ii; heat integration of the process steps [12] iii; anaerobic digestion of the stillage [13].
In this study, various process configurations of a corn-fibre-
based biorefinery having the potential to produce bioethanol, biomethane, xylitol, electricity and heat were investigated. Corn fibre is an abundant and inexpensive by-product of the corn wet milling process, which contributes to about 10% of the grain dry matter [14]. It is utilized mainly as low-value animal feed, however, as the market of animal feed is limited, the increase of the corn processing may result in market problems for the excess [15]. Many studies were carried out to find the feasible processing of the corn fibre to produce value-added products such as bioethanol, corn fibre gum, xylo-oligosaccharides, corn fibre oil, xylitol, and many other fermentable chemicals or enzymes [14], [16]-[20]. Regarding these publications, corn fibre seems to be promising feedstock for a biorefinery. The configurations of the corn-fibre-based biorefinery modelled in this study were compared in respect of energy efficiency and mass flows of the products.

2 Materials and methods

2.1 Simulation software

The process was modelled by Aspen Plus flow-sheeting software (Aspen Tech Inc, Cambridge, MA, USA), which is capable to solve mass and energy balances, and to calculate the thermodynamic properties of all the streams involved in the process. The accuracy of the target values adjusted by design specifications is ± 0.1%. The physical data for the components were obtained from the built-in database of Aspen Plus, or from databank of National Renewable Energy Laboratory (NREL, Golden, CO) on biomass components [21]. Data not directly available were estimated from data on similar components [22].

2.2 Raw material

Corn fibre is assumed to contain 21.3% starch, 15% cellulose, 22% xylan, 10.9% arabinan, 2.2% hemicellulosic glucan, 2% acetate, 14% protein, 5% lipids, 3% lignin and 1% inorganic compounds related to the dry matter. These data are based on the publication of Kálmán et al. [14]. To close the mass balance, other component is defined, which is not involved in any reaction during the process. The dry matter content of the raw material is assumed to be 40%.

2.3 Process description

The proposed biorefinery plant is assumed to process 95 000 dry metric ton corn fibre annually. Two base cases of the biorefinery plant – A and B showed in Figs. 1 and 2, respectively – were modelled and investigated in this study. It is defined as a base requirement for both base cases that the process can supply its own steam requisite. In the base case A, bioethanol, biomethane and in some scenarios also district heat are produced. In the base case B, beyond bioethanol and biomethane, crystalline xylitol is available as a value-added product. In both base cases electricity is produced during steam generation in considerable amount. The part of this electricity not consumed in the plant can be also realized as a product.

Fractionation

The first major step in the process is the fractionation, which is considered to be one of the most important steps in the biorefinery concept. The fractionation consists of two minor steps. The first step of fractionation is separation of the starch content stuck to the aleurone layer, which is carried out by hot water treatment at 120°C. The starch content is assumed to be released and solubilized completely [14]. The protein content is solubilized with the conversion factor of 0.236. Some xylose and arabinose are also released during the hot water treatment (conversion factors of the reactions: xylan to xylose: 0.059, arabinan to arabinose: 0.055). After hot water treatment the solubilized starch is removed from the solids in filterpress. The second step of fractionation is weak acid treatment by 1% sulphuric acid at 120°C to hydrolyze the hemicellulosic polymers into monomeric sugars. The assumed reactions and the conversion factors are the following: xylose to xylose 0.926, arabinan to arabinose 0.961, protein to soluble protein 0.455, glucan to glucose 1, acetate to acetic acid 0.561, lignin to soluble lignin 0.767. The acid-hydrolysed hemicellulosic sugars are separated from the solid residue in filterpress only in the base case B, while in the base case A, it is recovered with the distillation residue. In both base cases, the WIS (water-insoluble solid) contents before the hot water and the weak acid treatments were set to 30% and 22%, respectively. The same settings were applied in all filterpress units built in the model, which are the followings. The solid retention of the filterpress was assumed to be 0.99, and the dry matter content of the solid stream was 40% (or 30% in some scenarios). If it is necessary, washing liquid (water) is used to move 90% of the soluble component into the supernatant. The washing curve is based on a washing model developed for the recovery of lignin from pulp residue [22, 23]. The reactions and the conversion factors used in the model are based on the publication of Kálmán et al. [14]. Although the results reported in this article are based on fractionation experiments carried out at 10% WIS content, the conversion factors built in the model, where the treatments are performed at higher WIS content, were assumed to be the same.

Enzymatic hydrolysis and ethanol fermentation

The starch fraction is liquefied at 90°C by α-amylase and then saccharified by glucoamylase. The total amount of starch is assumed to be converted into glucose. The enzyme loadings of α-amylase and glucoamylase are 0.025 and 0.055 g enzyme preparation/g starch, respectively [24]. The cellulose fraction containing 10% WIS is directed to cellulose hydrolysis, which
is carried out by cellulase enzyme complex (5 filter-paper unit/g dry matter) at 50°C [14]. The cellulose fraction is referred to as lignocellulose fraction in the base case A, as the hemicellulose sugars are not separated in filterpress. According to Kálmán et al. [14] 92.7% of the cellulose is converted to glucose in both base cases. Residual solid of the cellulose hydrolysis (hydrolysis residue) contains mainly lignin, lipids, proteins and inorganic compounds, and is separated from the supernatant in filterpress after the hydrolysis. The sugar-rich liquors derived from the starch and cellulose hydrolysis are mixed and then subjected to the yeast cultivation and ethanol fermentation. Hence, the first and second generation ethanol productions are performed at the same time and place. This method can be referred to as integrated ethanol fermentation process [25]. Ordinary baker’s yeast is used to convert the glucose into ethanol at 35°C. The division of the sugar-rich liquors between the yeast cultivation and the fermentation was set to achieve 3 g DM/L yeast concentration in the fermentation. During ethanol fermentation the yeast converts 95% of the incoming glucose to ethanol, and growth of yeast cell mass is assumed to be negligible. By-product reactions are also assumed, like glucose to acetic acid, glucose to succinic acid and glucose to glycerol with the conversion factor of 0.1 for all reactions. Two percent of the glucose is remained in the fermented broth. As ordinary baker’s yeast is used for ethanol fermentation, the pentose sugars are not consumed.

**Distillation and dehydration**

Distillation and molecular sieve adsorption are used to produce pure (>99.8%) ethanol. The distillation step consists of two parallelly operating stripper columns (25 trays) to separate the ethanol from the fermented broth, and a rectification column (35 trays) to concentrate the ethanol to 94%. The columns operate at different pressures to be thermally coupled in order to reduce the energy demand. The feed is preheated in two steps, first by process streams and then by primary steam, before being divided between the two strippers. Overhead vapour from the first stripper (3 bar) is used as heating medium in the reboiler of the second stripper before being fed to the rectifier. The overhead vapour from the second stripper (1.25 bar) is used as heating medium in the reboiler of the rectifier, together with some primary steam, if it is necessary. The ethanol recovery is set to 99.5% in the three columns. Considering the presence of solids such as lignin and yeast in the feed stream, the strippers operate with a modest Murphree efficiency (50%). The rectifier operates without solids, so the Murphree efficiency was set to a higher value (75%). The remaining water in the overhead vapour (0.35 bar) leaving the rectifier is removed in the dehydration columns, which are then regenerated with pure ethanol stream. The regenerate is returned to the rectifier [26].
Anaerobic digestion
The feed streams of the anaerobic digestion (AD) are the stillage derived from the distillation and in some scenarios the separated hydrolysis residue. In the base case B, one more stream is directed to the AD, referred to as mother liquor, which is derived from the xylitol recovery. Anaerobic digestion is performed under mesophilic conditions, hence the inlet flows are cooled down to 37°C before being fed to the digester. The assumed degradation factors are 90% for soluble sugars, organic acids, sugar alcohols, ethanol, enzymes, yeast and water-soluble protein; 50% for polysaccharides, water-insoluble proteins, lipids and water-soluble lignin; 0% for water-insoluble lignin. The yields of the methane and the anaerobic sludge are assumed to be 0.35 Nm³/kg COD (chemical oxygen demand) removed and 0.03 kg sludge DM/kg COD fed, respectively. All organic components involved in the COD calculations, except for the water-insoluble lignin, as it was considered to be inert in respect of biogas and sludge production. The biogas is assumed to consist of approximately 50% methane, 46% carbon dioxide and 4% water [13].

Biogas upgrading
If biogas is expected as a marketable product like fuel for the transport sector, or support for the natural gas grid, it is needed to purify the methane. The removal of carbon dioxide and water is performed in the gas upgrading step, which is based on amine absorption-desorption, for which data were obtained from the literature [27]. The upgrading step consists of an absorption column to react the carbon dioxide with amine solution, and a desorption column to regenerate the carbon dioxide rich liquid leaving the absorption column. The raw biogas containing large amount of carbon dioxide is introduced at the bottom of the absorption column, and travels counter-current to the amine solution introduced at the top of the column. The absorption column is operated at high pressure (8 bar) and the product stream, which is taken away from the top of the tower, consists of more than 95 v/v % methane. Hence, it can be called as biomethane. The carbon dioxide rich amine solution is sent to the top of the desorption tower, which is equipped with a reboiler, and operates at atmospheric pressure. The carbon dioxide rich amine solution from the adsorption tower is pre-heated with the regenerated amine solution. Losses of amine and water are made up, and the lean amine solution is pumped back to the adsorption tower. The amine used is diethanolamine (DEA). The DEA load is 2.5 mol amine/mole carbon dioxide, and the DEA concentration is 15 w/w %. The boilup ratio in the reboiler was set to achieve 90% regeneration of the amine solution [28].

Aerobic waste water treatment
The whole effluent of the anaerobic digestion is driven to the aerobic waste water treatment to remove the remaining organic materials. The organic material is assumed to be removed en-
entirely, so the degradation factors of all organic compounds are unity. The aerobic sludge is presumed to form at a yield of 0.5 kg sludge DM/kg COD [29] (or 0.3 kg sludge DM/kg COD in some scenarios). The sludge is separated from the liquid fraction in filterpress. The liquid fraction can be treated e.g. in ozone treatment to produce clean water. After the aerobic treatment, however, no more water cleaning steps were considered in this study.

**Combined heat and power production**

Superheated steam (91 bar, 470°C) is generated in a steam boiler by burning one part of the raw biogas and in some scenarios also the hydrolysis residue, the sludge and the separated cell mass from the xylitol production. The heat losses in the steam boiler are assumed to be 1%. The generated steam is allowed to expand to 4 bar through a high-pressure turbine to produce electricity. The isentropic and the mechanical efficiency of the turbine are presumed to be 90% and 97%, respectively. The amount of the biogas incinerated in the burner is set to produce enough saturated steam of 4 bar, which can satisfy the process heat demand. The return liquid from the heating system of the process, which is 144°C and 4 bar, is used as the feed water for the superheated steam generation. The flue gases leaving the boiler are used to preheat the feed water to 220°C and then the air used for combustion. The temperature of the flue gases after the air heater is fixed at 150°C. At this temperature, the leaving flue gases consist of considerable amount of steam as the solid feeds of the burner have relatively low DM content (40% or 30%). Some of this latent heat can be utilized in the flue gas condenser, in which the temperature of the flue gases is reduced to 50°C. The recovered heat is consumed during the production of district heat in some scenarios of the base case A, and in the evaporation of the fermented broth derived from the xylitol production in the base case B. The temperature of the return water from the district-heating system is raised from 45°C to 90°C by passing the stream through the flue gas condenser and by the help of condensing some of the 4 bar steam withdrawn from the high-pressure turbine.

In one configuration the whole raw biogas is combusted in base case B, hence some steam is produced in excess. In this scenario a low-pressure turbine is built in the combined heat and power production (CHP) step to utilize the excess steam by producing more electricity. The pressure of steam after the low-pressure turbine is set to 1 bar. The isentropic and the mechanical efficiency of the low-pressure turbine are presumed to 85% and 97%, respectively [12].

**Xylitol fermentation and recovery**

The xylitol fermentation and recovery steps are implemented only in the base case B, in which the hydrolysed hemicellulosic sugars are separated from the solid fraction, before the ethanol fermentation. The hemicellulose fraction is utilized to produce xylitol from xylose and arabinose in two fermentation steps, operating sequential. In the first step the whole xylose content is consumed by a *Candida* yeast strain to produce xylitol and cell mass. The conversion factor of the xylose to xylitol reaction was set to 0.671 based on literature data [30]. The remaining xylose and the glucose fed are utilized by the yeast to form cell mass with a yield of 0.5 g DM/g sugar. After the first fermentation the cell mass is separated from the broth in filterpress to prevent the xylitol consumption by the *Candida* yeast, which may occur after the depletion of the other carbon sources [30]. In the second fermentation step, the xylitol formation from arabinose is carried out by a genetically engineered *Escherichia coli* strain, with the conversion factor of 0.709 [31]. This strain is assumed to use glycerol in order to maintain the redox balance in the cells, and to form cell mass. The amount of the added glycerol is half of the arabinose input, and is consumed entirely [31]. The cell yield is assumed to be 0.4 g DM/g glycerol. A by-product reaction takes into account that 4.7% of the arabinose is converted to ribitol [31]. Rest of the arabinose remains in the fermentation broth. The genetically modified *Escherichia coli* cell mass is separated from the xylitol-rich broth in filterpress. The downstream steps are clarification with activated charcoal, evaporation and crystallization [32] (Figure 3). The xylitol-rich broth is treated with charcoal at a concentration of 15 g/L to remove the impurities, which can color the xylitol crystals. During this step, 69% of the protein is removed, however, 5% of the sugars and sugar alcohols are also adsorbed to the charcoal surface. Separation of the solid and liquid fractions is performed in filterpress. To concentrate the liquid fraction until a xylitol concentration of 637 g/L, before the crystallization, vacuum evaporation (0.1 bar) is used. Hence, the side reactions and the thermal decomposition can be avoided. In the model xylitol is crystallized from the purified and concentrated broth in one step at 8°C. However, in the reality it is carried out in two steps (nucleation and growth of crystals) at different temperature (~20°C and 8°C, respectively) [32]. The crystallization yield is set to 47%, which can be achieved with recirculation of the mother liquor at least four times [32]. It is assumed that the xylitol losses during separation the crystals from the mother liquor are negligible. The purity of the recovered crystals is assumed to be more than 99%.

### 2.4 Comparison of the scenarios

The scenarios in the base case A were compared in terms of energy efficiency, which is defined as the energy output divided by the energy input, based on the higher heating values. During energy efficiency calculation raw material was considered as input, while the products (bioethanol, biomethane, district heat) and the electricity produced in the CHP were considered as output. In the base case B, the comparison of the scenarios is based on the mass flows of the products instead of their energy efficiencies, since xylitol is produced to be used as a food component and not as energy carrier. Within the two base cases 14 scenarios were investigated, which differed in process configuration and values of parameters. The configurations are shown
in Figs. 1 and 2, and the details of the scenarios are presented in Table 1.

3 Results and discussion

3.1 Base case A

Before the study of the configurations detailed above, a pre-investigation was carried out. In accordance with the laboratory experiments of Kálmán et al. [14] the WIS content in the steps of fractionation were set to 10%, which resulted in 5% WIS concentration in the following cellulose hydrolysis. In some cases of the pre-investigation, the biorefinery process was not able to produce enough steam to satisfy its own heat demand, although it had been defined as a base requirement. However, if the sludge, the hydrolysis residue and the whole amount of biogas were incinerated, and if the feed stream of the plant was preheated in the flue gas condenser, the appropriate amount of steam could be generated. Nevertheless, the energy efficiency was only 38%, which is considered not to be satisfactory on an industrial scale. This low energy efficiency was due to the low ethanol concentration in the fermented broth, which considerably raised the energy demand of the distillation (data not shown). To achieve higher energy efficiencies, higher WIS concentrations were set, namely 30% and 22% in the hot water and weak acid treatments, respectively. These values resulted in 10% WIS content in the cellulose hydrolysis step.

There are two options for the utilization of the hydrolysis residue: subjecting it to the AD to produce biogas, or incinerating it in the CHP to generate steam and electricity. Fig. 4 clearly shows that the scenarios, in which the hydrolysis residue is incinerated (A1, A2, A3, A7), have higher energy efficiencies, than the corresponding scenarios (A4, A5, A6, A8, respectively), in which the hydrolysis residue is digested anaerobically, while the other settings are equal. There is only one exception, namely the two scenarios in which the dry matter of the filter-pressed solid streams is set to 30% (cases A9 and A10). It is difficult to explain this exception, because the DM content of the filterpressed solid streams has effect on many process steps. The favourable influence of incinerating the hydrolysis residue can be explained as follows. The hydrolysis residue contains mainly water-insoluble lignin, which can not be utilized in the AD, water-soluble lignin, proteins and lipids, which have the conversion factors of 0.5 during the biogas production. Hence, one part of the hydrolysis residue is remained undigested after the AD, and is eliminated in the aerobic waste water treatment step, where aerobic sludge is produced with a yield of 0.5 kg DM/kg COD, however, part of the organic material is degraded to carbon dioxide and water. Thus, the energy from the incineration of the hydrolysis residue is more, than the energy from the incineration of the biogas and sludge, which are produced from the hydrolysis residue (data not shown). When the hydrolysis residue is subjected to AD instead of incineration, more raw biogas is produced (Table 2). Nevertheless, more raw biogas is needed in the burner to cover the process energy demand (Table 2), which results in decreased biomethane production (Table 2) in the cases of A4, A5, A6, A8 compared with their corresponding scenarios (A1, A2, A3, A7, respectively).

The differences between the cases A1 and A2, and between the cases A4 and A5 show the significant effect of sludge incineration (Fig. 4). The sludge replaces some biogas required by the burner, thus more biomethane can appear as product, resulting in higher energy efficiency. Processing of the sludge increases the energy efficiency to a larger extent, when the hydrolysis residue is subjected to anaerobic digestion, that is the difference between A4 and A5 is greater than that between A1 and A2. The reason is that during the aerobic treatment more sludge is produced from the lignin, proteins and lipids, remained from the hydrolysis residue after AD, in scenario A5, compared to case A2, in which the hydrolysis residue is incinerated.

If streams like the hydrolysis residue and the sludge, which contain water in considerable concentration, are subjected to the burner, large amount of energy is leaving with the flue gas in the form of steam. In the scenario A1, this waste energy is more than in the case A4 (Table 2), which is due to the water content of the incinerated hydrolysis residue. However, comparing case A2 to case A5, the energy leaving with the flue gas is smaller in case A5. In scenario A5 more sludge is produced, which results in more incoming water to the burner.

The energy leaving with the flue gas can be utilized in a flue gas condenser, which is implemented in the configurations of A3 and A6 (Table 2). However, to produce district heat it is also necessary to consume some primary steam. To achieve this goal, more biogas have to be incinerated compared the cases without flue gas condensation. At the same time, the increased steam production results in more electricity (Table 2). The highest en-

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**Fig. 3.** Process steps of xylitol recovery
Table 1. Process configurations and parameters set in the investigated scenarios.

<table>
<thead>
<tr>
<th>Scenarios</th>
<th>Processing of the</th>
<th>Aerobic sludge yield)</th>
<th>DM content of the filter (kg COD/kg COD)</th>
<th>Processing of the sludge</th>
<th>Utilizing the heat of consideration</th>
<th>Part of the hemicellulose fraction to produce Xylitol</th>
<th>produce biogas</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>CHP</td>
<td>0.5</td>
<td>40</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>A2</td>
<td>CHP</td>
<td>0.5</td>
<td>40</td>
<td>CHP</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>A3</td>
<td>CHP</td>
<td>0.5</td>
<td>40</td>
<td>CHP</td>
<td>District heating</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>A4</td>
<td>AD</td>
<td>0.5</td>
<td>40</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>A5</td>
<td>AD</td>
<td>0.5</td>
<td>40</td>
<td>CHP</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>A6</td>
<td>AD</td>
<td>0.5</td>
<td>40</td>
<td>CHP</td>
<td>District heating</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>A7</td>
<td>CHP</td>
<td>0.3</td>
<td>40</td>
<td>CHP</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>A8</td>
<td>AD</td>
<td>0.3</td>
<td>40</td>
<td>CHP</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>A9</td>
<td>CHP</td>
<td>0.5</td>
<td>30</td>
<td>CHP</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>A10</td>
<td>AD</td>
<td>0.5</td>
<td>30</td>
<td>CHP</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>B1</td>
<td>CHP</td>
<td>0.5</td>
<td>40</td>
<td>CHP</td>
<td>Evaporation</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>B2</td>
<td>CHP</td>
<td>0.5</td>
<td>40</td>
<td>CHP</td>
<td>Evaporation</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>B3</td>
<td>CHP</td>
<td>0.5</td>
<td>40</td>
<td>CHP</td>
<td>Evaporation</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>B4</td>
<td>CHP</td>
<td>0.5</td>
<td>40</td>
<td>CHP</td>
<td>Evaporation</td>
<td>0.3</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Base cases A and B are shown in Figs. 1 and 2, respectively. CHP: combined heat and power, AD: anaerobic digestion, DM: dry matter, COD: chemical oxygen demand.

Energy efficiency is achieved in the scenario A3, which contains flue gas condensation and incineration of both the hydrolysis residue and the sludge.

In case A2 less sludge is burnt compared with case A5, and in case A3 less energy can be recovered in the flue gas condenser in contrast with case A6 (Table 2). Hence, the positive effect of sludge utilization and flue gas condensation on the energy efficiency is greater in the cases, where the hydrolysis residue is digested anaerobically.

The yield of the aerobic sludge depends on the composition of the feed and some other environmental parameters [33]. There have not been any experimental data for the aerobic treatment of this kind of material in the literature, thus the assumption of the aerobic sludge yield is very uncertain. Therefore the effect of
3.2 Base case B

In the base case B the process is extended so that the biorefinery can produce an additional value-added product, that is xylitol. In these scenarios the hemicellulose fraction is separated in filterpress, and then it is subjected to xylitol fermentation. A preliminary study was also carried out in this base case, which elucidated the need for the division of hemicellulose fraction between the anaerobic digestion and the xylitol production. If the hemicellulose fraction was used exclusively for xylitol fermentation, there would not be enough steam available, which is due to both the reduced biogas production in the AD and the extra heat demand of the evaporation step in the xylitol production. Using 80% of the hemicellulose fraction for producing xylitol, the heat demand of the process can be covered by combusting the main part of raw biogas (cases B1 and B2, data not shown). The biogas excess is considered to be less than the amount needed for an economically-feasible gas upgrading system, thus the whole amount of raw biogas is subjected to incineration. Hence, steam excess is appeared, which operates a low-pressure turbine to make electricity.

In scenario B1, the feed stream of xylitol production contains 74 g/L xylose, 38 g/L arabinose, and 4 g/L glucose. The achieved xylitol and cell mass concentration after the first fermentation step is 51 g/L and 14 g/L, respectively. These concentrations are higher, than most of the data obtained in experimental works, as one of the highest xylitol yields was set in the simulation from the yields published [34]-[36], as the hemicellulose fraction, which is hydrolyzed with weak acid treatment at low temperature, does not contain any inhibitory compounds for the xylitol fermentation. After the second fermentation step the xylitol concentration increases to 78 g/L. It is presumed during this step, that the xylitol production of the genetically modified Escherichia coli is not repressed by the high xylitol concentration. The theoretical amount of the xylitol produced from the xylose and arabinose contents of the raw material is 36030 tonnes/year, however, the produced xylitol in case B1 is only 6733 tonnes/year (Table 4), which is 19% of the theoretical. It is due to the low yield of crystallization (47%) and the xylitol loss in the filterpress steps and during the treatment with activated charcoal. The achieved yield of ethanol production is 77% of the theoretical in cases B1, B3, and B4, as annually

<table>
<thead>
<tr>
<th>Scenarios</th>
<th>Energy flows of the CHP block (MW)</th>
<th>Energy flows of the products (MW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>24.67</td>
<td>14.64</td>
</tr>
<tr>
<td>A2</td>
<td>24.68</td>
<td>14.64</td>
</tr>
<tr>
<td>A3</td>
<td>24.70</td>
<td>14.64</td>
</tr>
<tr>
<td>A4</td>
<td>32.49</td>
<td>14.64</td>
</tr>
<tr>
<td>A5</td>
<td>32.45</td>
<td>14.64</td>
</tr>
<tr>
<td>A6</td>
<td>32.47</td>
<td>14.64</td>
</tr>
<tr>
<td>A7</td>
<td>24.68</td>
<td>14.64</td>
</tr>
<tr>
<td>A8</td>
<td>32.47</td>
<td>14.64</td>
</tr>
<tr>
<td>A9</td>
<td>24.44</td>
<td>14.64</td>
</tr>
<tr>
<td>A10</td>
<td>32.72</td>
<td>14.64</td>
</tr>
</tbody>
</table>

Summary of the scenarios is given in Table 1. CHP: combined heat and power. Waste energy leaving with the flue gas and energy of the flue gas condensation were determined by cooling the flue gas from 150˚C to 50˚C. Energy content of the raw material is 57.33 MW.
Tab. 3. Energy demand of the process steps related to the energy consumption of the whole process

<table>
<thead>
<tr>
<th>Scenarios</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
<th>A6</th>
<th>A7</th>
<th>A8</th>
<th>A9</th>
<th>A10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment</td>
<td>38</td>
<td>38</td>
<td>41</td>
<td>41</td>
<td>36</td>
<td>40</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>Distillation</td>
<td>42</td>
<td>42</td>
<td>46</td>
<td>41</td>
<td>45</td>
<td>42</td>
<td>43</td>
<td>42</td>
<td>43</td>
<td>42</td>
</tr>
<tr>
<td>Other</td>
<td>20</td>
<td>20</td>
<td>13</td>
<td>13</td>
<td>23</td>
<td>15</td>
<td>20</td>
<td>19</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

“Other” includes all process steps except the pretreatment and distillation.

Tab. 4. Process details of the scenarios investigated in base case B.

<table>
<thead>
<tr>
<th>Scenarios</th>
<th>B1</th>
<th>B2</th>
<th>B3</th>
<th>B4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part of hemicellulose fraction used for xylitol production</td>
<td>0.8</td>
<td>0.8</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>DM content of the filterpressed solid streams (%)</td>
<td>40</td>
<td>30</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Component flows of xylitol fermentation (kg/h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>xylose input</td>
<td>1698</td>
<td>1698</td>
<td>1061</td>
<td>637</td>
</tr>
<tr>
<td>arabinose input</td>
<td>875</td>
<td>875</td>
<td>547</td>
<td>328</td>
</tr>
<tr>
<td>xylitol derived from xylose</td>
<td>1155</td>
<td>1155</td>
<td>722</td>
<td>433</td>
</tr>
<tr>
<td>xylitol derived from arabinose</td>
<td>614</td>
<td>607</td>
<td>384</td>
<td>230</td>
</tr>
<tr>
<td>added glycerol</td>
<td>427</td>
<td>422</td>
<td>267</td>
<td>160</td>
</tr>
<tr>
<td>yeast cell mass (Candida strain)</td>
<td>319</td>
<td>319</td>
<td>199</td>
<td>120</td>
</tr>
<tr>
<td>bacterial cell mass (Escherichia coli strain)</td>
<td>169</td>
<td>167</td>
<td>106</td>
<td>63</td>
</tr>
<tr>
<td>Biogas streams (kg/h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>methane obtained from mother liquor</td>
<td>440</td>
<td>432</td>
<td>275</td>
<td>165</td>
</tr>
<tr>
<td>methane obtained from hemicellulose fraction</td>
<td>244</td>
<td>244</td>
<td>609</td>
<td>853</td>
</tr>
<tr>
<td>total produced methane in the AD</td>
<td>1197</td>
<td>1197</td>
<td>1398</td>
<td>1532</td>
</tr>
<tr>
<td>methane incinerated in the CHP</td>
<td>1197</td>
<td>1197</td>
<td>754</td>
<td>554</td>
</tr>
<tr>
<td>Produced electricity in the CHP (TJ/year)</td>
<td>209</td>
<td>202</td>
<td>156</td>
<td>138</td>
</tr>
<tr>
<td>Process heat demand (TJ/year)</td>
<td>591</td>
<td>612</td>
<td>556</td>
<td>495</td>
</tr>
<tr>
<td>Products (tonne/year)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>15089</td>
<td>14885</td>
<td>15089</td>
<td>15089</td>
</tr>
<tr>
<td>Methane</td>
<td>–</td>
<td>–</td>
<td>5599</td>
<td>8520</td>
</tr>
<tr>
<td>Xylitol</td>
<td>6733</td>
<td>6611</td>
<td>4208</td>
<td>2525</td>
</tr>
</tbody>
</table>

Summary of the scenarios is given in Table 1. AD: anaerobic digestion, CHP: combined heat and power, DM: dry matter.

19587 tonnes of ethanol can be produced from the starch and cellulose inputs theoretically. 65% of the xylitol produced in the scenarios B1, B3 and B4 is obtained from xylose, however, this ratio is higher in the case B2 (Table 4). The comparison of the cases B1 and B2 establishes the conclusion that the dry matter content of the filterpressed solid streams is of significant effect. Both the ethanol and xylitol productions are diminished by reducing the DM content of the filterpressed solid streams from 40% to 30%, as in the latter case less sugar is available in the supernatant utilized for ethanol fermentation and in the liquid fraction used for fermenting xylitol.

If the part of hemicellulose fraction used for xylitol fermentation is set to 0.5 (case B3), the biorefinery can produce bioethanol, biomethane and xylitol, synergistically (Table 4). As, larger part of the hemicellulose fraction is subjected to anaerobic digestion in case B3 than in cases B1 and B2, less xylitol is available as product. In scenario B4, 30% of the hemicellulose fraction is utilized in the xylitol fermentation and recovery step, which results in further decrease of the xylitol production, however, more biomethane is produced (Table 4). By decreasing the amount of hemicellulose subjected to xylitol production less bacterial cell mass is needed in the second step of xylitol fermentation. Thus, less glycerol is added in this step (Table 4). The methane obtained from the mother liquor is 37%, 20% and 11% of the total methane produced in the AD in scenario B1, B3 and B4, respectively (Table 4). This tendency is due to both the decrease of the methane obtained from mother liquor and the increase of the total methane produced in the AD.
The process heat demand is reduced due to the decrease of the mass flow of xylitol fermentation broth subjected to evaporation, which results in decreased heat demand of this step. The process heat demand is larger in scenario B2 than in scenario B1 (Table 4) because of the increased xylitol fermentation broth volume, which is due to the increased volume of washed hemicellulose fraction.

4 Conclusions

Two base cases of a biorefinery processing corn fibre were modelled with Aspen Plus flow-sheeting program. In the base case A the plant is able to produce bioethanol, biomethane and electricity. Several scenarios were simulated and compared with each other in point of energy efficiency, as this is of great importance of a process producing energy carriers. If a DM content of 40% can be achieved in the solid fraction of filterpressing, it is more favourable to combust the hydrolysis residue, than each other in point of energy efficiency, as this is of great importance of a process producing energy carriers. If a DM content of 40% can be achieved in the solid fraction of filterpressing, it is more favourable to combust the hydrolysis residue, than subjecting it to anaerobic digestion. When the DM content of the filterpresssed solid streams is changed from 40% to 30%, the overall energy efficiency is not affected considerably in the scenarios of anaerobic digestion of the hydrolysis residue, but significant change occurs in the cases of incineration of the hydrolysis residue. There is also significant influence of the aerobic sludge yield. Energy efficiency can be improved by incinerating the sludge, and by implementing district heating with the aid of flue gas condensation. The highest energy efficiency is achieved in that scenario, which contains the following settings: incineration of the hydrolysis residue and sludge, production of district heat, aerobic sludge yield of 0.5 kg DM/kg COD and DM content of the filterpressed solid streams of 40%. The energy efficiency of all scenarios could be increased by decreasing the energy demands of distillation and pretreatment, as these steps have the largest energy consumption in the process.

In the base case B, the process is able to produce bioethanol, biomethane, electricity and xylitol, parallelly. The scenarios were compared in terms of mass flows of the products, as in this base case, beside the energy carriers, a food component is also produced. To produce bioethanol, biomethane and xylitol at the same time, the hemicellulose fraction has to be shared between anaerobic digestion and xylitol fermentation. By the division of hemicellulose fraction, the amounts of produced biomethane and xylitol can be varied and adjusted to the market conditions. Although it was not considered in this study, the amount of the bioethanol can also be varied by dividing the sugar-rich liquid obtained in enzymatic hydrolysates of starch and cellulose between ethanol fermentation and anaerobic digestion. Hence, by decreasing the bioethanol production more biomethane can be produced, which is also a possibility of market adaptation.

References
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Investigation of commercial enzyme preparations for selective release of arabinose from corn fibre

Csaba Fehér,* Boglárka Gál, Anikó Fehér, Zsolt Barta and Kati Réczey

Abstract

BACKGROUND: Lignocellulosic materials have great potential in the production of L-arabinose in a new cost-effective way. Selective enzymatic liberation of L-arabinose from agricultural residues could be a promising method if purification of specific enzymes and extraction of pure polysaccharides could be omitted from the process. To achieve this goal, investigation of commercial multi-component enzyme preparations for selective release of L-arabinose from destarched corn fibre and soaking in aqueous ammonia (SAA) treated destarched corn fibres were performed.

RESULTS: Hemicellulase NS22002 (Novozymes) has a relative xylanase activity of 1% (of measured highest) and 9%, and a relative arabinoxyylan-arabinofuranohydrolase (AX-AFH) activity of 71% and 100% at pH 3 and 4, respectively. At pH 6 its relative xylanase and AX-AFH activities are 93% and 52%, respectively. Hydrolysates 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 solubilised.

CONCLUSION: SAA pretreatment has been found to be an appropriate method to make the structure of destarched corn fibre accessible for hemicellulose-degrading enzymes. Hemicellulase NS22002 has high endo-xylanase activity over a broad pH range, and its $\alpha$-L-arabinofuranosidase can release arabinose from solubilised hemicellulosic oligomers derived from corn fibre. Enzymatic hydrolysis of SAA pretreated destarched corn fibre using Hemicellulase NS22002 is suitable to solubilise the hemicellulose fraction, however it cannot selectively release arabinose monomers.

Keywords: arabinofuranosidase; xylanase; enzyme activity; hemicellulases; enzymatic hydrolysis; corn fibre arabinoxylan

INTRODUCTION

In the so-called bio-based economy, it is expected that industrial biotechnology will play a key role in the development of biorefinery, a term which describes the integrated conversion of plant biomass into a wide array of fuels, chemicals and materials. Globally large amount of agricultural residues are produced, part of which is burnt to generate energy or as waste management and used for mulching or as fodder. However, these kinds of lignocelluloses could be raw materials for producing pentose and/or hexose sugars as platform intermediates in a lignocellulosic biorefinery. Production of L-arabinose from lignocelluloses is of great importance, as the actual production of high quality L-arabinose on an industrial scale results in high price, and it is limited by the availability of gum arabic. Corn fibre, a low-value by-product of the corn wet milling process, is an appropriate raw material for producing sugars including L-arabinose. Corn fibre has been used as a low-grade animal feed ingredient or as solid fuel in pelletized form, however it can also be converted into value-added products in a biorefinery concept. Corn fibre dry matter contains about 20% starch, 14% cellulose and 35% hemicellulose. The hemicellulose fraction consists of a xylan backbone, which is highly substituted with monomeric side-chains of L-arabinose, acetic acid and D-glucuronic acid, and with oligomeric side-chains containing L-arabinose, D-xylose and D-galactose. Ferulic acid and p-coumaric acid can be ester-linked to the arabinose moieties present at the end of the side chains. Owing to the presence of many different components and the branched structure of the corn fibre hemicellulose, several types of enzymes are required for complete degradation, such as endo-$\beta$-1,4-xylanase, $\beta$-xylosidase, $\alpha$-L-arabinofuranosidase, $\alpha$-glucuronidase, acetylxylan esterase, ferulic acid esterase, and p-coumaric acid esterase. Alpha-L-arabinofuranosidases (EC 3.2.1.55) can liberate L-arabinose moieties from the non-reducing end of the side-chains. Several types of $\alpha$-L-arabinofuranosidases have been discovered showing different substrate specificities and operational conditions. Activity of $\alpha$-L-arabinofuranosidases is influenced by the degree of substitution of the hemicellulose polymers with arabinosyl residues, the position of the

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arabinose moieties and the presence of other substitutions.\textsuperscript{24–26} 

Utilization of \( \alpha-L\)-arabinofuranosidases and endo-arabinanases to produce arabinose from lignocellulose- or pectin-derived materials is of growing importance in the field of biotechnology.\textsuperscript{27–32} \( L\)-arabinose has been reported to have beneficial properties regarding blood sugar reduction and for the treatment of human diabetes.\textsuperscript{33,34} \( L\)-arabinose can also be utilised for analytical purposes, for bacteriological diagnostics,\textsuperscript{34} as a starting material in the synthesis of non-ionic surfactants\textsuperscript{35} and as intermediate for antivirus drug synthesis.\textsuperscript{36} Lim et al. and Kim et al. investigated arabinose production from linear arabinan and branched arabinan derived from sugar beet using thermostable \( \alpha-L\)-arabinofuranosidase and endo-\( \alpha\)-1,5-arabinanase from \textit{Caldicellulosiruptor saccharolyticus} simultaneously.\textsuperscript{28,29} At the selected conditions 16 g L\textsuperscript{−1} arabinose was obtained from 20 g L\textsuperscript{−1} arabinan with a productivity of 8 g arabinose L\textsuperscript{−1} h\textsuperscript{−1},\textsuperscript{28} which was increased to 9.9 g arabinose L\textsuperscript{−1} h\textsuperscript{−1} in a packed-bed reactor containing immobilised enzymes.\textsuperscript{29} Kurakake et al. investigated arabinose production from corn hull arabinoylan using \( \alpha-L\)-arabinofuranosidase purified from \textit{Arthrobacter aurescens} MKS.\textsuperscript{30} The enzyme released only arabinose from the arabinoylan, and an arabinose yield of 45% was achieved in the case of a substrate concentration of 2% (w/w).\textsuperscript{30} The advantages of these methods are the mild reaction conditions applied, and the pure arabinose solution obtained regarding the solubilised monosaccharides. The drawbacks are that the production of the starting materials investigated (purified arabinan and arabinoylan) and the purification of the applied enzymes require complex and expensive processes. Moreover, the purification and recovery of arabinose from the hydrolysates can be challenging, as the starting materials are also soluble in water. To avoid these problems the possibility of selective arabinose solubilisation from destarched corn fibre and soaking in aqueous ammonia pretreated destarched corn fibre using commercial enzyme preparations is investigated in this study.

**MATERIALS AND METHODS**

**Destarched corn fibre**

Destarched corn fibre was used as a raw material for enzymatic hydrolysis experiments. The corn fibre was kindly donated by Hungrana Ltd. (Szabadegyháza, Hungary). It was dried and stored at room temperature. A destarching process was performed as follows: ground (particle size less than 1 mm) corn fibre was suspended in sodium acetate buffer (pH = 4.8, 100 mmol L\textsuperscript{−1}) at 3% dry matter content, and treated by thermostable \( \alpha\)-amylase (5 g kg\textsuperscript{−1} dry matter) in 1 L closed glass-flasks at 90 °C for 3 h with continuous agitation (250 rpm) in a water bath. The solid fraction was separated by vacuum filtration through a 150 μm nylon filter, and washed with distilled water at 80 °C to completely remove the soluble substances. The volume of distilled water used in the washing step was three times that of the liquid volume of the corn fibre suspension. Destarched corn fibre was dried at 40 °C, and stored at room temperature until the next treatments.

**Enzymes**

Xylanase NS22083, Enzyme complex NS22119, Hemicellulase NS22002 and Celtic CTeC2 enzyme cocktails, which are dedicated to hydrolysis of lignocellulosic materials, were generously provided by Novozymes A/S ( Bagsvaerd, Denmark). Alpha-L-arabinofuranosidase E-APAM2 (91201a) enzyme preparation was purchased from Megazyme (Bray, Ireland). The main characteristics of these enzyme preparations are summarized in Table 1. Thermostable \( \alpha\)-amylase enzyme preparation was kindly donated by Hungrana Ltd.

**Soaking in aqueous ammonia (SAA) pretreatment**

The ammonia steeping of destarched corn fibre 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 solid fraction was separated by vacuum filtration through a nylon filter (150 μm), washed with distilled water (80 °C) until neutral pH, and immediately processed in the enzymatic hydrolysis experiments.

**Determination of monosaccharides**

Glucose, arabinose and other hemicellulosic sugars (OHS) in liquid samples were determined by high-performance liquid chromatography (HPLC). OHS consist of xylose and galactose as these sugars appeared as one peak in the chromatogram. The HPLC analysis was performed using a BioRad (Hercules, CA, USA) Aminex HPX-87H column (300 × 7.8 mm) at 65 °C. The eluent was 5 mmol L\textsuperscript{−1} sulphuric acid at a flow rate of 0.5 mL min\textsuperscript{−1}, and the sample volume was 40 μL. The monosaccharides were detected and quantified by refractive index.

**Determination of total sugars**

Total sugars of the liquid samples include monomer sugars and sugar oligomers. To determine the total sugar content the samples were mixed with 8% (w/v) sulphuric acid at a volume ratio of 1:1, and treated at 120 °C in an autoclave for 15 min. Hence, the sugar oligomers were hydrolysed into monomers, which were analysed according to the method of monosaccharide determination.

**Xylanase activity**

Xylanase activity of the enzyme cocktails from Novozymes was investigated as a function of pH within the range 3 to 10. The xylanase activity was assayed in a reaction mixture (1.5 mL) containing 0.1 mL of appropriately diluted enzyme solution and 1.4 mL of 1% (w/w) birch wood xylan (Sigma) solution, at least in duplicate. The xylan solution was prepared with 0.1 mol L\textsuperscript{−1} sodium acetate buffer (pH = 3, 4, 5) or 0.1 mol L\textsuperscript{−1} phosphate buffer (pH = 6, 7, 8, 9, 10). The enzyme preparations were diluted with distilled water. Reducing sugar content of the reaction mixture was measured colorimetrically using dinitrosalicylic acid reagent according to Miller’s method.\textsuperscript{39} After incubation at 90 °C for 5 min with continuous agitation, the reaction was stopped by adding 3 mL of 3,5-dinitrosalicylic acid reagent. The reaction mixture was boiled for 5 min, then cooled to room temperature and mixed with 16 mL of distilled water. The adsorption value was measured at a wavelength of 550 nm. Reducing sugar content was determined in xylose equivalent from a calibration curve prepared using pure xylose solution. Xylanase activity was expressed in xylanase unit g\textsuperscript{−1} enzyme preparation. One xylanase unit (XU) was defined as the amount of released reducing sugar in xylose equivalent (μmol) per minute under the assay conditions.

**Arabinofuranosyl-arabinobiofuranohydrolase activity**

To determine the optimal pH regarding the release of arabinose, arabinobiofuranosyl-arabinobiofuranohydrolase (AX-AFH) activity of the enzyme preparations from Novozymes were determined within...
the range from 3 to 10. The AX-AFH activity was assayed in a reaction mixture (2 mL) containing 1 mL of appropriately diluted enzyme solution and 1 mL of 2% (w/w) water-insoluble wheat arabinoxylan (Megazyme) suspension, at least in duplicate. The enzyme solution and the arabinoxylan suspension were prepared with 0.1 mol L\(^{-1}\) sodium acetate buffer (pH = 3, 4, 5) or 0.1 mol L\(^{-1}\) phosphate buffer (pH = 6, 7, 8, 9, 10). After incubation at 50°C for 1 h with continuous agitation, the reaction was stopped by adding 2 mL of 1 mol L\(^{-1}\) disodium carbonate solution, and the supernatant was separated by filtration (0.45 μm). The arabinose concentration of the supernatant was measured using an HPLC method. The AX-AFH activity of the purified \(\alpha\)-L-arabinofuranosidase E-AFAM2 was determined at 40°C and pH 6, otherwise the method was the same as described above. AX-AFH activity was expressed in AX-AFH unit (AU). One AX-AFH unit (AU) was defined as the amount of released arabinose (μmol) per min under the assay conditions.

### Table 1. Main characteristics of the enzyme preparations

<table>
<thead>
<tr>
<th>Enzyme complex</th>
<th>Source</th>
<th>Optimal pH regarding the main activities</th>
<th>Optimal temperature regarding the main activities (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylanase NS22083</td>
<td>Endo-xylanase</td>
<td>n.a.</td>
<td>4.5-6.0</td>
</tr>
<tr>
<td>Endo-hemi NS22002</td>
<td>β-glucanase</td>
<td>Humicola insolens</td>
<td>5.0-6.5</td>
</tr>
<tr>
<td>CellcTce2</td>
<td>Cellulase</td>
<td>n.a.</td>
<td>5.0-5.5</td>
</tr>
<tr>
<td>α-L-arabinofuranosidase E-AFAM2</td>
<td>α-L-arabinofuranosidase</td>
<td>Bifidobacterium adolescentis</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Information on Xylanase NS22083, Enzyme complex NS22119, Hemicellulase NS22002 and Cellc Tce2 supplied by product sheets from Novozymes A/S ( Bagsvaerd, Denmark). Information on \(\alpha\)-L-arabinofuranosidase E-AFAM2 supplied by product sheet from Megazyme (Bray, Ireland).

n.a. – not available

### Determination of recoverable sugars

The amount of recoverable sugars (glucose, arabinose, OHS) of destarched corn fibre, 6 h SAA pretreated destarched corn fibre and 24 h SAA pretreated destarched corn fibre were determined using the NREL method with minor modifications. Half a gram of dry matter was mixed with 2.5 mL 72% (w/w) sulphuric acid and was kept at room temperature for 2 h. Then 75 mL of distilled water was added and the suspension was treated at 120°C in an autoclave for 1 h. The supernatant was analysed with HPLC to determine its monosaccharide content. The determination of recoverable sugars was carried out in duplicate.

### Enzymatic hydrolysis

Enzymatic hydrolysis of destarched corn fibre, 6 h SAA pretreated destarched corn fibre and 24 h SAA pretreated destarched corn fibre were carried out at 3% dry matter content and at three different pH values (3, 4, 6) with Hemicellulase NS22002 (0.02 g enzyme preparation g\(^{-1}\) dry matter for 4 days. The suspensions were prepared with 0.1 mol L\(^{-1}\) sodium acetate buffer (pH = 3, 4) or 0.1 mol L\(^{-1}\) phosphate buffer (pH = 6) supplemented with a small amount of Thimerosal (Sigma) to avoid microbial infection. The suspensions were incubated at 50°C in a rotary shaker (175 rpm). Homogenous samples were taken daily and the supernatants were separated by centrifugation (10 min, 1000 g). Determination of monosaccharides and total sugars in the supernatants was performed according to methods described previously. The sugar yields were expressed as percentage of theoretical based on the recoverable sugars of destarched corn fibre, 6 h SAA pretreated destarched corn fibre and 24 h SAA pretreated destarched corn fibre.

### RESULTS AND DISCUSSION

Xylanase and AX-AFH activities of four commercial enzyme preparations from Novozymes were investigated as a function of pH within the range from 3 to 10. These enzyme mixtures have one or more main activities given by the manufacturer (Table 1), however they can also contain several types of side activities such as \(\alpha\)-L-arabinofuranosidase. Different enzymes can act synergistically during the hydrolysis of complex natural substrates, which include poly- and oligosaccharides derived from lignocellulose- or pectin-containing materials. For example, some \(\alpha\)-L-arabinofuranosidases are more active on xylanase-digested arabinoxylan substrates, however the xylan chain, from which arabinose moieties are removed, are more accessible for these xylanases. Hence, during the determination of AX-AFH activity of multi-component enzyme cocktails by using complex polysaccharide (e.g. arabinoxylan) as a substrate, the activity depends on the \(\alpha\)-L-arabinofuranosidase activity and the other activities present in the mixture. By this means the AX-AFH activity characterizes the capability of the enzyme preparations to release arabinose from complex polysaccharides. In many cases the \(\alpha\)-L-arabinofuranosidase activity is determined on \(p\)-nitrophenyl-\(\alpha\)-L-arabinofuranoside (pNPA) model substrate, however the activity on pNPA does not guarantee the ability to liberate arabinose from natural substrates. Hence, investigation of AX-AFH activity on water-insoluble wheat arabinoxylan was chosen in this study. Multi-component enzyme preparations contain different enzyme activities, which can have different pH and temperature optima. That implies the possibility of controlling an enzymatic hydrolysis process by shifting the pH or the temperature. It could also allow, for example, performing selective hydrolysis reactions without using purified enzymes. The pH dependence of xylanase and AX-AFH activities of the selected enzyme mixtures were determined to investigate the possibility of selective arabinose release during the hydrolysis of corn fibre. AX-AFH activity of a purified \(\alpha\)-L-arabinofuranosidase was also studied to produce arabinose from corn fibre.
Determination of xylanase and AX-AFH activities

Figure 1 shows the relative xylanase and AX-AFH activities of Xylanase NS22083 (Fig. 1(A)), Enzyme complex NS22119 (Fig. 1(B)), Cellic CTeC2 (Fig. 1(C)) and Hemicellulase NS22002 (Fig. 1(D)) enzyme preparations as a function of pH. Relative activity is expressed as percentage of the highest activity value obtained for a given enzyme preparation and type of activity (Table 2). Xylanase NS22083 had maximum xylanase activity at pH 5. The optimal pH range regarding endo-xylanase activity (pH 4.5–6), which is defined by the manufacturer (Table 1) corresponds with our results, as 96% and 82% of the maximum xylanase activity was observed at pH 4 and 6, respectively. However, by increasing the pH from 5 to 10, it continuously decreased to 8%. At pH 3 a relative activity of 44% was obtained. In the case of the AX-AFH activity, two maxima were observed, one at pH 5 (acidic maximum), which is considered as the absolute maximum, and another at pH 8 (alkaline maximum) with 78% relative activity. However, at pH 6 and 7 no AX-AFH activity was observed. The existence of two maximum values can be explained by the presence of two different types of α-L-arabinofuranosidase in the enzyme mixture, however further investigation is required to confirm this hypothesis. The highest AX-AFH and xylanase activities were obtained at the same pH, furthermore at pH 8 – where the alkaline maximum of AX-AFH activity – the relative xylanase activity was 46%. These results imply that Xylanase NS22083 is not applicable to selectively release arabinose from an arabinoxylan-containing substrate. The optimum pH of xylanase activity of Enzyme complex NS22119 was 6. At pH from 7 to 10 and from 5 to 3 more than 30% and 60%, respectively, of its xylanase activity remained. Regarding AX-AFH activity it also had an acidic maximum at pH 4 with 100% relative activity and an alkaline maximum at pH 9 with a relative activity of 48%. This implies

Table 2. Measured highest activities of xylanase and arabinoxylan-arabinofuranohydrolase (AX-AFH)

<table>
<thead>
<tr>
<th>Enzyme preparation</th>
<th>AX-AFH activity (AU/g enzyme preparation)</th>
<th>Xylanase activity (XU/g enzyme preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme complex NS22119</td>
<td>1.66 (0.12)</td>
<td>538 (40)</td>
</tr>
<tr>
<td>Xylanase NS22083</td>
<td>6.66 (0.06)</td>
<td>18862 (794)</td>
</tr>
<tr>
<td>Hemicellulase NS22002</td>
<td>7.30 (0.29)</td>
<td>1893 (54)</td>
</tr>
<tr>
<td>Cellic CTeC2</td>
<td>3.28 (0.43)</td>
<td>14803 (121)</td>
</tr>
<tr>
<td>α-L-arabinofuranosidase E-AFAM2</td>
<td>0.44 (0.03)</td>
<td>n.m.</td>
</tr>
</tbody>
</table>

AU – arabinoxylan-arabinofuranohydrolase unit, XU – xylanase unit, n.m. – not measured. Standard deviations are calculated from triplicates, and are indicated in parenthesis.

Figure 1. Relative activities of xylanase and arabinoxylan-arabinofuranohydrolase (AX-AFH) as a function of pH. A - Xylanase NS22083, B - Enzyme complex NS22119, C - Cellic CTeC2, D - Hemicellulase NS22002. Numbers in bold and italic are the average values of relative AX-AFH and xylanase activities, respectively. Standard deviation is calculated from duplicates at least.
the presence of two different types of α-L-arabinofuranosidase. At pH 7 no AX-AF activity was detected, hence in the case of the presence of two different α-L-arabinofuranosidases neither the acidic- nor the alkaline-active ones can act. At the pH values of acidic and alkaline maximum of AX-AF activity, 70% and 32% relative xylanase activities were obtained, respectively. Hence, enzyme complex NS22119 is considered to be inappropriate for selective release of arabinose. The pH optimum of xylanase activity of Cellic Ctc2 was 4. By increasing the pH from 4 to 10, the xylanase activity continuously decreased to 7% of the maximum value. At pH 3 a relative xylanase activity of 81% was obtained. Regarding the AX-AF activity the optimum pH was found to be 5 and 96% of that was obtained at pH 4. At other pH values Cellic Ctc2 did not show AX-AF activity. Hence, Cellic Ctc2 is also considered to be inappropriate to selectively release arabinose moieties from arabinoxylan polymers. Xylanase activity of Hemicellulase NS22002 as a function of pH had a broad optimum. The optimum pH was 7, however, it kept around 90% of its maximum activity within the pH range from 5 to 8. At pH 4 and 3 significant decrease occurred resulting in 9% and 1% relative xylanase activities, respectively. However, regarding AX-AF activity, pH 4 was found to be the optimum, and at pH 3 it decreased only by 29%. More than 50% relative AX-AF activity of Hemicellulase NS22002 was retained over the whole pH range investigated. The considerable difference between the relative activities of xylanase and AX-AF at pH 4 and 3 implies the possibility to selectively release arabinose by using Hemicellulase NS22002 from arabinoxylan-containing raw materials. Comparing the maximum absolute values of xylanase activity of the four commercial enzyme preparations Xylanase NS22083 had the largest one followed by Cellic Ctc2e2 (Table 2). The xylanase activity of Xylanase NS22083 is 10-fold and 35-fold of the Hemicellulase NS22002 and Enzyme complex NS22119, respectively (Table 2). Low absolute values of AX-AF activity of the commercial enzyme preparations were obtained (Table 2), which is due to the fact that AX-AF is side activity, and it was measured on water-insoluble substrate. The highest value was observed in Hemicellulase NS22002 (Table 2). AX-AF activity of purified α-L-arabinofuranosidase E-APAM2 was also determined, however it was lower than those of the enzyme cocktails (Table 2). This can be explained by the insoluble nature of the substrate, which requires additional enzymes that make the arabinose moieties accessible for α-L-arabinofuranosidases. Considering that Hemicellulase NS22002 had the highest AX-AF and low xylanase activities compared with the other enzyme preparations (Table 2), and at the pH optimum of its AX-AF activity it had relatively low xylanase activity (Fig. 1(D)), this enzyme preparation was selected to investigate selective arabinose solubilisation from destarched corn fibre and SAA pretreated destarched corn fibre. In preliminary experiments purified α-L-arabinofuranosidase E-APAM2 was also tested on destarched corn fibre, however it could not release arabinose. Enzymatic hydrolyses of destarched corn fibre and SAA pretreated destarched corn fibre with Hemicellulase NS22002 were carried out at pH 3 and 4, and as a control experiment at pH 6, where AX-AF activity is minimal and the xylanase activity is close to the maximum (Fig. 1(D)).

### Pretreatment

Table 3 shows the amount of recoverable sugars from destarched corn fibre and from destarched corn fibres pretreated by the SAA method for 6 and 24 h. The total amount of recoverable sugars from destarched corn fibre was 743 mg g⁻¹ dry matter. Following 6 h SAA pretreatment and 24 h SAA pretreatment, the total amount of recoverable sugars were increased to 842 and 855 mg g⁻¹ dry matter, respectively. The ratio of hemicellulosic sugars (arabinose and OHS) to the total sugar content decreased from 67% to 59% when increasing the duration of SAA pretreatment from 6 to 24 h, which indicates the partial solubilisation of the hemicellulose fraction during the 24 h SAA pretreatment.

<table>
<thead>
<tr>
<th>Recoverable sugars (mg/g dry material)</th>
<th>DCF</th>
<th>6hSAA-DCF</th>
<th>24hSAA-DCF</th>
</tr>
</thead>
<tbody>
<tr>
<td>arabinose</td>
<td>179 (1)</td>
<td>196 (4)</td>
<td>173 (8)</td>
</tr>
<tr>
<td>OHS</td>
<td>335 (4)</td>
<td>364 (7)</td>
<td>328 (11)</td>
</tr>
<tr>
<td>glucose</td>
<td>229 (2)</td>
<td>282 (6)</td>
<td>354 (13)</td>
</tr>
</tbody>
</table>

### Enzymatic hydrolysis

Enzymatic hydrolysis experiments were performed on destarched corn fibre and on destarched corn fibres pretreated by SAA method for 6 and 24 h. The aim of the SAA pretreatment was to increase the amount of solubilised arabinose. The hydrolyses were evaluated in terms of the yields of monosaccharides and total sugars (oligomers and monomers), which were expressed as a percentage of theoretical. Glucose, arabinose, and OHS were considered during the evaluations. Selectivity of the hydrolyses, which was evaluated by the ratio of OHS (g) to arabinose (g) (OHS/arabinose) was also calculated.

### Enzymatic hydrolysis at pH 4

Figure 2(A-C) shows the hydrolysis profiles of the enzymatic hydrolyses of destarched corn fibre and SAA pretreated destarched corn fibres at pH 4 using Hemicellulase NS22002. The sugar yields and the OHS/arabinose values are presented in Table 4. In the case of destarched corn fibre the amount of recovered monosaccharides in the supernatant was negligible, as the yields of monomer glucose, arabinose, and OHS were less than 5% (Table 4). Regarding total sugars an arabinose yield of 17%, glucose and OHS yields of 8% were obtained (Table 4). The yields of total sugars reached their maximum within 1 day (Fig. 2(A)). The low yields of total sugars imply that the lignocellulose structure of destarched corn fibre is recalcitrant for the efficient enzymatic hydrolysis with Hemicellulase NS22002 at pH 4. To make the hemicellulose fraction of destarched corn fibre more accessible for enzymatic digestion, SAA pretreatment was performed. Following 6 h SAA pretreatment the monomer and total arabinose yields were significantly increased (Fig. 2(B) vs Fig. 2(A)). The monomer arabinose yield continuously increased during enzymatic hydrolysis up to 17% (Fig. 2(B)). The monomer glucose yield and OHS yield also continuously increased to 9% (Table 4). The amount of released monomer OHS was negligible (Table 4). The yields of total arabinose and total OHS reached their maximums within 1 day (Fig. 2(B)), and were 63% and 57%, respectively.
Figure 2. Enzymatic hydrolysis of destarched corn fibre and soaking in aqueous ammonia (SAA) pretreated destarched corn fibres using Hemicellulase NS22002. A – hydrolysis of destarched corn fibre at pH 4, B – hydrolysis of 6 h SAA pretreated destarched corn fibre at pH 4, C – hydrolysis of 24 h SAA pretreated destarched corn fibre at pH 4, D – hydrolysis of 6 h SAA pretreated destarched corn fibre at pH 6. OHS – other hemicellulosic sugars (xylose and galactose). Standard deviations are calculated from triplicates.

Table 4. Yields of monomer and total sugars and ratios of other hemicellulosic sugars (OHS) to arabinose at the end of the hydrolysis of destarched corn fibre and soaking in aqueous ammonia (SAA) pretreated destarched corn fibres using Hemicellulase NS22002

<table>
<thead>
<tr>
<th></th>
<th>Hydrolysis at pH 4</th>
<th>Hydrolysis at pH 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DCF</td>
<td>6hSAA-DCF</td>
</tr>
<tr>
<td>monomer total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>arabinose (% of theoretical)</td>
<td>4.2 (0.2)</td>
<td>16.5 (0.9)</td>
</tr>
<tr>
<td>OHS/arabinose (g/g)</td>
<td>0.12 (0.01)</td>
<td>0.93 (0.01)</td>
</tr>
</tbody>
</table>

(Yarabinose – yield of arabinose; YOHS – yield of other hemicellulosic sugars (xylose and galactose); Yglucose – yield of glucose; DCF – destarched corn fibre; 6hSAA-DCF – 6 h SAA pretreated destarched corn fibre; 24hSAA-DCF – 24 h SAA pretreated destarched corn fibre. The hydrolysis was performed for 4 days. Standard deviations are calculated from triplicates, and are indicated in parenthesis.)

(Table 4). The yield of total glucose increased continuously to 16% (Table 4).

The high yields of total arabinose and OHS and the low yields of monomer arabinose and OHS indicate that the hemicellulose fraction was solubilised mainly in the form of oligosaccharides. The fast liberation and accumulation of oligosaccharides can be explained by high endo-xylanase and low β-xyllosidase activities of Hemicellulase NS22002 at pH 4. This combination of endo-xylanase and β-xyllosidase activities could also be the reason for the relatively low xylanase activity determined by the measurement of reducing sugars at pH 3 and 4. High endo-xylanase activity results in xylo-oligosaccharides from the birch wood xylan used in the xylanase activity assay. Xylo-oligosaccharides contain relatively few reducing-ends resulting in low xylanase activity.

The presence of glucose monomers and oligomers in the supernatant of enzymatic hydrolysis indicates that Hemicellulase NS22002 contains cellulase and β-glucosidase enzymes with considerable activity at pH 4. During the enzymatic hydrolysis of 24 h SAA pretreated destarched corn fibre the curves of the sugar yields showed similar trends to those obtained in the case of the 6 h SAA pretreatment (Fig. 2(C) and 2(B)). The yields of monomer sugars increased until the end of hydrolysis (Fig 2(C)). The OHS yield regarding monosaccharides was negligible (5%) (Table 4). The monomer glucose yield increased to 9%, which was
the same as that obtained in the case of the 6 h SAA pretreatment (Table 4). However, significant improvement was achieved in the monomer arabinose yield, which was raised from 17% to 27% by increasing the duration of SAA pretreatment from 6 to 24 h (Table 4). Regarding the total sugars, the glucose yield increased continuously until the end of hydrolysis and reached 16%, which is equal to that obtained by 6 h SAA pretreatment (Table 4). The yields of total arabinose and OHS reached their maximums within 2 days (Fig. 2(C)), 59% and 51%, respectively (Table 4). These yields are significantly lower than the yields achieved by the 6 h SAA pretreatment.

According to these results, during the enzymatic hydrolysis less hemicellulosic sugars are released after the 24 h SAA pretreatment than after the 6 h SAA pretreatment, however, the solubilised oligosaccharides are more accessible for the α-L-arabinofuranosidases. To find an accurate explanation further investigation is required. The selectivity of the hydrolyses was evaluated by the ratio of OHS (g) to arabinose (g) present in the supernatant at the end of the hydrolyses. In desirable cases mainly the arabinose moieties are released resulting in an OHS/arabinose value close to 0, however in undesirable cases the whole hemicellulose structure is solubilised resulting in an OHS/arabinose value of approximately 1.9, which is calculated from the recoverable sugars of destarched corn fibre and SAA pretreated destarched corn fibres. In our approach OHS/arabinose value was considered to be unacceptable above 1.2, satisfactory between 1.2 and 0.4 and good below 0.4. Regarding the monomer sugars the selectivity of hydrolyses of destarched corn fibre and destarched corn fibres pretreated by SAA method for 6 and 24 h were found to be good (Table 4). The hydrolysis selectivity concerning monosaccharides decreased with increasing the reaction time of SAA pretreatment (Table 4). Regarding the total sugars appearing in the supernatant, the hydrolysis selectivity was satisfactory in the case of destarched corn fibre and unacceptable in the case of 6 h and 24 h SAA pretreated destarched corn fibres, however the unacceptable selectivities were less than 1.9 (Table 4). There was no significant difference between the hydrolysis selectivities obtained after 6 h and 24 h SAA pretreatments.

**Enzymatic hydrolysis at pH 3**

Enzymatic hydrolysis of 6 h SAA pretreated destarched corn fibre was performed at pH 3 with Hemicellulase NS22002. Monomer glucose, arabinose and OHS were not detected in the supernatant. The yields of total arabinose and total OHS were 14% and 12%, respectively, and the yield of total glucose was 3% at the end of hydrolysis (data not shown). The results show that without formation of the appropriate amount of solubilised oligosaccharides the liberation of monomer arabinose is retarded. This implies that the α-L-arabinofuranosidase of Hemicellulase NS22002 can act only on solubilised substrates.

**Enzymatic hydrolysis at pH 6**

Enzymatic hydrolysis of 6 h SAA pretreated destarched corn fibre was performed at pH 6 with Hemicellulase NS22002. Higher yields of monosaccharides and total sugars were achieved than at pH 3 and 4. The yields of monomer sugars increased until the end of hydrolysis (Fig. 2(D)), where 36% monomer arabinose yield and 12% monomer glucose yield were obtained (Table 4). The yield of monomer OHS was only 6% (Table 4), whereas the xylose selectivity of Hemicellulase NS22002 at pH 6 is close to the maximum (Fig. 1(D)). This could be due to the highly substituted structure of the oligosaccharides derived from corn fibre hemicellulose, which can make them recalcitrant against enzymatic decomposition [44]. The yields of total arabinose and OHS reached their maximums within 2 days, in contrast with the yield of total glucose, which increased until the end of hydrolysis (Fig. 2(D)). The yields of total arabinose and OHS were 88% and 83%, respectively (Table 4). The yield of total glucose increased to 20% (Table 4). Although the AX-AFH activity of Hemicellulase NS22002 – determined on water-insoluble wheat arabinoxylan – at pH 6 was only half that at pH 4, at the end of hydrolysis of 6 h SAA pretreated corn fibre, approximately two times more monomeric arabinose was released at pH 6 than at pH 4 (Fig. 2(B) and 2(D)). After 2 days the yield of monomer arabinose was the same in the case of enzymatic hydrolysis at pH 4 and pH 6, however the yield of total arabinose was much higher at pH 6 (Fig. 2(B) and 2(D)). This means that much more arabinose-containing oligosaccharides were liberated at pH 6 than at pH 4. The higher amount of oligosaccharides can contribute to obtain higher yield of monomer arabinose. It is also possible that the hydrolytic behaviour of the multi-component enzyme preparation Hemicellulase NS22002 is different in water-insoluble wheat arabinoxylan and SAA pretreated corn fibre. Obtaining higher yield of monomer arabinose at pH 6 than at pH 4 despite the higher AX-AFH activity at pH 4 could be due to the different structure of the water-insoluble wheat arabinoxylan used in AX-AFH activity assay and the SAA pretreated corn fibre used in the hydrolysis experiments. To find the exact explanation further investigation is required. At the end of hydrolysis at pH 6 the OHS/arabinose value regarding solubilised monosaccharides was 0.34 (Table 4), which is considered to be good hydrolysis selectivity. The OHS/arabinose value regarding total sugars present in the supernatant was 1.78 (Table 4), which is close to the OHS/arabinose ratio of the starting material (6 h SAA pretreated destarched corn fibre).

**CONCLUSIONS**

Xylanase and AX-AFH activities of four commercial enzyme preparations from Novozymes (Xylanase NS22083, Enzyme complex NS22119, Cellic Ctec2 and Hemicellulase NS22002) were investigated as a function of pH (3–10). In the case of Xylanase NS22083, Enzyme complex NS22119 and Cellic Ctec2 considerable relative xylanase activity was obtained at pH values of the maximum AX-AFH activity. AX-AFH activity of Xylanase NS22083 and enzyme complex NS22119 had two maximum values, one at acidic and another at alkaline pH range indicating the possibility of the presence of two different types of α-L-arabinofuranosidase in these enzyme mixtures. In the case of Hemicellulase NS22002 at pH 3 and 4 low relative xylanase and high relative AX-AFH activities were obtained, hence this enzyme mixture was selected to investigate the possibility to selectively release arabinose from corn fibre. Enzymatic hydrolysis of destarched corn fibre and destarched corn fibres pretreated by the SAA method for 6 and 24 h were performed at pH 4, and enzymatic hydrolysis of 6 h SAA pretreated destarched corn fibre was carried out at pH 3 and also at pH 6. SAA pretreatment has been found to be an appropriate method to make the structure of destarched corn fibre accessible to the hemicellulose-degrading enzymes, as a significant part of the hemicellulose fraction was solubilised during hydrolysis with Hemicellulase NS22002 at pH 4 and 6. Long residence time in the SAA pretreatment (24 h) resulted in increased yield of monomer arabinose, hence it could improve the accessibility of arabinose moieties to the α-L-arabinofuranosidase present in Hemicellulase.
Enzymatic hydrolysis of 6h SAA pretreated destarched corn fibre using Hemicellulase NS22002 at pH 6 was found to be a promising method to separate hemicellulosic sugars from the cellulose fraction, as it resulted in yields of total arabinose and OHS higher than 80% and a total glucose yield of 20%. The highest yield of monomer arabinose (36%) was obtained in the case of hydrolysis of 6h SAA pretreated destarched corn fibre with Hemicellulase NS22002 at pH 6. This yield of monomer arabinose is comparable with the yields achieved on purified corn hull arabinan by Kurakake et al.30 The results of the investigation of enzymatic activities (xylanase and AX-AFH) and hydrolysis of corn fibre imply that Hemicellulase NS22002 has high endo-xylanase activity over a broad pH range, negligible β-xylanosidase activity at pH 3 and 4, and its α-L-arabinofuranosidase is hypothesized to act only on solubilised substrates. During the enzymatic hydrolysis of 6h SAA pretreated destarched corn fibre at pH 6, negligible amounts of monomer OHS were released indicating the recalcitrance of the solubilised oligosaccharides against enzymatic decompositions. Xylo-oligomers derived from corn fibre that resist the hydrolytic enzymes available currently were published by Hespell et al.45 and Appeldoorn et al.46,47 The hydrolyses of SAA pre-treated destarched corn fibre with Hemicellulase NS22002 result in high amounts of hemicellulosic oligomers, considerable amount of monomer arabinose and negligible amounts of monomer OHS. According to the results there is no pH value allowing the selective solubilisation of arabinose from arabinoxylan-containing insoluble materials using the multi-component enzyme preparations investigated in this study. Selective liberation of arabinose from corn fibre using enzymatic hydrolysis could be a promising method for arabinose production, however finding the appropriate enzyme or enzyme mixtures and reaction conditions is still a challenge.

ACKNOWLEDGEMENT

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Investigation of commercial enzymes for selective release of arabinose


Investigation of selective arabinose release from corn fibre by acid hydrolysis under mild conditions

Csaba Fehér, a* Zita Gazsó, a Patomwat Tatijarern, b Máté Molnár, a Zsolt Barta a and Kati Réczey a

Abstract

BACKGROUND: The high cost of pure arabinose has resulted in a growing demand to develop new cost-effective methods of arabinose production from lignocellulosic and pectin-rich materials. Corn fibre is an inexpensive by-product of the corn wet-milling process, and is a promising raw material for producing arabinose and other value-added products. In this study fast dilute sulphuric acid hydrolysis of corn fibre was investigated for selective arabinose solubilization. Acid concentration and reaction time of hydrolysis of destarched ground corn fibre and raw corn fibre were 0.25–5 w/w% at 5–15 min and 0.25–1.25 w/w% at 25–75 min, respectively. Results were evaluated statistically and model equations were developed to predict the yields of sugars.

RESULTS: In the case of raw corn fibre the most favourable conditions were 1.1 w/w% sulphuric acid concentration and 51 min reaction time at 90°C, where a total arabinose yield of 75.9% was achieved with satisfactory selectivity. In the case of destarched ground corn fibre at a sulphuric acid concentration of 4.6 w/w%, a reaction time of 5 min and a temperature of 90°C, a total arabinose yield of 80.5% could be achieved with satisfactory selectivity, according to the model equations.

CONCLUSION: Arabinose-rich liquid fractions were produced by mild sulphuric acid treatments of corn fibre. Based on the models developed the mono- and oligosaccharide contents of the hydrolysates can be controlled under the conditions investigated.

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Keywords: biomass pre-treatment; lignocellulose fractionation; hemicellulose hydrolysis; arabinose production; biorefinery

NOTATION

- mY A: monomer arabinose yield (% of theoretical)
- mY OHS: monomer OHS yield (% of theoretical)
- tY A: total arabinose yield (% of theoretical)
- tY OHS: total OHS yield (% of theoretical)
- oY A: oligomer arabinose yield (% of theoretical)
- oY OHS: oligomer OHS yield (% of theoretical)
- m(OHS/A): OHS/A ratio for monosaccharides (g/g)
- t(OHS/A): OHS/A ratio for total sugars (g/g)
- o(OHS/A): OHS/A ratio for oligosaccharides (g/g)

INTRODUCTION

Corn fibre is an inexpensive by-product of the corn wet-milling process, and contributes to about 8–12% of the grain dry matter.1–3 Wet-milling of corn involves steeping the grain in water and sulphur dioxide to increase the moisture content and to soften the grain structure, which facilitates separation of the components: starch, gluten, fibre and germ. The major marketable products of wet-milling are the starch and the oil derived from germ. Co-products of wet-milling include corn fibre, corn gluten and steeping solids.4–6 Corn fibre consists of thick-walled cells originated from the aleurone layer, testa, pericarp, cell wall material from the endosperm and a significant amount of residual starch. The walls of the various cell types are mainly composed of hemicellulose and cellulose, however, they also contain phenolic acids, proteins, lipids and low amounts of lignin.7–9 Focusing on the polysaccharide content, corn fibre contains around 20% starch, 35% hemicellulose and 15% cellulose (dry matter).10–12 However, the exact chemical composition depends on the origin of the corn, the applied technology and it also varies year by year. The hemicellulose fraction of corn fibre is a heteroxylan referred to as glucurono-arabinoxylan. It consists of homopolimeric backbone chains of 1,4-linked β-D-xylopyranose units highly substituted with monomeric side-chains of arabinose and acetic acid linked to O-2 and/or O-3 positions, glucuronic acid linked to O-3 position and/or O-2 positions.

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acid and 4-O-methyl-glucuronic acid linked to O-2 position and also by oligomeric side-chains mainly containing arabinose, xylose, galactose and different type of hydroxycinnamic acids linked to O-5 position of arabinose moieties.\textsuperscript{13–16} The hemicellulose fraction of corn fibre contains around 48–54\% xylose, 33–35\% arabinose, 5–11\% galactose, and 3–6\% glucuronic acid.\textsuperscript{16} The heteropolysaccharide matrix is highly cross-linked by di-, tri-, and tetaferulic bridges contributing to create an insoluble network.\textsuperscript{17,18} Corn fibre hemicellulose is considered one of the most complex heteropolysaccharides recalcitrant to enzymatic hydrolysis.\textsuperscript{19} However, it can be easily solubilized at relatively mild conditions by mineral acids.\textsuperscript{1,2,11,20}

Corn fibre is utilized mainly as low-value animal feed, or solid fuel in pelletized form, however, it is a promising raw material for producing value-added products.\textsuperscript{21–23} The main fields of research concerning corn fibre utilization are the production of bioethanol\textsuperscript{24} and food additives like cellulose fibre gel,\textsuperscript{25} corn fibre gum,\textsuperscript{26} xyl-o-glicosaccharide prebiotics,\textsuperscript{27} corn fibre oil,\textsuperscript{28} ferulic acid,\textsuperscript{29} xylitol\textsuperscript{30–32} and vanillin.\textsuperscript{33} Moreover, considering the high arabinose content of the corn fibre hemicellulose, corn fibre could be a raw material for arabinose production.\textsuperscript{34,35}

In nature arabinose occurs in L-configuration in hemicellulose and pectin polymers. Traditionally, it is used for the production of flavours in the food industry. It can be used as a natural and non-caloric sweetener, their taste is similar to sucrose, however with half sweetness.\textsuperscript{36} Moreover, physiological experiments have revealed that L-arabinose inhibits the sucrase activity of intestinal mucosa. Addition of L-arabinose in combination with sucrose results in suppressed blood glucose and insulin responses in humans.\textsuperscript{37} Hence, L-arabinose is a promising additive for functional foods. Arabinose can also be used as an intermediate molecule for antivirus drug synthesis or as precursor to the drug intermediate (R)-3,4-dihydroxybutyric acid, to carnitine and agrichemicals.\textsuperscript{38} However, due to the high cost of pure arabinose, it is not common yet in the food and pharmaceutical industry.

On an industrial scale it 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 result in the high cost of pure arabinose, which is around 50 EUR kg\textsuperscript{-1}.\textsuperscript{39} Therefore, there is biotechnological and commercial interest in the development of new cost-effective methods for producing high purity grade L-arabinose from lignocellulosic and pectin-containing materials.\textsuperscript{39} Different strategies investigated involve enzymatic hydrolysis of purified arabinan\textsuperscript{40} or arabinoxylan,\textsuperscript{41} biopurification of hemicellulosic hydrolysate\textsuperscript{39} and acidic hydrolysis of agricultural residues containing significant amount of arabinose under mild conditions.\textsuperscript{34,42} Acid hydrolysis under mild conditions seems to be an appropriate method to selectively release arabinose as the a\textleftarrow{}1−2/3 bonds connecting arabinose moieties to the xylan backbone are more sensitive to the effects of pH and temperature than b\textleftarrow{}1−4 bonds of the xylan.\textsuperscript{42} However, 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 aim of the present study is to investigate the production of monosaccharides and oligosaccharides during fast dilute acid treatments of destarched ground corn fibre and raw corn fibre to determine the most favourable conditions for obtaining arabinose-rich liquid fractions.

**MATERIALS AND METHODS**

**Corn fibre**

Corn fibre (CF) was kindly donated by Hungrana Ltd (Szabadegháza, Hungary). It was dried and stored at room temperature. Before destarching corn fibre was ground to a particle size less than 1 mm.

**Destarching method**

Ground, air-dried corn fibre was suspended in acetic buffer (pH = 4.8, 100 mmol L\textsuperscript{-1}) at 3\% dry matter content, and then treated by thermostable a-amylase (5 g kg\textsuperscript{-1} dry matter) in 1 L closed glass-flasks at 90\°C for 3 h with continuous agitation (250 rpm) in a water bath in quadruplicate. The thermostable a-amylase enzyme preparation was donated by Hungrana Ltd. The solid fraction was separated by vacuum filtration through a 150 \μm pore sized nylon filter, and washed with distilled water at 80\°C to completely remove adsorbed substances. The volume of distilled water used in the washing step was three times that of the liquid volume of the corn fibre suspension. Destarched corn fibre (DCF) was dried at 40\°C and stored at room temperature until the next treatments.

**Soaking in aqueous ammonia (SAA) pretreatment**

The ammonia steeping of DCF was performed using the method of Nghiem et al. with minor modifications.\textsuperscript{4} DCF was treated at 10\% dry matter content in closed glass-flasks using 15 w/w\% ammonia solution for 6 h at 55\°C in rotary shaker (175 rpm). The solid fraction was separated by vacuum filtration through nylon filter (150 \μm), and washed by distilled water (80\°C) until neutral pH. The solid fraction (pretreated destarched corn fibre – PDCF) was immediately processed in dilute sulphuric acid treatment.

**Dilute sulphuric acid treatment**

DCF or PDCF was suspended in appropriately diluted sulphuric acid solution at 3\% dry matter content (50 g total weight). Treatments were carried out in 100 mL closed glass-flasks incubated in water bath at 90\°C or in an autoclave at 120\°C and 140\°C without agitation. The reaction times and the sulphuric acid concentrations were set according to the experimental design. The warm-up period was 15 min at 140\°C and 10 min at 90\°C and 120\°C. After treatments at 90\°C the flasks were cooled in cold water for 1 min and then the solid residues were separated by vacuum filtration through a nylon filter (150 \μm). At 120\°C and 140\°C the autoclave was cooled to 100\°C in 19 and 37 min, respectively. After that the flasks were cooled in cold water for 1 min and then the solid residues were separated by vacuum filtration through a nylon filter (150 \μm). The supernatants were analysed to determine monosaccharide and total sugars content.

**Monosaccharide determination**

Glucose, arabinose and other hemicellulosic sugars (OHS) in liquid samples were determined by high-performance liquid chromatography (HPLC). OHS consist of xylose and galactose as these sugars appeared as one peak in the chromatogram. The HPLC analysis was performed using a BioRad (Hercules, CA, USA) Aminex HPX-87H column (300 x 7.8 mm) at 65\°C. The eluent was 5 mmol L\textsuperscript{-1} sulphuric acid at a flow rate of 0.5 mL min\textsuperscript{-1}, and the sample volume was 40 \μL. The monosaccharides were detected and quantified by refractive index.
Determination of total sugars
Total sugars in the liquid samples include monomer sugars and sugar oligomers. To determine the total sugars content the samples were mixed with 8 w/w% sulphuric acid at a volume ratio of 1:1, and treated at 120°C in the autoclave for 15 min. The sugar oligomers were hydrolysed into monomers, which were analysed according to the method of monosaccharide determination.

OHS/A value
To evaluate the selectivity of hydrolysis in terms of the selective arabinose release the ratio of OHS (g) and arabinose (g) (OHS/A) appearing in the supernatant during the dilute sulphuric acid treatments was defined. In the most suitable cases much more arabinose is released than other sugars resulting in an OHS/A value close to 0, however in undesirable cases all the hemicellulosic sugars are solubilized resulting in an OHS/A value of approximately 1.9, which comes from the sugar composition of DCF.

Compositional analysis
The amount of recoverable sugars (glucose, arabinose, OHS) and the acid-insoluble material of ground CF, DCF and PDCF were determined using the NREL method with minor modifications.43 A half gram of dry matter was mixed with 2.5 mL of 72 w/w% sulphuric acid and was kept at room temperature for 2 h. Then, 75 mL of distilled water was added and the suspension was treated at 120°C in the autoclave for 1 h. The acid-insoluble fraction was separated by filtration through a G4 glass filter, washed with hot distilled water, dried at 105°C and measured gravimetrically. The supernatant was analysed using HPLC to determine its monosaccharide content. The compositional analysis was carried out in triplicate.

Experimental design and statistical analysis
Dilute sulphuric acid treatments were carried out according to a full factorial orthogonal design (3²) in quadruplicate at the centre point to determine the effects of the independent variables (sulphuric acid concentration and reaction time), the interactions between the variables, and to reduce the number of experiments. The sulphuric acid concentrations and reaction times were set according to the following: 0.25, 0.5, 0.75 w/w% or 1, 3, 5 w/w% and 5, 10, 15 min. The monomer arabinose yield (mY A), monomer OHS yield (mY OHS), total arabinose yield (tY A), total OHS yield (tY OHS), OHS/A for monosaccharide (mOHS/A) and OHS/A for total sugars (tOHS/A) were chosen as response variables in the experimental design. Statistica® v.11 (Statsoft®, Tulsa, USA) software was used to fit a second-order polynomial model for the measured data, and to enable the analysis of variance (ANOVA). The quadratic model was expressed as:

\[ Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 \]  

(1)

where Y represents the response variable, b0 is the intercept, b1 and b2 are the linear coefficients, b_{11} and b_{22} are the quadratic terms and X1 and X2 represent the independent variables studied. The independent variables were expressed in original physical values. Where possible, the model was simplified by eliminating statistically insignificant terms.

The goodness of the reduced model was checked by the value of lack of fit and its statistical significance was evaluated by F-test at 5% significance level. The statistical significance of the effect of variables was checked by Pareto chart and half normal probability plot.

In order to determine the optimum condition in terms of t(OHS/A) and tY A simultaneously, a desirability function approach was applied.44 The desirability function D involves transformation of each estimated response variable Yi to a desirability value di, where 0 ≤ di ≤ 1, and i = 1,2,…,k corresponded to the number of estimated response variables. The individual desirabilities are then combined using the geometric mean:

\[ D = (d_1 \times d_2 \times \ldots \times d_k)^{1/k} \]  

(2)

This single value of D gives the overall assessment of the desirability of the combined response levels,44 in our study d1 and d2 functions were set to change linearly by changing t(OHS/A) and tY A, respectively. The desired interval for the response variables were the following: 1.2 ≥ t(OHS/A) ≥ 0 and 50 ≤ tY A ≤ 100, where t(OHS/A) = 0 and tY A = 100 corresponded to d1 and d2 values of 1, and t(OHS/A) = 1.2 and tY A = 50 corresponded to d1 and d2 values of 0.

RESULTS AND DISCUSSION

Compositional analysis
The amount of the recoverable sugars and acid-insoluble solid from CF, DCF and PDCF are listed in Table 1. In the case of CF the recoverable glucose is derived from its cellulose and starch content. The amount of glucose liberated in the destarching step was around 162 mg g⁻¹ dry matter. Assuming that the destarching step completely removed the starch fraction, 46% and 54% of the CF glucan were derived from starch and cellulose, respectively. Based on the results of Benkő et al. it is estimated that the recoverable OHS of CF consists of 24% galactose and 76% xylose.45 According to Gáspár et al. the acid-insoluble fraction contains lignin, protein, oil and ash.23 In the SAA pretreatment the acid-insoluble matter of DCF decreased by 41%, which can be explained by removal of the lignin content,4 and the lignocellulosic structure was swollen.

Fast dilute sulphuric acid treatments
The liberation of arabinose has been found to be much faster than that of xylose in the early period of dilute acid hydrolysis,3,4 thus, in this study short reaction times were chosen to obtain arabinose-rich supernatants. Destarching of the ground corn fibre was performed to avoid starch hydrolysis during the acidic treatments aiming at production of pure arabinose solution. Targets for the arabinose solubilized and the selectivity of hydrolysis were defined as the following: total arabinose yield unacceptable below 50%, satisfactory between 50% and 70%, and good above 70%. The t(OHS/A) unacceptable above 1.2, satisfactory between 1.2 and 0.4 and good below 0.4. Sugar yields were expressed in percentage of the theoretical.

Acid hydrolysis of DCF at 140°C
During the fast dilute sulphuric acid hydrolysis of DCF at 140°C the major part of the arabinose was solubilized immediately, however, a great amount of OHS also appeared in the supernatant in all cases (Table 2). At an acid concentration of 0.15% and a reaction time of 5 min more than 40% of OHS and 80% of arabinose were released in monomer form (Table 2). Regarding the total sugars present in the liquid fraction the OHS yield was more than

1.9, which comes from the sugar composition of DCF.
Arabinose release from corn fibre by acid hydrolysis under mild conditions


Table 1. Recoverable sugars and acid-insoluble solid of starting materials, CF – corn fibre, DCF – destarched corn fibre, PDCF – pretreated destarched corn fibre, OHS – other hemicellulosic sugars (xylose and galactose). Standard deviations are indicated in parenthesis

<table>
<thead>
<tr>
<th>Components</th>
<th>Starting materials</th>
<th>CF</th>
<th>DCF</th>
<th>PDCF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recoverable sugars</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>glucose</td>
<td></td>
<td>352 (2)</td>
<td>273 (2)</td>
<td>290 (5)</td>
</tr>
<tr>
<td>OHS</td>
<td></td>
<td>238 (1)</td>
<td>343 (2)</td>
<td>354 (5)</td>
</tr>
<tr>
<td>arabinose</td>
<td></td>
<td>131 (1)</td>
<td>181 (1)</td>
<td>188 (3)</td>
</tr>
<tr>
<td>Acid-insoluble solid</td>
<td></td>
<td>75 (0)</td>
<td>99 (8)</td>
<td>58 (6)</td>
</tr>
</tbody>
</table>

90%, when the arabinose yield was 101% (Table 2). Yields which exceed 100% can be explained by sugar decomposition during the method of compositional analysis. At the most severe reaction condition (0.75% acid concentration, 15 min reaction time) both OHS and arabinose were completely recovered in the supernatant exceeding 100% can be explained by sugar decomposition during the method of compositional analysis.

Acid hydrolysis of DCF at 120°C

The sugar yields and OHS/A values obtained in fast dilute sulphuric acid treatments of DCF at 120°C are listed in Table 3. Statistical analysis of the significance of independent variables and their interactions were carried out, and model equations were fitted to the measured response variables (yields and OHS/A values of monosaccharides and total sugars). The yields of sugars obtained in oligomer form were calculated as the difference between total sugars and monomers. However, these were not involved in the statistical analysis. The model equations were expressed in the form of Equation (1) reduced by the statistically insignificant terms (Equations (3)–(8)), in which the abbreviations and the dimensions of the independent and response variables are given in Table 3:

\[
mY_A = 46.5 + 75.75 - 46.75^2 + 0.647T
\]

\[
mY\text{OHS} = -0.436 + 48.05 + 0.143T + 2.90ST^2
\]

\[
tY_A = 66.9 + 88.05 - 70.25^2
\]

\[
tY\text{OHS} = -3.10 + 2495 - 1745^2 + 2.09T - 3.06ST
\]

\[
m\left[\text{OHS/A}\right] = -0.193 + 1.725 - 0.6775^2 + 0.041T
\]

\[
- 0.002T^2 + 0.050ST
\]

\[
t\left[\text{OHS/A}\right] = -0.203 + 6.065 - 4.785 + 0.127T - 0.003T^2
\]

\[
- 0.371ST + 0.0095T^2 + 0.2825T^2 - 0.0065T^2
\]

The monomer arabinose yield increased continuously from 57% to 87% as a quadratic function of acid concentration and as a linear function of reaction time. However, by raising the acid concentration the slope of the monomer arabinose yield curve decreased (Equation (3)). The monomer yield of OHS increased linearly from 8% to 68% with increasing acid concentration and reaction time (Equation (4)). Under more severe reaction conditions (increasing the acid concentration and/or the reaction time) the m[OHS/A] value also increased continuously from 0.26 to 1.49, indicating a decline in the selectivity of hydrolysis. The standardized effects of the independent variables and the interactions were investigated by Pareto chart and half-normal probability plot, which indicated that the linear term of acid concentration has the biggest effect on all the response variables. A Pareto chart of monomer arabinose yield is demonstrated in Fig. 1. Regarding the total sugars the reaction time has no significant effect on the arabinose yield (Equation (5)). By increasing the acid concentration the total arabinose yield increased from 74% to 93%. By raising the acid concentration the slope of the total arabinose yield curve decreased (Fig. 2). The total OHS yield depends on the acid concentration in a quadratic way, however the linear term of reaction time and the interaction of the two independent variables (ST) also have significant effects on it (Equation (6) and Fig. 2). The total OHS yield increased from 37% to 84% with

Table 2. Reaction conditions, sugar yields and OHS/A values of DCF hydrolysis at 140°C. DCF – destarched corn fibre, OHS – other hemicellulosic sugars (xylose and galactose), A – arabinose, m – monomer, o – oligomer, t – total, Y – yield expressed in percentage of the theoretical, S – sulphuric acid concentration, T – reaction time

<table>
<thead>
<tr>
<th>Reaction conditions</th>
<th>Monomer</th>
<th>Oligomer**</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S (w/w%) T (min)</td>
<td>mY\text{OHS} (%)</td>
<td>mY_A (%)</td>
<td>m{OHS/A} (g/g)</td>
</tr>
<tr>
<td>0.15 5</td>
<td>41.6</td>
<td>81.2</td>
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</tr>
<tr>
<td>0.15 10</td>
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<td>85.4</td>
<td>1.22</td>
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<td>0.15 15</td>
<td>59.0</td>
<td>84.2</td>
<td>1.33</td>
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<tr>
<td>0.45 5</td>
<td>87.3</td>
<td>90.5</td>
<td>1.83</td>
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<tr>
<td>0.45 10</td>
<td>93.5*</td>
<td>93.3*</td>
<td>1.90*</td>
</tr>
<tr>
<td>0.45 15</td>
<td>97.8</td>
<td>96.0</td>
<td>1.93</td>
</tr>
<tr>
<td>0.75 5</td>
<td>92.4</td>
<td>91.3</td>
<td>1.92</td>
</tr>
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<td>0.75 10</td>
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<td>1.94</td>
</tr>
<tr>
<td>0.75 15</td>
<td>99.9</td>
<td>98.4</td>
<td>1.93</td>
</tr>
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</table>

*Average values. **Variables related to oligomers were calculated as the difference of total sugars and monomers. Yields which exceed 100% can be explained by sugar decomposition during the method of compositional analysis.
Table 3. Reaction conditions, sugar yields and OHS/A values of DCF hydrolysis at 120°C. DCF – destarched corn fibre, OHS – other hemicellulosic sugars (xylose and galactose), A – arabinose, m – monomer, o – oligomer, t – total, Y – yield expressed in percentage of the theoretical, S – sulphuric acid concentration, T – reaction time

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<th>Monomer</th>
<th>Oligomer**</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mY_{OHS}</td>
<td>mY_A</td>
<td>m[OHS/A]</td>
</tr>
<tr>
<td>0.15 (w/w%)</td>
<td>5</td>
<td>8.0</td>
<td>57.3</td>
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<td>0.15</td>
<td>10</td>
<td>11.9</td>
<td>65.5</td>
</tr>
<tr>
<td>0.15</td>
<td>15</td>
<td>13.8</td>
<td>66.9</td>
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<tr>
<td>0.45</td>
<td>5</td>
<td>28.2</td>
<td>75.8</td>
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<td>5</td>
<td>44.9</td>
<td>80.4</td>
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<tr>
<td>0.75</td>
<td>10</td>
<td>59.3</td>
<td>83.3</td>
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<tr>
<td>0.75</td>
<td>15</td>
<td>68.1</td>
<td>86.8</td>
</tr>
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</table>

*Average values. **Variables related to oligomers were calculated as the difference of total sugars and monomers.

Figure 1. Pareto chart of standardized effects of independent variables and their interactions regarding the monomer arabinose yield of DCF hydrolysis at 120°C. DCF – destarched corn fibre, S – sulphuric acid concentration, T – reaction time, L – linear term, Q – quadratic term, ‘x’ refers the interactions between the terms.

Figure 2. Response surfaces of the total arabinose and OHS yields of acid hydrolysis of DCF at 120°C. The response surfaces represent the reduced model equations (Equations (5) and (6)). DCF – destarched corn fibre, OHS – other hemicellulosic sugars (xylose and galactose).

Acid hydrolysis of DCF at 90°C

The sugar yields and the OHS/A values obtained in fast dilute sulphuric acid treatments of DCF at 90°C are listed in Table 4. The model equations for the response variables are presented in Equations (9) – (14), in which the abbreviations and the dimensions of the independent and response variables are given in Table 4:

\[
m_{Y_A} = -0.729 + 15.45 + 0.102T + 1.12ST \quad (9)
\]
The hemicellulose structure was attacked at 90°C of the starting material, which suggests that only the side chains of oligomers was always larger than the amount of OHS monomers, as a first-order function of the acid concentration and the reaction time (Equations (9), (10)). The m[OHS/A] values remained between 0.10 and 0.11, which means that approximately nine times more arabinose monomers were obtained in the liquid fraction of OHS monomers, which is considered to be good selectivity of hydrolysis. The total OHS yields were low in all cases, even under the most severe conditions it was only 7%. The total arabinose yield varied between 3% and 24%. Monomer OHS and arabinose yields increased from 26% to 98% and from below 0.15 indicating good hydrolysis selectivity. Regarding the acid concentration the slope of the monomer arabinose yield curve decreased (Equation (15)). The m[OHS/A] values remained below 0.15 showing). The amount of monomer OHS released was negligible, it was less than 4% of the OHS content of PDCF. The monomer OHS yield increased linearly with increasing acid concentration and/or reaction time (Equation (16)). Compared with the hydrolysates of DCF at 90°C, more arabinose was obtained in both monomer and oligomer forms. The monomer arabinose yield varied between 6% and 45% as a first-order function of reaction time and, in contrast with hydrolysis without pretreatment, as a second-order function of the acid concentration. However, by increasing the acid concentration the slope of the monomer arabinose yield curve decreased (Equation (15)). The m[OHS/A] values remained below 0.15 indicating good hydrolysis selectivity. Regarding the monosaccharides, moderate arabinose yield was obtained with satisfactory selectivity by using ammonia pretreatment. The total arabinose and OHS yields increased from 26% to 98% and from 17% to 82%, respectively. In both cases the increase is linear in terms of the reaction time and quadratic regarding the acid concentration (Fig. 3(b)). By increasing the acid concentration the m[OHS/A] values remained below 0.15 indicating good hydrolysis selectivity. The total arabinose yields were unacceptable. When increasing the temperature from 90°C to 100°C, no significant differences were observed in terms of sugar yields and selectivity of hydrolysis (data not shown).

\[ mY_{\text{OHS}} = -0.033 + 0.6995 - 0.0001T + 0.0865ST \]  
\[ tY_A = -7.52 + 35.55 + 0.893T \]  
\[ tY_{\text{OHS}} = -2.35 + 7.575 + 0.206T \]  
\[ m[\text{OHS/A}] = 0.096 - 0.0015T - 0.0025T^2 \]  
\[ t[\text{OHS/A}] = 0.246 + 0.0795 + 0.001T + 0.0085T^2 \]  

Acid hydrolysis of PDCF at 90°C

To increase the sugar yields the option of SAA pretreatment performed before the acidic hydrolysis of 90°C was investigated. The sugar yields and the OHS/A values obtained by the dilute sulphuric acid treatments of PDCF at 90°C are listed in Table 5. The model equations for the response variables are presented in Equations (15)–(20), in which the abbreviations and the dimensions of the independent and response variables are given in Table 5.

The amount of monomer OHS released was negligible, it was less than 4% of the OHS content of PDCF. The monomer OHS yield increased linearly with increasing acid concentration and/or reaction time (Equation (16)). Compared with the hydrolysates of DCF at 90°C, more arabinose was obtained in both monomer and oligomer forms. The monomer arabinose yield varied between 6% and 45% as a first-order function of reaction time and, in contrast with hydrolysis without pretreatment, as a second-order function of the acid concentration. However, by increasing the acid concentration the slope of the monomer arabinose yield curve decreased (Equation (15)). The m[OHS/A] values remained below 0.15 indicating good hydrolysis selectivity. Regarding the monosaccharides, moderate arabinose yield was obtained with satisfactory selectivity by using ammonia pretreatment. The total arabinose and OHS yields increased from 26% to 98% and from 17% to 82%, respectively. In both cases the increase is linear in terms of the reaction time and quadratic regarding the acid concentration (Fig. 3(b)). By increasing the acid concentration the m[OHS/A] values remained below 0.15 indicating good hydrolysis selectivity. The total arabinose yields were unacceptable. When increasing the temperature from 90°C to 100°C, no significant differences were observed in terms of sugar yields and selectivity of hydrolysis (data not shown).

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<th>Monomer</th>
<th>Oligomer**</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S (w/w%)</td>
<td>T (min)</td>
<td>mY_{\text{OHS}} (%)</td>
<td>mY_A (%)</td>
</tr>
<tr>
<td>0.15</td>
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<tr>
<td>0.75</td>
<td>15</td>
<td>1.5</td>
<td>24.4</td>
</tr>
</tbody>
</table>

*Average values. **Variables related to oligomers were calculated as the difference of total sugars and monomers.
slope of the curves of total arabinose and OHS yield decreased (Equations (17), (18) and Fig. 3(B)). Therefore, almost the whole amount of OHS was solubilized as oligomers. In contrast with any other experiments, in the acidic treatments of PDCF more arabinose was released as oligomers than as monosaccharides. The amount of oligomers of both arabinose and OHS increased with increasing acid concentration and/or reaction time. The OHS/A value for oligomers clearly shows that this fraction was enriched in OHS compared with the starting material. According to the evaluation of standardized effects of the independent variables and the interactions by Pareto chart and half-normal probability plot the linear term of acid concentration has the biggest effect on all the response variables except for the m[OHS/A] value, where the linear term of reaction time does. The amount of glucose recovered in the supernatant during these experiments was negligible, it was less than 4% of the glucan content of PDCF in all cases.

Table 5. Reaction conditions, sugar yields and OHS/A values of PDCF hydrolysis at 90°C. PDCF – pretreated destarched corn fibre, OHS – other hemicellulosic sugars (xylose and galactose), A – arabinose, m – monomer, o – oligomer, t – total, Y – yield expressed in percentage of the theoretical, S – sulphuric acid concentration, T – reaction time

<table>
<thead>
<tr>
<th>Reaction conditions</th>
<th>Monomer</th>
<th>Oligomer**</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S (w/w%)</td>
<td>T (min)</td>
<td>mY_A (%)</td>
<td>mY_O (%)</td>
</tr>
<tr>
<td>0.15</td>
<td>5</td>
<td>0.3</td>
<td>5.9</td>
</tr>
<tr>
<td>0.15</td>
<td>10</td>
<td>0.7</td>
<td>11.6</td>
</tr>
<tr>
<td>0.15</td>
<td>15</td>
<td>1.1</td>
<td>16.3</td>
</tr>
<tr>
<td>0.45</td>
<td>5</td>
<td>1.4</td>
<td>25.1</td>
</tr>
<tr>
<td>0.45</td>
<td>10</td>
<td>2.4*</td>
<td>33.2*</td>
</tr>
<tr>
<td>0.45</td>
<td>15</td>
<td>2.7</td>
<td>41.8</td>
</tr>
<tr>
<td>0.75</td>
<td>5</td>
<td>2.0</td>
<td>35.1</td>
</tr>
<tr>
<td>0.75</td>
<td>10</td>
<td>3.1</td>
<td>46.0</td>
</tr>
<tr>
<td>0.75</td>
<td>15</td>
<td>3.6</td>
<td>44.5</td>
</tr>
</tbody>
</table>

*Average values. **Variables related to oligomers were calculated as the difference of total sugars and monomers.
Significant increase in total sugar yields was observed during the acidic hydrolysis of PDCF compared with the acidic hydrolys-
isis of DCF, which proves that SAA pretreatment can facilitate the solubilization of the hemicellulose fraction under mild acidic treat-
ment. Furthermore, SAA pretreatment was found to be favourable to produce oligomers containing mainly OHS under mild acidic hydrolysis. SAA treatment can remove the lignin part of DCF and eliminate the ferulic acid cross-linkages in the hemicellulose fraction,\(^1\) hence it makes the hemicellulose part less recalcitrant during acid hydrolysis. Although almost all of the recoverable arabi-
lose could be obtained in the supernatant, the large amount of solubilized oligomers resulted in unacceptable selectivity.

**Acid hydrolysis of DCF at 90°C with increased sulphuric acid concentration**

The aim of increasing the sulphuric acid concentration applied during the treatments at 90°C was to increase the arabinose yield, since in all previous designs acid concentration has been found to play the major role in terms of sugar yields. The acid concentrations were increased from 0.25%, 0.45% and 0.75% to 1%, 3% and 5%, respectively. The sugar yields and the OHS/A values obtained are listed in Table 6. The model equations for the response variables are presented in Equations (21)–(26), in which the abbreviations and the dimensions of the independent and response variables are given in Table 6.

\[
\begin{align*}
    mY_A &= -5.09 + 25.05 - 2.585S^2 + 1.37T \quad (21) \\
    mY_{OHS} &= 0.424 + 0.544S - 0.062T + 0.162ST \quad (22) \\
    tY_A &= 2.49 + 27.75 - 2.665S + 1.64T \quad (23) \\
    tY_{OHS} &= 1.60 + 3.36S + 0.035ST + 0.618ST \quad (24) \\
    m[OHS/A] &= 0.092 + 0.008S - 0.002T + 0.003ST \quad (25) \\
    t[OHS/A] &= 0.340 + 0.038S - 0.0001T + 0.010ST \quad (26)
\end{align*}
\]

The monomer arabinose yield varied from 25% to 75% as a second-order function of the acid concentration and as a first-order function of the reaction time. By increasing the acid concentration the slope of the monomer arabinose yield curve decreased (Equation (21)). The monomer OHS yields depended on both the reaction time and the acid concentration linearly (Equation (22)). It changed from 1% to 14% resulting in good hydrolysis selectivity. Although the m[OHS/A] increased with increasing acid concentration and/or reaction time, it was satis-
factory (less than 0.4) at all points of the experimental design. The total arabinose yield also changed as a second-order function of the acid concentration and as a first-order function of the reaction time, and the total OHS yield also depended linearly on both the reaction time and the acid concentration (Fig. 3(C)). By raising the acid concentration the slope of the total arabinose yield curve decreased (Equations (23), (24) and Fig. 3(C)). The total arabinose and OHS yields varied from 36% to 97% and from 8% to 64%, respectively. Except under the most severe conditions (5% acid concentration and 15 min reaction time), where almost the whole arabinose content of DCF was solubilized, the t[OHS/A] was within the range considered to be satisfactory. The yields of both arabinose and OHS oligomers increased with increasing acid concentra-
tion and/or reaction time. The amount of OHS in oligomer form was higher than the amount of monomer OHS in all cases. However, the monomer arabinose content of the hydrolysates was always higher than the amount of arabinose present as oligomers. By increasing the acid concentration and/or the reaction time the OHS proportion in the oligomers was increased. At this experimental design the linear term of acid concentration has the biggest effect on all response variables. A minor amount of glucose was also recovered in the supernatant during acid treatments, but this was less than 6% of the glucan content of DFC in all cases. The total arabinose yield and t[OHS/A] were in the satisfactory range. According to the optimization of D-function, the optimum conditions for our targets are 4.6% acid concentration and 5 min reaction time. At this point the model assumed a total arabinose yield of 80.5% and a t[OHS/A] value of 0.74.

**Acid hydrolysis of CF at 90°C**

Non-ground CF was also tested in fast dilute acid treatments, since at an industrial scale milling of corn fibre should be omitted due to the high energy demand. In these experiments the acid concentrations were 0.25%, 0.75% and 1.25% and the reaction times were 25, 50 and 75 min. The hydrolysates were performed at 10% dry matter content (100 g total weight in 250 mL closed
Table 7. Reaction conditions, sugar yields and OHS/A values of CF hydrolysis at 90°C. CF – corn fibre, OHS – other hemicellulosic sugars (xylose and galactose), A – arabinose, m – monomer, o – oligomer, t – total, Y – yield expressed in percentage of the theoretical, S – sulphuric acid concentration, T – reaction time.

<table>
<thead>
<tr>
<th>Reaction conditions</th>
<th>Monomer</th>
<th>Oligomer**</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S (w/w%) T (min)</td>
<td>mY&lt;sub&gt;OHS&lt;/sub&gt; (%)</td>
<td>mY&lt;sub&gt;A&lt;/sub&gt; (%)</td>
<td>m(OHS/A) (g/g)</td>
</tr>
<tr>
<td>0.25 25</td>
<td>5.4</td>
<td>4.5</td>
<td>2.18</td>
</tr>
<tr>
<td>0.25 50</td>
<td>5.6</td>
<td>8.3</td>
<td>1.22</td>
</tr>
<tr>
<td>0.25 75</td>
<td>5.8</td>
<td>11.8</td>
<td>0.89</td>
</tr>
<tr>
<td>0.75 25</td>
<td>6.7</td>
<td>27.5</td>
<td>0.44</td>
</tr>
<tr>
<td>0.75 50</td>
<td>8.2</td>
<td>42.3</td>
<td>0.35</td>
</tr>
<tr>
<td>0.75 75</td>
<td>9.9</td>
<td>53.2</td>
<td>0.34</td>
</tr>
<tr>
<td>1.25 25</td>
<td>8.4</td>
<td>46.2</td>
<td>0.33</td>
</tr>
<tr>
<td>1.25 50</td>
<td>13.1</td>
<td>61.8</td>
<td>0.39</td>
</tr>
<tr>
<td>1.25 75</td>
<td>17.2</td>
<td>70.3</td>
<td>0.44</td>
</tr>
</tbody>
</table>

*Average values. **Variables related to oligomers were calculated as the difference of total sugars and monomers.

Figure 4. Response surfaces of the total arabinose and OHS yields of acid hydrolysis of CF at 90°C. The response surfaces represent the reduced model equations (Equations (29) and (30)). CF – corn fibre, OHS – other hemicellulosic sugars (xylose and galactose).
Arabinose release from corn fibre by acid hydrolysis under mild conditions

investigated, it was less than 4% of the glucan content of CF. The total glucose yield was between 35% and 40% in the cases of low acid concentrations (0.25%) and it changed between 45% and 50% in the cases of higher acid concentrations (0.75% and 1.25%). It implies that at 0.75% and 1.25% acid concentrations almost the whole amount of CF starch was obtained in the supernatant as oligomers.

According to the optimization of the D-function, the optimum conditions for our targets are 1.1% acid concentration and 51 min reaction time. At this point the model predicted a total arabinose yield of 73.6% and a t(OHS/A) value of 0.79. To verify the optimum, CF was treated under these conditions in triplicate. The averaged results were in line with the model prediction, as a total arabinose yield of 75.9% and a t(OHS/A) value of 0.77 were achieved. Both values are inside the prediction interval, which proves the goodness of the model.

CONCLUSIONS

Model equations have been 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 are obtained with good hydrolysis selectivity, however at high arabinose yields significant amounts of other sugars are 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 is released in monomeric form – except when using ammonia-pretreated corn fibre – when OHS are mostly present in oligosaccharides. The effects of acid concentration and reaction time on the sugar yields and OHS/A 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 has the main effect on sugar yields. Up to a limit the sugar yields depend linearly on the acid concentration, however, above that they change 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 OHS. Both the monomer and total arabinose yields change linearly until around 30%, while the monomer and total OHS yields increase linearly until around 60%. In the case of acid hydrolysis of raw corn fibre similar trends are obtained except that increasing the acid concentration increases the OHS 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 can be easily hydrolysed by dilute acid treatments. The most favourable conditions in terms of the selective arabinose solubilization were determined by a D-function approach. In the case of destarched ground corn fibre at a sulphuric acid concentration of 4.6 w/w%, a reaction time of 5 min and a temperature of 90°C a total arabinose yield of 80.5% and a t(OHS/A) value of 0.74 could be achieved. In the case of raw corn fibre the most favourable conditions are estimated to be 1.1 w/v% sulphuric acid concentration and 51 min reaction time at 90°C, where a total arabinose yield of 75.9% and a t(OHS/A) value of 0.77 were achieved. Hence, arabinose-rich liquid fractions were produced by mild sulphuric acid treatments of corn fibre. However, to obtain all sugars in monomeric form a second hydrolysis step is needed.

Based on the models developed in this study the monosaccharide and oligosaccharide contents of the supernatant can be controlled under the conditions investigated. The major part of OHS was retained in the solid residue, from which a xylose-rich supernatant and a cellulose-rich solid fraction could be produced by a second dilute acid hydrolysis step. It was proved in this study that at 120°C and 140°C the hemicellulose fraction could be sharply separated from the cellulose part. Separation of corn fibre hemicellulose after acidic treatment under mild conditions has been reported in several studies.11,24 Production of arabinose-rich and xylose-rich liquid fractions separately could result in more effective sugar utilization in the biofermentation process.

ACKNOWLEDGEMENT

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Paper IV
Integrated process of arabinose biopurification and xylitol fermentation based on the diverse action of Candida boidinii

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Budapest University of Technology and Economics, Department of Applied Biotechnology and Food Science, Hungary, H-1111 Szent Gellért tér 4.

Abstract

Hemicellulosic hydrolysates of agro-residues are promising raw materials for xylitol and arabinose production through biotechnological methods. 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 Candida boidinii NCAIM Y.01308 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 of arabinose with a purity of 90%, based on total sugars. Xylitol fermentation under microaerobic condition resulted in a xylitol yield of 53% of theoretical and a xylitol concentration of 10.4 g/L 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 to produce xylitol.

Keywords: biorefinery, hemicellulosic hydrolysate, corn fibre, arabinose, xylitol, Candida boidinii

Introduction

Agricultural and agro-industrial residues (agro-residues) containing high amount of carbohydrates, such as cellulose and hemicellulose are produced in large amounts annually world-wide. Agro-residues are attractive raw materials of biorefinery processes producing value-added products due to their relatively low commercial value, renewability and abundant availability. Biorefining of agro-residues for biofuel and bioproducts does not compete with food production and is considered to be advantageous from the environmental point of view, as it contributes to waste management, and its products can replace fossil-derived ones.

One of the most important steps in lignocellulose-based biorefining technologies is the fractionation of the lignocellulosic biomass into its core constituent (hemicellulose, cellulose and lignin). Several studies proved the applicability of dilute acid-catalysed hydrolysis to selectively solubilise the hemicellulose fraction, therefore it is widely used as part of lignocellulose fractionation. Hemicellulosic hydrolysates containing high amount of xylose have great potential as a raw material for microbial xylitol production, while hemicellulosic hydrolysates with considerable amount of arabinose might be suitable for arabinose production. L-arabinose can be used as a non-caloric sweetener in the food industry, moreover it strongly inhibits intestinal sucrase uncompetitively and consequently inhibits the
absorption of sucrose in the small intestine, which makes it a promising sugar substitute in treatment of diabetes. L-arabinose can be utilized for bacterial diagnostics, as a starting material in the synthesis of non-ionic surfactants and as intermediate for antiviral drug synthesis. Commercial production of L-arabinose is a complex process consisting of acid hydrolysis of gum arabic, followed by multiple procedures of purification. The relatively high cost of gum arabic and the expensive purification steps result in the high cost of pure arabinose, which evoked an increasing research effort to develop new cost-effective methods of arabinose production from agro-residues rich in hemicellulose or pectin, for example from sugar beet pulp, corn hull, xylose-mother liquor, wheat bran and corn fibre. 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) using the adequate microorganisms. The drawbacks of biopurification process are wasting the potential of other sugars by converting them into value-added products and the formation of considerable amount of cell mass as by-product.

Xylitol has a potential use in pharmaceutical, odontological and food industries as an alternative sweetener. Besides its anticariogenic properties, xylitol can be used for diabetes treatments, since its metabolism is partially independent of insulin. On an industrial scale xylitol is produced through chemical reduction of xylose derived from hemicellulosic hydrolysate of birchwood or other xylose-rich materials. The purification and separation steps required to remove other by-products from xylose and xylitol makes the process relatively expensive. As an alternative method microbial production of xylitol is becoming more attractive, since the downstream processing is expected to be cheaper and, unlike the chemical route, mild reaction conditions are required. 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 using microbial processes. Xylitol can be produced by some bacteria and filamentous fungi, but the best producers are yeasts, especially species of genus Candida such as C. guilliermondii, C. pelliculosa, C. parapsilosis, C. tropicalis and C. boidinii. Microbial production of xylitol from hemicellulosic hydrolysate is influenced by several factors including the strain, the fermentation conditions (pH, dissolved oxygen concentration, initial cell concentration, temperature) employed in the process and the composition of the fermentation medium (initial xylose concentration, concentrations of other sugars and the ratios of these sugars, concentrations of inhibitor compounds and nutrients). The use of high initial cell concentration results in increased volumetric productivity and reduces the toxic effect of inhibitors, however considerable amount of carbon source is required to obtain an inoculum of high cell density.

In this study, arabinose biopurification and xylitol fermentation were investigated on semidefined medium and real hemicellulosic hydrolysate derived from corn fibre, and an integrated process was developed, in which the cell mass obtained as by-product in the biopurification step was utilized to inoculate the xylitol fermentation step. This process resulted in a more effective carbon utilization, as the cell propagation for the xylitol fermentation did not require additional carbon source or it did not consume xylose convertible into xylitol in the xylitol fermentation.
Materials and Methods

Corn fibre

Corn fibre was kindly donated by Hungrana Starch and Isosugar Manufacturing and Trading Co. Ltd. (Szabadegyháza, Hungary). It was dried and stored at room temperature.

Yeast strains and inoculum preparation

*Candida boidinii* NCAIM Y.01308, *Candida parapsilosis* NCAIM Y.01011, *Candida guilliermondii* (*Pichia guilliermondii*) NCAIM Y.01050, *Hansenula anomala* (*Pichia anomala*) Y.01499 were purchased from the National Collection of Agricultural and Industrial Microorganisms (Budapest, Hungary). Yeast strains were stored and maintained on agar slants containing 1% (w/w) glucose, 1% (w/w) peptone, 0.3% (w/w) yeast extract and 2% (w/w) agar at room temperature and at 4°C, respectively. The medium used for inoculum preparation (pH=6) contained 10 g/L yeast extract, 15 g/L KH₂PO₄, 1 g/L MgSO₄×7H₂O, 3 g/L (NH₄)₂HPO₄ and 30 g/L xylose. The solutions containing xylose and the other components were sterilized separately at 120°C for 15 min in autoclave. Cells were cultivated in 750-mL cotton-plugged Erlenmeyer flasks containing 150 mL inoculum medium at 220 rpm rotation speed in a rotary shaker at 30°C for 72 h, subsequently recovered by centrifugation (1000×g, 5 min), washed with sterile distilled water and the adequate amount of cell mass was directly resuspended in the xylitol fermentation and biopurification media.

Two-step acidic fractionation of corn fibre

The fractionation process of corn fibre includes two sequential hydrolyses catalysed by sulphuric acid (Fig. 1). The first acidic hydrolysis was carried out in 1000-mL closed glass-flasks containing 800 g corn fibre suspensions at 90°C for 51 min (plus 15 min warm-up period) without agitation in water bath. The corn fibre suspensions contained 10% (w/w) dry matter and 1.1% (w/w) sulphuric acid. Subsequently, the flasks were cooled in cold-water (10°C) for 1 min and the solid fractions were separated by vacuum filtration through a 150 µm-pore size nylon filter (*Rimóczi és Társa Ltd.*, Hungary). The supernatants were stored at -10°C (first hydrolysate). The solid fractions were washed with distilled water until neutral pH, collected, then dried and stored at 40°C (first solid residue). The first hydrolysate was treated at 120°C for 60 min to decompose oligosaccharides. The solution obtained is referred to as glucose- and arabinose-rich hydrolysate. The first solid residue was used in the second acidic hydrolysis step, which is performed in 500-mL glass-flasks containing 300 g suspensions at 120°C for 30 min in autoclave. The suspensions of the first solid residue contained 10% (w/w) dry matter and 1.1% (w/w) sulphuric acid. After the flasks were removed from the autoclave, they were cooled in cold-water (10°C) for 1 min, and the solid fractions were separated by vacuum filtration through nylon filter (150 µm). The supernatants were collected and stored at -10°C, and called as xylose-rich hydrolysate. The solid fractions were washed with distilled water until neutral pH, collected, then dried and stored at 40°C (cellulose-rich solid fraction).
Biopurification, medium preparation

Biopurifications were carried out at 30°C in rotary shaker (220 rpm) for 96 h in 100-mL cotton-plugged Erlenmeyer flasks containing 20 mL semidefined medium or glucose- and arabinose-rich hydrolysate of corn fibre, and monitored through daily sampling. Semidefined biopurification medium (pH=6) contained 10 g/L yeast extract, 15 g/L KH2PO4, 1 g/L MgSO4×7H2O, 3 g/L (NH4)2HPO4, 15 g/L arabinose, 7.5 g/L xylose and 7.5 g/L galactose. The solution of the sugars and that of the other components were sterilized separately at 120°C for 15 min in autoclave. The glucose- and arabinose-rich hydrolysate was sterilized using the same conditions. Before sterilization, the pH of the glucose- and arabinose-rich hydrolysate (pH=1) was adjusted to 6 by addition of calcium hydroxide (powder). The precipitated gypsum was removed by filtration with folding filter.

Xylitol fermentation, medium preparation

Xylitol fermentations were performed on semidefined medium or xylose-rich hydrolysate of corn fibre at 30°C in rotary shaker for 96 h, and monitored through daily sampling. Semidefined fermentation medium (pH=6) contained 10 g/L yeast extract, 15 g/L KH2PO4, 1 g/L MgSO4×7H2O, 3 g/L (NH4)2HPO4, and 30 g/L or 70 g/L xylose. The solution of the xylose and that of the other components were sterilized separately at 120°C for 15 min in autoclave. The xylose-rich hydrolysate was sterilized using the same conditions. Before sterilization, pH adjustment and clarification of the xylose-rich hydrolysate were performed. The pH of xylose-rich hydrolysate (pH=1) was adjusted to 6 by adding calcium hydroxide (powder). The precipitated gypsum was removed by filtration with folding filter. After pH adjustment, the xylose-rich hydrolysate was clarified by activated carbon (0.05g/100g hydrolysate) at room temperature for 30 min with continuous agitation, subsequently the activated carbon was removed by filtration with folding filter. Activated carbon (Norit DX ULTRA 8005.3) was kindly donated from Cabot Norit Activated Carbon (Amersfoort, The Netherlands). Fermentations were carried out in 100-mL Erlenmeyer flasks closed with cotton plugs. In order to simulate different aeration conditions three levels of medium volume (35, 50 and 65 mL) were used at two levels of rotation speed (125 and 220 rpm). Xylitol yields were considered to evaluate the experiments. Xylitol yield was calculated from the highest xylitol concentration obtained during the fermentation and expressed as percentage of theoretical. Theoretical xylitol yield was calculated from the initial xylose concentration by assuming a complete (stoichiometric) conversion. Xylitol volumetric productivities were also calculated at the time of the maximum xylitol concentration.

Compositional analysis

Composition of the carbohydrate fractions of corn fibre and the solid residues (first solid residue and cellulose-rich solid fraction) derived from fractionation of corn fibre were determined using the method of National Renewable Energy Laboratory with minor modifications. Half a gram of dry matter was mixed with 2.5 mL of 72% (w/w) sulphuric acid and the mixture was kept at room temperature for 2 h. Then, 75 mL of distilled water were added and the suspension was treated at 120°C in the autoclave for 1 h in 100-mL glass-flasks. The supernatant was analysed using high-performance
liquid chromatography (HPLC) to determine its monosaccharide content. Starch content of the corn fibre was determined using thermostable α-amylase. Ground, air-dried corn fibre was suspended in sodium acetate buffer (pH=4.8, 100 mmol/L) at 3% (w/w) dry matter content, and then treated by thermostable α-amylase (20 (mmol/min)/kg dry matter) in 1-L closed glass-flasks at 90°C for 3 h with continuous agitation (250 rpm) in a water bath. The thermostable α-amylase enzyme preparation with a volumetric enzyme activity of 4 mmol/(min×mL) (defined on starch substrate at 25°C) was donated by Hungrana Starch and Isosugar Manufacturing and Trading Co. Ltd. The supernatant was separated by vacuum filtration through nylon filter (150 μm), mixed with 8% (w/w) sulphuric acid at a volume ratio of 1:1 and treated at 120°C in autoclave for 15 min to decompose oligosaccharides. Then it was analysed for glucose by HPLC. The cellulose content was calculated as the difference of the total glucan and starch content. The compositional analysis was carried out in triplicate.

Analytical methods

Concentrations of glucose, xylose, arabinose, xylitol, ethanol, methanol and acetic acid were determined by HPLC using BioRad (Hercules, CA, USA) Aminex HPX-87H (300 × 7.8 mm) column equipped with Micro-Guard Cation H⁺ Refill Cartridge (30 × 4.6 mm) pre-column at 65°C, and a refractive index detector. The eluent was 5 mmol/L sulphuric acid at a flow rate of 0.5 mL/min. Galactose was determined by using Phenomenex (Torrance, CA, USA) Rezex RPM-Monosaccharide Pb⁺² (300 × 7.8 mm) column equipped with Carbo-Pb Security Guard Cartridge (4 × 3.0 mm) pre-column at 80°C, and a refractive index detector. The eluent was ultra-pure (milli-Q) filtered water at a flow rate of 0.5 mL/min. The sample volume was 40 μL.

Cell concentration in the inoculum and fermentation samples was calculated from the optical density of the sample using a calibration curve based on the relationship of optical density and cell dry weight. Cell dry weight was determined gravimetrically after separation a certain volume of inoculation broth (48 hour) by centrifuge (1000×g, 5 min), washing it with distilled water and drying the cells at 105°C. Optical density was determined by spectrophotometer (Ultrospec III, Pharmacia LKB, Uppsala, Sweden) at a wavelength of 600 nm.

Total phenolics content was estimated using Folin-Ciocalteu reagent according to the method described by Guo et al. Gas-liquid mass transfer coefficients (K_La) of oxygen from the headspace of the flask to the media of xylitol fermentation (semidefined medium and xylose-rich hydrolysate) were determined by using a non-fermentative gassing-out method. A 100-mL Erlenmeyer flask was equipped with an optical oxygen sensor (VisiFerm DO 120, HAMILTON Bonaduz AG, Switzerland) to measure dissolved oxygen concentration (C). After gassing out of the fermentation media with nitrogen, the increase of the dissolved oxygen concentration due to the shaking of the flask was measured until constant level of the dissolved oxygen concentration (C’). The measurements were performed at 30°C by using different levels of medium volume (35, 50 and 65 mL) and rotation speed (125 and 220 rpm). The values of the expression lnn(1-(C/C’)) was plotted as a function of time. The slope of the fitted linear curve gave the value of K_La.
Maximum oxygen transfer rate (OTR) was calculated by multiplying \( K_{La} \) (1/h) and \( C^* \)(mmol/L).

Results and Discussions

Arabinose biopurification on semidefined medium

The capability of the investigated yeast strains for arabinose biopurification was tested on semidefined medium containing sugars typically present in hemicellulosic hydrolysates, such as xylose, arabinose and galactose. The aim of the biopurification is the selective depletion of other sugars beside the arabinose by using the adequate microorganism, which results in pure arabinose solution. The absence of other sugars enables the effective crystallization of pure arabinose. During aerobic biopurification, the undesired sugars are utilized mainly to produce cell mass, which occurs as byproduct of the process. Biopurification offers an inexpensive method to purify arabinose from hemicellulosic hydrolysates, however due to the loss of the other valuable sugars, this method might be reasonable only in the case of utilization of arabinose-rich hemicellulosic hydrolysates. Hence, the concentration of arabinose in the semidefined medium used in this study was two times higher than the concentrations of xylose and galactose. Four xylitol fermenting yeast strains, namely \( C. boidinii \), \( C. guilliermondii \), \( C. parapsilosis \) and \( H. anomala \) were cultured in shake flasks under aerobic conditions to investigate their sugar consumption from mixed sugar solution. \( C. guilliermondii \), \( C. parapsilosis \) and \( H. anomala \) utilized xylose, galactose and arabinose simultaneously to produce cell mass. \( C. parapsilosis \) consumed all of the sugars in two days, \( C. guilliermondii \) and \( H. anomala \) depleted xylose and galactose in two days and arabinose in three days (data not shown). Hence, these strains are found to be inappropriate for arabinose biopurification from hemicellulosic hydrolysate. \( C. boidinii \) metabolized almost all of the xylose in one day (Fig. 2). The galactose concentration was continuously decreased, 85% of the initial was consumed in two days and 7% of the initial (0.4 g/L) was remained after four days (Fig. 2). \( C. boidinii \) did not consume arabinose, even if the other carbon sources were present in negligible quantities (Fig. 2). The cell concentration increased from 0.5 g/L to 5.5 g/L during the biopurification (Fig. 2), which corresponds to a cell yield of 0.38 g dry cell mass/g sugar consumed. The purity of the arabinose solution was defined as the ratio of arabinose content to total sugar content, in percentage. After four days, the biopurificated medium contained 13 g/L arabinose and 0.4 g/L galactose, which resulted in a purity of 97%. As \( C. boidinii \) was found to be appropriate for arabinose biopurification on semidefined media, it was selected for further investigations.

Xylitol fermentation on semidefined medium

Xylitol fermentations were performed on semidefined medium to determine the most favourable conditions for xylitol production by \( C. boidinii \). One of the most important factors affecting microbial production of xylitol is the aeration. In order to simulate different aeration conditions in shake flasks, the fermentations were performed at three levels of filling ratio (0.35, 0.5 and 0.65) each of them at two levels of rotation speed (125 and 220 rpm) (Table 1). Filling ratio is defined as the ratio of the medium volume to the flask volume. The higher filling ratio results in lower oxygen supply, while the
higher rotation speed intensifies the dissolution of oxygen, during shake flask fermentations. In order to confirm that, OTR values of the different settings of filling ratio and rotation speed were determined (Table 1). The effects of initial cell concentration, initial xylose concentration and methanol addition were also investigated. The different sets of the variables are shown in Table 1. Fermentations were carried out for four days. The investigation of different aeration conditions was performed with 1 g/L initial cell concentration and 30 g/L initial xylose concentration (Table 1). Xylitol fermentations under these initial conditions using *C. boidinii* followed the same trends. One example is demonstrated in Fig. 3a. Xylitol concentration keeps increasing as long as xylose is available in the fermentation broth. After xylose is depleted, *C. boidinii* starts to consume xylitol, hence xylitol concentration has a maximum value during the fermentation. Therefore, in the case of industrial implementation, it is crucial to terminate the process at the highest xylitol concentration, for which continuous monitoring of xylitol and xylose concentrations is necessary. Small amount of ethanol is always produced simultaneously with xylitol however, after xylose depletion the ethanol is also consumed. The cell mass continuously increases, which indicates that xylitol and ethanol are used to form cell mass after xylose depletion. Similar behaviour was observed by Walther et al. using *C. tropicalis*.30

At the rotation speed of 220 rpm, the OTR value decreased from 6.6 mmol/(L×h) to 5.2 mmol/(L×h) by increasing the filling ratio from 0.35 to 0.65 (Table 1). At the filling ratios of 0.35, 0.5 and 0.65 xylitol yields of 22%, 25% and 28% were obtained in two days (Table 1), which resulted in volumetric productivities of 0.14 g/(L×h), 0.16 g/(L×h) and 0.18 g/(L×h), respectively. In the case of 125 rpm rotation speed the highest xylitol yield, 44%, was achieved at the filling ratio of 0.5, where an OTR value of 2.8 mmol/(L×h) was measured (Table 1). The maximum xylitol concentration was observed in two days, which resulted in 0.28 g/(L×h) volumetric productivity. At 0.35 and 0.65 filling ratios, 4.2 mmol/(L×h) and 1.9 mmol/(L×h) OTR was observed, respectively (Table 1). At 0.35 filling ratio a xylitol yield of 36% was obtained in one day (Table 1), resulting in a volumetric productivity of 0.46 g/(L×h). At the filling ratio of 0.65 the xylitol yield was 27%, which was obtained in two days (Table 1). The volumetric productivity was 0.17 g/(L×h). In terms of the xylitol yields achieved, the most favourable aeration condition in shake flask fermentation using *C. boidinii* was obtained at the filling ratio of 0.5 with 125 rpm rotation speed, hence that condition was set for the subsequent experiments. That condition of aeration can be considered as microaerobic condition according to Walther et al.30 The xylitol fermentation performed at 1 g/L initial cell concentration and 30 g/L initial xylose concentration under microaerobic condition is referred to as base case in the following sections.

The effect of high initial xylose concentration for the fermentative capacity of *C. boidinii* was investigated using 70 g/L initial xylose concentration (Fig. 3b). The high initial xylose concentration resulted in a xylitol yield of 40% (Table 1), which is slightly lower compared to that obtained in the base case (44%). The xylitol concentration continuously increased until the end of the fermentation (Fig. 3b), resulting in a volumetric productivity of 0.3 g/(L×h). The continuous increase of xylitol concentration through four days was due to the presence of xylose during the whole fermentation. After four days, 10 g/L xylose was remained in the medium, hence the cells did not consume any accumulated xylitol (Fig. 3b). The ethanol formation showed similar trend than that of xylitol (Fig. 3b). The ethanol concentration increased until 6 g/L (Fig. 3b). The cell mass also continuously increased during the fermentation, which resulted in a
The effect of high cell density on the fermentation process was investigated using 5 g/L NRRL Y-17213 by varying xylose concentration from 20 g/L to 200 g/L, and it was investigated the effect of initial xylose concentration on xylitol production of final cell concentration of 4.5 g/L (Fig. 3b). Although it has been published in many studies that high initial xylose concentration increases the xylitol yield, it was not observed in this study. The relatively high amount of ethanol accumulated during the fermentation could negatively affect the xylitol production. Vandeska et al. investigated the effect of initial xylose concentration on xylitol production of C. boidinii NRRL Y-17213 by varying xylose concentration from 20 g/L to 200 g/L, and it was found that 150 g/L initial xylose concentration is the most favourable, as it results in a xylitol yield of 0.47 g/g xylose consumed. In our study, similar xylitol yield (0.46 g/g xylose consumed) was obtained using 70 g/L initial xylose concentration and 1 g/L initial cell concentration. However, Vongsuvanlert and Tani reported that increasing xylose concentration up to 150 g/L resulted in lower xylitol production than that of 100 g/L of xylose, using C. boidinii no. 2201. High initial xylose concentration negatively affects the xylitol production, which might be due to the osmotic stress on the cells of C. boidinii.

The effect of high cell density on the fermentation process was investigated using 5 g/L initial cell concentration. High initial cell density resulted in significantly higher xylitol yield within shorter fermentation time, compared to the base case, as 58% xylitol yield was achieved in one day (Table 1). The volumetric productivity of the fermentation was 0.73 g/(L×h). The cell concentration was almost constant through the fermentation, less than 1 g/L was formed (Fig. 3c), in contrast with the cases of low (1 g/L) initial cell density (Fig. 3a and 3b). Due to the reduced cell formation, more xylose was available in the cells for xylitol production, which probably contributed to obtain higher xylitol yield. The ethanol formation was also reduced, only 1 g/L was produced in two days (Fig. 3c), which is the half of that obtained in the base case (Fig. 3a). Hence, high initial cell concentration was found to be favourable in terms of the xylitol yield, the xylitol productivity and the ratio of xylitol to ethanol. The improvement of xylitol yield (g xylitol/g xylose consumed) by increasing the initial cell density of C. boidinii NRRL Y-17213 was reported by Vandeska et al.

Methanol addition is considered to be favourable for polyols production using methylotrophic yeasts, since the oxidation of methanol results in formation of NADH, which is needed for the reduction of sugars. However, addition of methanol as a co-substrate did not result in significant increase of xylitol yield (Fig. 3d vs. 3c). A xylitol yield of 60% was obtained when 12 g/L methanol was added to the fermentation broth containing 30 g/L initial xylose and 5 g/L initial cell concentrations (Table 1). However, longer time was needed (2 days) to reach the highest xylitol concentration compared to that of without methanol addition (1 day) (Fig. 3d vs. 3c), which resulted in a volumetric productivity of 0.38 g/(L×h). The methanol concentration decreased until 10 g/L in four days (Fig. 3d). The cell concentration slowly increased during the fermentation and 6 g/L of final cell concentration was obtained (Fig. 3d). Only 0.5 g/L ethanol, half of that obtained without methanol addition, was produced simultaneously with xylitol, which indicates that methanol addition can repress the formation of ethanol (Fig. 3d). In contrast, Vongsuvanlert and Tani reported that xylitol production using C. boidinii no. 2201 was significantly increased by adding methanol, and the methanol addition resulted in increased ethanol formation.

According to our investigation of xylitol production on semidefined media using C. boidinii, aeration and initial cell density have the greatest effects on xylitol yield. Xylitol production is favoured under microaerobic condition (2.8 mmol/(L×h) OTR) using increased (5 g/L) initial cell concentration.
Fractionation of corn fibre

The fractionation process of corn fibre includes two sequential hydrolyses catalysed by sulphuric acid (Fig. 1). Through the fractionation steps, xylose and galactose were determined as one component, which is referred to as xylose (+galactose). In the first step of the fractionation, corn fibre was treated under mild acidic conditions to solubilise the starch content and liberate most of the arabinose. After the first hydrolysis, considerable amount of oligosaccharides was obtained in the supernatant, thus an oligomer hydrolysis step was required to recover the sugars in monomeric form (Fig. 1). According to preliminary results, additional acid is not required to decompose oligomers of the first hydrolysate, when the oligomer hydrolysis step is carried out at 120°C for 1 h (data not shown). After the first and oligomer hydrolyses, glucose, arabinose and xylose (+galactose) were recovered in the supernatant with the yields of 103%, 74% and 32% of theoretical based on the raw material composition (Table 2), respectively. In this case, glucose yield is calculated from the starch content of corn fibre as the cellulose is assumed to be intact under mild conditions applied in the first hydrolysis. Glucose yield of 103% could be explained by the presence of small amount of glucose in the hemicellulose fraction, which can be easily hydrolysed under mild acidic treatments.14 The glucose- and arabinose-rich hydrolysate contained 15 g/L glucose, 8.4 g/L xylose (+galactose), 10.5 g/L arabinose and 0.9 g/L acetic acid. It was utilized as the fermentation medium for biopurificaton after pH adjustment (pH=6) and sterilization.

The solid residue of the first step was utilized in the second acidic hydrolysis to produce the xylose-rich hydrolysate (Fig. 1). Glucose, xylose (+galactose) and arabinose were recovered in the supernatant with the yields of 10%, 88% and 63% of theoretical based on the composition of first solid residue, respectively. The lower yield of arabinose than that of xylose might be due to arabinose degradation. The low glucose recovery during the second hydrolysis resulted in a cellulose-rich solid fraction (Fig. 1) containing 50% cellulose based on dry weight. The xylose-rich liquid fraction contained 3 g/L glucose, 28 g/L xylose (+galactose), 6.6 g/L arabinose and 2.3 g/L acetic acid. The total phenolics content was 5.3 g/L. The xylose-rich hydrolysate was utilized for xylitol production after pH adjustment (pH=6), treatment with activated carbon and sterilization.

Arabinose biopurification on glucose- and arabinose-rich hydrolysate of corn fibre

The glucose- and arabinose-rich hydrolysate derived from the fractionation process of corn fibre was utilized to produce pure arabinose solution through biopurification using *C. boidinii*. Biopurification was carried out for three days. The biopurification medium contained 13.5 g/L glucose, 9.2 g/L arabinose, 5.8 g/L xylose and 2.3 g/L galactose after the inoculation, and the initial cell concentration was 0.5 g/L (Fig. 4). It also contained 0.8 g/L acetic acid. During the biopurification of glucose- and arabinose-rich hydrolysate of corn fibre, the consumptions of glucose, xylose and galactose occurred simultaneously, however with different consumption rates. The consumption rate of glucose was the highest, followed by that of xylose, while the galactose consumption was the slowest (Fig. 4). Glucose and xylose were totally depleted in one day and two days, respectively (Fig. 4). Galactose was not consumed completely, 32% and 57% of...
the initial amount were removed in one day and four days, respectively (Fig. 4). Acetic acid was consumed within one day (data not shown). Most of the cell mass (4.5 g/L) was formed in one day, however cell concentration increased continuously until 6.5 g/L (Fig. 4). Beside the cell formation, considerable amount of ethanol was produced (2 g/L) in one day, however it was consumed within the next day (Fig. 4). In the case of biopurification of glucose free semidefined medium, ethanol formation was not observed, which might indicate that the ethanol was formed from glucose during biopurification of corn fibre hydrolysate. To find the exact explanation of ethanol production under aerobic biopurification, further investigation of the metabolism of C. boidinii is required. The arabinose concentration was constant through the whole biopurification process (Fig. 4). After three days of biopurification the medium contained 9.2 g/L arabinose and 1 g/L galactose (Fig. 4), that is the purity of arabinose was 90% of total sugars, which exceeds the purity requirement for crystallization of L-arabinose from a solution. These results prove the applicability of C. boidinii to produce arabinose-rich solution from crude hemicellulosic hydrolysates through aerobic biopurification.

Yeast mediated biopurification of arabinose from hemicellulosic hydrolysate was published by Cheng et al. (2011) and Park et al. (2001). Cheng et al. performed arabinose biopurification on xylose mother liquor using P. anomala. The purity of arabinose obtained under optimized conditions of biopurification was 86% of total sugars, the optimal time of biopurification was 75 hours. Park et al. developed a method to produce arabinose from purified corn fibre arabinoxylan by enzymatic hydrolysis and arabinose biopurification. Williopsis saturnus yeast strain was selected to perform the arabinose biopurification on enzymatic hydrolysate of corn fibre arabinoxylan. Almost all of the xylose was consumed in three days without any loss of arabinose, however data of the concentrations of other components were not reported. Compared to these methods, arabinose biopurification from corn fibre hydrolysate using C. boidinii is a competitive strategy.

**Xylitol fermentation on xylose-rich hydrolysate of corn fibre, integration of xylitol fermentation and arabinose biopurification**

The xylose-rich hydrolysate derived from the fractionation of corn fibre was utilized to produce xylitol, however it contained significant amount of phenolic compounds, which are strong inhibitors of microbial xylitol production. In order to remove phenolic substances the xylose-rich hydrolysate was treated with activated carbon before xylitol fermentation (Fig. 1). Activated carbon treatment is widely considered as an inexpensive and efficient method to reduce the content of inhibitory compounds in hemicellulosic hydrolysates. After the activated carbon treatment, the total phenolic content reduced to 0.3 g/L. Xylose concentration during activated carbon treatment did not change. The initial composition of the xylitol fermentation medium was the following: 2.2 g/L glucose, 24.1 g/L xylose (+galactose) and 6.1 g/L arabinose. The acetic acid concentration (2.3 g/L) was not changed significantly through the treatments of the xylose-rich hydrolysate.

The fermentation was performed under microaerobic condition (2.8 mmol/(L×h) OTR) using 5 g/L initial cell concentration. The C. boidinii cell mass used to inoculate the fermentation was harvested from the arabinose biopurification (Fig. 1), in contrast with the fermentations on semidefined medium, in which cell mass for inoculation was

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**Note**: The text is a transcription of the content of the image. It includes corrections and additions to ensure readability and coherence. The author's intent is to describe the process of biopurification and fermentation, focusing on the use of C. boidinii and the conditions under which it was performed. The text includes references to previous work by Cheng et al. (2011) and Park et al. (2001), highlighting the progress made in the field of biopurification and fermentation. The results show the applicability of C. boidinii in producing arabinose-rich solutions from hemicellulosic hydrolysates, which is a significant advancement in the biorefinery process.
propagated on semidefined inoculum medium. This kind of integration of the xylitol fermentation and the arabinose biopurification (Fig. 1) 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. Utilization of on-site by-products and integration of different production routes are crucial to develop a viable biorefinery process.

The profiles of utilization of sugars and formation of products through xylitol fermentation of the integrated process are shown in Fig. 5. Glucose was present in the medium in small concentration and it was depleted within one day (Fig. 5). The arabinose concentration was unaltered through the whole fermentation (Fig. 5). The cell concentration was almost constant, less than 0.5 g/L was produced in four days (Fig. 5). Xylitol concentration increased until the third day, when it was 10.4 g/L (Fig. 5), resulting in a volumetric productivity of 0.14 g/(L×h). This concentration corresponds to a xylitol yield of 43% of theoretical based on the initial concentration of xylose (+galactose). However, according to our previous results, 80% of the component measured as xylose (+galactose) in the xylose-rich hydrolysate was xylose, thus the initial xylose concentration can be assumed to be 19.3 g/L. By using this assumption during the calculation of the concentration of theoretically achievable xylitol, a xylitol yield of 53% was obtained. It is only 5% less, than the xylitol yield of the case performed under the same conditions (microaerobic aeration, 5 g/L initial cell concentration) on semidefined media, however, on semidefined media the xylitol yield was close to the maximum in one day (Fig. 3c). During the fermentation of xylose-rich hydrolysate, higher amount of ethanol (3 g/L) was produced in two days, than that obtained under the same conditions on semidefined medium (1 g/L) (Fig. 5 vs Fig. 3c). The increased ethanol formation might contribute to the reduced yield and slow accumulation of xylitol. After the third day of xylitol fermentation on xylose-rich hydrolysate of corn fibre, the fermentation broth contained 10.4 g/L xylitol, 6.1 g/L arabinose, 4.1 g/L xylose (+galactose) and 2.7 g/L ethanol (Fig. 5). As the xylitol concentration is approximately the same as the concentration of the residual sugars, further purification steps might be required to enable xylitol crystallization from the broth.

Leathers and Dien developed a two-stage, sequential fermentation process for xylitol and arabitol production from neutralized and deionized corn fibre hydrolysate using *P. guilliermondii.*33 This strategy resulted in a xylitol yield of 0.27 g xylitol/g initial xylose within four days, which yield is around half of that achieved in our process. Rao et al. investigated xylitol production from corn fibre hydrolysate, which was neutralized, treated with activated charcoal and ion exchange resins.24 *C. tropicalis* cells were adapted by sub-culturing in hydrolysate containing medium for 20 cycles. This method resulted in a xylitol yield of 0.58 g/g xylose utilized, within two days. In our process, a xylitol yield of 0.54 g/g xylose utilized was obtained within three days by assuming that all of the initial xylose was utilized, however deionization step was not included. Buhner and Agblevor investigated different detoxification methods to produce xylitol from concentrated corn fibre hydrolysate by using *C. tropicalis.*34 The highest xylitol yield, 0.4 g/g xylose utilized, was obtained within four days in the case of the highest concentrations (three times of the original hydrolysate) that had been partially neutralized by adding calcium hydroxide and treated with activated charcoal prior to the
fermentation. Hence, xylitol production from corn fibre obtained in our integrated process is competitive with those methods found in the literature.

Conclusions

Two step acidic fractionation of corn fibre was developed to produce a glucose- and arabinose-rich hydrolysate containing 15 g/L glucose, 10.5 g/L arabinose, 8.4 g/L xylose (+galactose), and a xylose-rich hydrolysate containing 28 g/L xylose (+galactose), 6.6 g/L arabinose, 3 g/L glucose. C. boidinii NCAIM Y.01308, a natural isolate yeast strain, was found to be suitable to produce arabinose solution with a purity of 90% from the glucose- and arabinose-rich hydrolysate through aerobic biopurification, and to be appropriate for xylitol production from the xylose-rich hydrolysate using high initial cell concentration (5 g/L) under microaerobic condition (2.8 mmol/(L×h) OTR). In order to eliminate the separate cell propagation step prior to xylitol fermentation and to solve the utilization of cell mass by-product obtained in biopurification, an integrated biorefinery process based on the diverse action of C. boidinii was developed, in which cell mass produced in biopurification was used to perform the xylitol fermentation. Xylitol fermentation resulted in a xylitol yield of 53% of theoretical in three days. Hence, an integrated biorefinery process was developed to purify arabinose and to produce xylitol from hemicellulosic hydrolysates by using C. boidinii yeast strain.

Acknowledgements

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References

Tables

Table 1
Fermentation conditions, maximal xylitol yields achieved on semidefined media and times required to achieve the maximal yield. Standard deviations are calculated from triplicates and indicated in parenthesis.

<table>
<thead>
<tr>
<th>Rotation speed (rpm)</th>
<th>Filling ratio</th>
<th>OTR (mmol/Lh)</th>
<th>Initial cell concentration (g/L)</th>
<th>Initial xylose concentration (g/L)</th>
<th>Co-substrate</th>
<th>Xylitol yield (% of theoretical)</th>
<th>Time (day)</th>
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<td>220</td>
<td>0.35</td>
<td>6.6</td>
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<td>30</td>
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<td>22 (1)</td>
<td>2</td>
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<tr>
<td>220</td>
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<td>6.1</td>
<td>1</td>
<td>30</td>
<td></td>
<td>25 (0)</td>
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<td>220</td>
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<td>1</td>
<td>30</td>
<td></td>
<td>28 (0)</td>
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<td>30</td>
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<td>36 (1)</td>
<td>1</td>
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<td></td>
<td>44 (4)</td>
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<td>1.9</td>
<td>1</td>
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<td></td>
<td>40 (1)</td>
<td>4</td>
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<tr>
<td>125</td>
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<td>5</td>
<td>30</td>
<td>12 g/L MeOH</td>
<td>58 (5)</td>
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Table 2
Carbohydrates and acetate content of corn fibre. Standard deviations are calculated from quintuplicate and indicated in parenthesis.

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage of dry weight</th>
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<tr>
<td>Starch</td>
<td>13 (0.6)</td>
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<tr>
<td>Cellulose</td>
<td>17 (0.8)</td>
</tr>
<tr>
<td>Xylan</td>
<td>19 (0.7)</td>
</tr>
<tr>
<td>Arabinan</td>
<td>12 (0.5)</td>
</tr>
<tr>
<td>Galactan</td>
<td>4 (0.1)</td>
</tr>
<tr>
<td>Acetate</td>
<td>3 (0.4)</td>
</tr>
</tbody>
</table>
Figures

Figure 1
Process scheme of fractionation of corn fibre and integration of arabinose biopurification and xylitol fermentation. Process steps and material streams are indicated in capital letters and in italics, respectively.

Figure 2
Arabinose biopurification on semidefined medium using Candida boidinii NCAIM Y.01308. Standard deviations are calculated from duplicates.
Fermentation profiles of xylitol production on semidefined medium using *Candida boidinii* NCAIM Y.01308 under different conditions. Fermentations were carried out under microaerobic conditions (0.5 filling ratio, 125 rpm) using (a) 1 g/L initial cell concentration and 30 g/L initial xylose (base case), (b) 1 g/L initial cell concentration and 70 g/L initial xylose, (c) 5 g/L initial cell concentration and 30 g/L initial xylose and (d) 5 g/L initial cell concentration, 30 g/L initial xylose and 12 g/L methanol. Standard deviations are calculated from triplicates.
Figure 4
Arabinose biopurification on glucose- and arabinose-rich hydrolysate of corn fibre using *Candida boidinii* NCAIM Y.01308. Standard deviations are calculated from triplicates.

Figure 5
Xylitol fermentation on xylose-rich hydrolysate of corn fibre in the integrated process using *Candida boidinii* NCAIM Y.01308. In the integrated process, the cell mass used for inoculation of xylitol fermentation is obtained in the arabinose biopurification step. Standard deviations are calculated from triplicates.