Dissertationes Forestales 248

Hemicellulosic sugars to biobutanol via acid catalyzed pretreatment and acetone-butanol-ethanol fermentation

Suvi Kuittinen

School of Forest Sciences
Faculty of Science and Forestry
University of Eastern Finland

Academic dissertation

To be presented, with the permission of the Faculty of Science and Forestry of the University of Eastern Finland, for public examination in the Auditorium C2 of the University of Eastern Finland, Yliopistokatu 4, Joensuu, on 23rd February 2018, at 12 o'clock noon.

Title of dissertation: Hemicellulosic sugars to biobutanol via acid catalyzed pretreatment and acetone-butanol-ethanol fermentation

Author: Suvi Kuittinen

Dissertationes Forestales 248

http://dx.doi.org/10.14214/df.248

Thesis Supervisors:
Prof. Ari Pappinen, School of Forest Sciences, University of Eastern Finland, Finland
Prof. Jouko Vepsäläinen, Department of Pharmacy, University of Eastern Finland, Finland
Prof. Markku Keinänen, Department of Biology, University of Eastern Finland, Finland
Doc. Ossi Turunen, School of Forest Sciences, University of Eastern Finland, Finland
D.Sc. Matti Siika-aho, Division of Bio- and Chemical Processes, VTT, Finland
D.Sc. Ossi Pastinen, School of Chemical Technology, Aalto University

Pre-examiners:
Professor Jianxin Jiang
Beijing Forestry University, China
Assistant Professor Sandip P. Bankar
Department of Bioproduct and Biosystems, Aalto University, Finland

Opponent:
Professor (emeritus) Heikki Ojamo
School of Chemical Technology, Aalto University

ISSN 1795-7389 (online)
ISBN 978-951-651-586-4 (pdf)

ISSN 2323-9220 (print)
ISBN 978-951-651-587-1 (paperback)

Publishers:
Finnish Society of Forest Science
Faculty of Agriculture and Forestry at the University of Helsinki
School of Forest Sciences at the University of Eastern Finland

Editorial Office:
The Finnish Society of Forest Science
Viikinkaari 6, FI-00790 Helsinki, Finland
https://dissertationesforestales.fi/

Kuittinen S. (2018). Hemicellulosic sugars to biobutanol via acid catalyzed pretreatment and acetone-butanol-ethanol fermentation. Dissertationes Forestales 248. 54 p. Available at: http://dx.doi.org/10.14214/df.248

ABSTRACT

The aim of this study was to use acid catalyzed pretreatment for efficient solubilization of hemicellulosic sugars from lignocellulosic materials and test the fermentability of the liquid prehydrolysate via acetone-butanol-ethanol (ABE) fermentation. Three different lignocellulosic materials were chosen: barley straw (*Hordeum vulgare*), a willow species (*Salix schwerinii),* and a spruce species (*Picea abies).* The aim of the pretreatment was to clarify the most optimal conditions to liberate hemicellulosic sugars into a fermentable monomeric form without serious degradation and leave the cellulose as intact as possible.

With the barley straw, xylan was completely extracted into the liquid prehydrolysate with the combined severity (CS) 1.27 (120°C, 1% H_2SO_4 and 60 min) and with willow, approximately 65% of xylan was extracted as monosaccharidic xylose with the CS 2.29 (0.1% H_2SO_4, 200°C, 30 min). Microwave pretreatment was shown to be effective with Norway spruce, with almost complete extraction of mannan, galactan, and xylan to the liquid prehydrolysate. Additionally, low concentrations of degradation products including furfural, HMF, formic acid, and levulinic acid were produced during acid-catalyzed pretreatments.

On the other hand, results showed that the dilute acid catalyzed pretreatments tested gave incomplete enzymatic saccharification of the willow and Norway spruce pretreated solid materials. Results showed, however, that with the optimization of pretreatment conditions based on the lignocellulosic biomass used, hemicelluloses could be extracted more selectively to fermentable sugars and cellulose preserved for further biorefining.

The liquid prehydrolysate of willow without detoxification but supplemented with starch was successfully fermented to butanol using *Clostridium acetobutylicum,* with butanol and ABE yields of 0.22 g/g and 0.35 g/g monosaccharide, respectively. It was also found that starch from barley grain ensured the essential nutrients for ABE fermentation. For efficient utilization of hemicellulose for butanol production, combining starch-containing side-streams to the hemicellulosic side-streams would offer an option for industrial ABE production.

Keywords: Lignocelluloses, Hemicellulose, Pretreatment, Microwaves, Enzymatic hydrolysis, Acetone-butanol-ethanol

ACKNOWLEDGEMENTS

This work has been ongoing for a long time, contributing many happy as well as desperate moments to my life. Also, during this period, I have met and worked with many skilled and intelligent people, and I hope that I have been able to show my appreciation to all of them. I feel the greatest gratitude to my main supervisor, Prof. Ari Pappinen. Thank you for providing support, advice, and opportunities for my professional development. Also, thank you for the thorough discussions on research work, project planning and management, and even on the question about the meaning of life. I would like to also express special appreciation to my other supervisors, Prof. Jouko Vepsäläinen and Prof. Markku Keinänen. Without Markku's help and kind advice in the beginning of this work, we would not have the opportunity to begin these research studies. With the help of Jouko, especially in the final part of this work, I have realized how many possibilities and viewpoints there are in the same research area. I also want to show my sincere appreciation to my other supervisors, Ossi Turunen, Matti Siika-aho, and Ossi Pastinen. Your help, advice, and comments have been priceless to me.

Together with my supervisors, I want to thank my colleague and coauthor, Ming Yang. I can only hope that our cooperation has been as informative, beneficial, and enjoyable to you as it has been for me. My other coauthors, Junhua Zhang, Yohama Puentes-Rodriquez, Aki Villa, and Erik Kaipiainen, are appreciated for the valuable comments and discussions. I want also thank the pre-examiners of my work Prof. Jianxin Jiang (Beijing Forestry University, China) and Assistant Prof. Sandip P. Bankar (Aalto University, Finland). There are many other people to thank for participating in, commenting on, and criticizing my work during this period. So, Aate, Aifang, Anas, Antti, David, Eero, Eino, Esa, Helena P.-T., Helena S., Hossain, Jarmo, Junko, Kaisa, Kamrul, Karthik, Kirsi, Laura, Leena, Maini, Markku H., Markku R., Mir, Mohsin, Pauliina, Piia, Riikka, Risto, Ritva, Saila, Sandra, Sylas, Tanja, Veli, Victoria and Yasuhito, thank you very much!

This work has been part of the projects "New business models for bioenergy entrepreneurship" financed by European Fund for Rural Development, "Business from Biorefining" financed by European Regional Development Fund and "Sustainable production concepts on integrated biorefining industry (SusBioRef)" financed by Academy of Finland. I am grateful to all of these for financial support.

I am most grateful to my family for all the support during this period of my life. My parents, Ulla and Jouko, my sister, Saara, and my brother, Ville, thank you for helping and being there for me and my boys. Also, I want to thank my parents-in-law, Pirkko and Martti, and other members of my family, especially Outi, Hellu, Raikka, Titi, Hannu, Aila, Tuuli, and Jarkko for all their support during this period. I also want to thank my dear friend, Paula, and her husband, Pekka, for the close connection despite the distance between us. But, there is no doubt that the most sincere gratitude belongs to my husband Petteri, for standing by me during this time. You and our sons, Jaakko and Panu, mean everything to me.

I am very proud of my roots in North Karelia, Finland, and finally, I want to honor my two grandmothers: Annikki (who I would really like to know also as an adult) and Maila (who I have privilege to meet almost every day). From your courage and guts in everyday life and from your way to treat other people, I only can learn.

Kunnasniemi, January 2018,
Suvi

LIST OF ORIGINAL ARTICLES

The thesis is based on the following articles, which are referred to in the text by the Roman numerals I-IV. Articles I-IV are reproduced with the kind permission of publishers.

I Yang M., Kuittinen S., Zhang J., Keinänen M., Pappinen A. (2013). Effect of dilute acid pretreatment on the conversion of barley straw with grains to fermentable sugars. Bioresource Technology 146: 444-450. http://dx.doi.org/10.1016/j.biortech.2013.07.107

II Kuittinen S., Yang M., Keinänen M., Vepsäläinen J., Pappinen A. (2018). Non-detoxified acetone-butanol-ethanol fermentation of dilute acid extracted hemicellulosic monosaccharides from *Salix schwerinii* E. wolf. Manuscript submitted to BioResources

III Kuittinen S., Puentes Rodriguez Y., Yang M., Keinänen M., Pastinen O., Siika-aho M., Turunen O. and Pappinen A. (2015). Effect of microwave-assisted pretreatment conditions on hemicellulose conversion and enzymatic hydrolysis of Norway spruce. BioEnergy Research 9(1): 344-354. http://dx.doi.org/10.1007/s12155-015-9696-9

IV Yang M., Kuittinen S. Vepsäläinen J., Zhang J., Pappinen A. (2017). Enhanced acetone-butanol-ethanol production from lignocellulosic hydrolysates by using starchy slurry as supplement. Bioresource Technology 243: 126-134. http://dx.doi.org/10.1016/j.biortech.2017.06.021

The author's contribution

Suvi Kuittinen contributed to Article I by designing the research, executing laboratory work, analyzing sugars, and participating in writing the manuscript. Suvi Kuittinen was responsible for designing the study, executing the experiments, analyzing data, and writing Articles II and III. Suvi Kuittinen contributed to Article IV by designing the research, executing laboratory work, and writing the manuscript. Research ideas for Articles I-IV were developed by Ari Pappinen, Ming Yang, and Suvi Kuittinen.

Ming Yang contributed to Articles II and IV by executing ABE fermentation experiments and was the main writer for Articles I and IV. Yohama Puentes-Rodriquez participated in executing the experiments for Article III and commented on the manuscript. On Article III, Ossi Pastinen contributed by conducting HPLC analyses and commenting on the manuscript, and Matti Siika-aho and Ossi Turunen contributed by commenting on the manuscript. Markku Keinänen participated by commenting on manuscripts I-IV and conducting the GC-MS analysis of sugars and degradation products for Articles I-III. Jouko Vepsäläinen contributed by performing the NMR analysis of the samples and commenting on the manuscripts for Articles I, II and IV. Junhua Zhang provided advice for designing the experiments for Articles I and IV.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND TERMS

C5 sugars	Sugar molecules containing 5 carbons, pentoses (xylose, arabinose)
C6 sugars	Sugar molecules containing 6 carbons, hexoses (glucose, mannose, galactose)
LCC	Lignin-carbohydrate complex
ILs	Ionic liquids
OS	Organic solvents
AFEX	Ammonia fiber expansion
ABE	Acetone-butanol-ethanol
EMP	Embden-Mayerhof-Parnas (EMP) glycolysis
GC-MS	Gas chromatography – mass spectrometry
NMR	Nuclear magnetic resonance spectroscopy
HPLC	High performance liquid chromatography
FPU	Filter paper unit
nkat	Nanokatal
BSA	Bovine serum albumin
PEG	Polyethyleneglycol
AIL	Acid insoluble lignin
ASL	Acid soluble lignin
CS	Combined severity

Liquid prehydrolysate	Hemicellulose-rich liquid obtained after pretreatment
Pretreated solid material	Solid material separated from liquid prehydrolysate by filtration
Solid residual material	Remaining solid after enzymatic hydrolysis

” Voisi olla parempi, vaikka toisaalta kylläkin.*”*

Rauli Badding Somerjoki

1. INTRODUCTION

Sustainably utilizing biomass for the replacement of fossil-based carbon products with renewable substitutes has demanded and will also demand in the future the continuous development of biorefineries, where biofuels, biochemicals and biomaterials, bioenergy, and even food and feed are coproduced (Kamm and Kamm 2004; Cherubini et al. 2009; de Jong and Jungmeier 2015). Producing biomaterials, biochemicals, and biofuels is possible from all the major components in the lignocellulosic biomass, hemicellulose, cellulose, lignin, and extractables via different platforms such as syngas, hydrogen, pulp, lignin, C5, and C6 sugars (Anugwom et al. 2012; Taylor et al. 2015). According to Cherubini et al. (2009), biorefineries can be divided into energy-driven biorefinery systems and material-driven biorefinery systems. Energy-driven biorefinery systems utilize biomass to produce transportation biofuels, heat, and power, whereas material-driven systems primarily produce bio-based products such as chemicals, food, feed, and biomaterials, as well as process residues which can be used for energy production.

Forest biomass biorefineries can produce several products from lignocellulosic feedstock, especially pulp, paper, and energy. Cellulose is efficiently utilized and refined for fiber products, and lignin combined with the hemicellulose and its remains is most often combusted to produce energy. However, properties of hemicelluloses as a carbohydrate-rich material makes it interesting from the viewpoint of valorizing industrial residues to more valuable products and increasing biorefineries' profitability (Rissanen et al. 2014). In addition to forest lignocellulosic biomass, there are agricultural lignocellulosic feedstocks such as barley and wheat straw, which are residues from agricultural processes and have valuable properties worth utilizing in biorefining. Recently, biochemical refining of both hemicellulose and cellulose of cereal straws, like barley straw, to bioethanol has also been developed for use on an industrial scale in Europe (Larsen et al. 2008; Larsen et al. 2012; Zech et al. 2016). However, despite intensive research and development, utilizing hemicelluloses in cellulosic ethanol plants is still inefficient and unprofitable due to the low fermentability of hemicellulosic sugars, although it has been expected that genetically modified microbes utilizing a wide variety of sugars with equal effectiveness to overcome this challenge (Girio et al. 2010; Ko et al. 2016; Zhao et al. 2016). There is also a demand to produce more valuable products than only ethanol from lignocellulosic biomass, since refining lignocellulosic sugars is energy demanding and costly with many process stages (de Jong and Jungmeier 2015).

As a biofuel, butanol has advantages over ethanol such as higher energy content per molecule, lower vapor pressure, and lower corrosiveness (Patakova et al. 2011; Procentece et al. 2015; Algayyim et al. 2018). Butanol is also a platform chemical which can be utilized as a solvent and a precursor for polymers and plastics. Currently, butanol is produced mainly from oil, but can be obtained alternatively by fermentation using some natural *Clostridium* species, genetically modified *Clostridium* strains, or various other genetically modified microorganisms with a cloned butanol metabolic pathway. From a lignocellulose refining standpoint, *Clostridia* produce a wide spectrum of hydrolytic enzymes, and in this sense, it can ferment a range of carbohydrates such as xylose and arabinose (Peng et al. 2012).

In producing bioalcohols and biochemicals from lignocellulosic materials, biochemical refining refers to processes where fermentable carbohydrates are fractionated via a sugar platform and then refined via microbial fermentation. Three main stages are involved in converting lignocellulosic sugars to value-added products via biochemical refining:

pretreatment, enzymatic hydrolysis, and fermentation (Peng et al. 2012). Generally, the most important purpose for pretreatment is to affect the biomass structure efficiently enough to fulfill fractionation purposes but be gentle in the means of producing fermentable sugar yields as high as possible (Bhutto et al. 2017). However, instead of whole raw material used for one purpose like the saccharification of lignocellulosic carbohydrates to fermentable sugars, the goal of the modern biorefinery concept is utilizing lignocellulosic fractions (hemicellulose, cellulose, lignin) to manufacture an array of value-added products (Bozell 2010; Rissanen et al. 2014). This, of course, requires process design and valorizing all fractions to the bioproducts to maintain a biorefinery's economic and ecological sustainability (Rissanen et al. 2014). To achieve valorization, there is a need to optimize the conditions of each stage of the process, like hemicelluloses extraction, according to the various raw materials used. Also, there is an option to join platform intermediates (like C5 and C6 sugars) from different biorefinery systems for processing (de Jong and Jungmeier 2015). In the petrochemical industry, this idea of fractionation and valorization is successfully used to convert crude oil into various products, and it could also provide the basis for a robust biorefining industry (Bozell 2010).

2. BACKGROUND OF THE WORK

2.1 General composition of lignocellulosic biomass

Lignocellulosic biomass is a term used for herbaceous plants, softwood and hardwood (Ragauskas 2014). The structure of lignocellulose is unique to each plant species, but it consists mainly of cellulose (30-50%), hemicellulose (15-35%) and lignin (15-35%) (Pedersen and Meyer 2010; Mori et al. 2015; Chen et al. 2017). In addition to these three main fractions, minor components of lignocellulosic biomass are extractives, pectin, proteins and inorganic ingredients. Cellulose and hemicellulose are polysaccharides composed from sugar units, cellulose from glucose or more specifically, cellobiose (consisting of two glucose units) and hemicellulose having a more heterogenous structure composed of different monosaccharidic units. Pectins are also heteropolysaccharides by their structure (Alén 2000).

Cellulose is a linear polymer of glucose units connected to each other by β-(1→4)-glycosidic bonds and with the high degree of polymerization (Goring and Timell 1962; Alén 2000). In the native type of cellulose, the molecules are oriented to microfibrils by intra- and intermolecular hydrogen bonds and hydrophobic interactions forming crystalline (highly ordered) and non-crystalline (more amorphous) celluloses (Alén 2000; Rowell et al. 2013) (Figure 1). Crystalline cellulose is accessible only via its surfaces, but non-crystalline cellulose is more largely accessible by microbial enzymes. The special structure of lignocellulose, however, also makes part of non-crystalline cellulose inaccessible since it is covered with hemicelluloses and lignin. Cellulose's ordered fiber structure forms the basis for the lignocellulosic recalcitrant cell wall structure. These features of cellulose are important from the viewpoint of pulping, chemical modification, extractions, and microbial refining.

Unlike cellulose, which is composed of uniform glucose (or cellobiose) units, hemicellulose composition depends on the plant species and cell tissue (Chundawat et al. 2011). The structure of hemicellulose is branched polysaccharides built with different

monosaccharide units, especially pentoses, hexoses, and uronic acids (Figure 1). More specifically, the pentoses found in hemicellulose are D-xylose, D-arabinose, and L-arabinose; the hexoses are D-glucose, D-mannose, and D-galactose, with minor amounts of L-rhamnose and L-fucose (deoxyhexoses); the uronic acids are D-glucuronic acid, 4-O-methyl-D-glucuronic acid, and D-galacturonic acid (Fengel and Wegener 1983; Alén 2000). Compared to cellulose, polymerization degrees of hemicelluloses are low, and lack of crystalline regions make the structure more easily degraded into the monosaccahridic form (Sun et al. 2004; Ragauskas 2014).

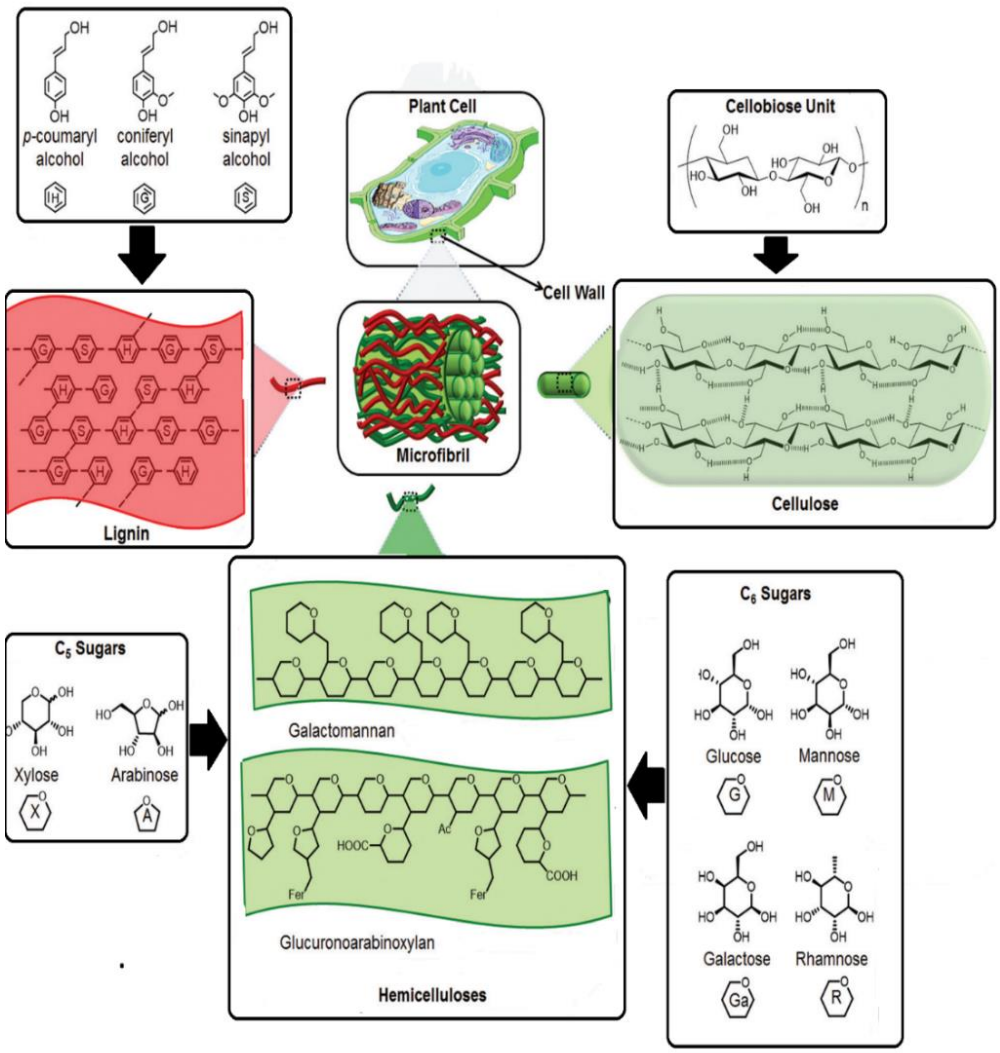

Figure 1. General structures and arrangement of lignocellulosic biomass components cellulose, hemicelluloses and lignin (According to Isikgor and Remzi Becer 2015).

The classification of hemicellulosic polysaccharides is most often based on the main component of the polymer; for example, xylans and mannans are based on xylose and mannose, respectively (Fengel and Wegener 1983). Lignocellulosic materials differ from each other by their percentages of hemicellulosic content in wood but also by variations in the individual hemicellulosic polysaccharides. For the herbaceous lignocellulosic biomasses (such as cereal straws) and hardwood materials, the main hemicellulosic polysaccharides are xylans, with a β-1,4-linked D-xylose as a backbone substituted with L-arabinose, D-galactose, and glucuronic acid residues, for example (Girio et al. 2010). In hardwoods, xylan occurs mainly as glucuronoxylan, constituting 15-30% of biomass, while in herbaceous biomass, arabinoglucuronoxylan and glucuronoarabinoxylan dominate. In softwood material, however, mannans such as galactoglucomannan are the major hemicellulosic polysaccharides.

Lignin is an amorphous, cross-linked aromatic polymer synthesized by the monomeric structural units (precursors) *trans*-coniferyl, *trans*-sinapyl, and *trans*-p-coumaryl alcohol via ether linkages and carbon-carbon bonds (Rowell 2013, Alén 2000) (Figure 1). Hardwood lignin mainly consists of coniferyl and sinapyl units, and the lignin formed is called guaiacyl-syringyl lignin. Softwood lignin, however, consists mainly of coniferyl units, and the lignin formed is called guaiacyl lignin. Lignin in herbaceous (monocots) plants are also called guaiacyl-syringyl lignin, consisting of coniferyl units, sinapyl units, and other precursors. Lignin and polysaccharides of hemicellulose are linked together via benzyl ether, benzyl ester, and glycosidic bonds forming lignin-carbohydrate complexes (LCC) (Alén 2000; Lai 2001). LCCs, together with high crystallinity and the degree of cellulose polymerization, make lignocellulosic cell walls stable and challenging in degradation processes. The reactivity of these complexes is highly dependent on the chemical bonds and reaction media; in alkaline conditions, ester linkages are easily hydrolyzed but ether linkages remain relatively stable, for example (Lai 2001).

2.2 Effect of acid catalyzed pretreatment on lignocellulose

2.2.1 Acid catalyzed pretreatment, among other pretreatment methods

Plant cell wall hemicelluloses, cellulose, and lignin are naturally strictly bound to each other, so isolation processes are required to separate these components from feedstock material for biochemical refining. This separation is commonly started with the pretreatment of the lignocellulosic biomass and followed by saccharification (hydrolysis with enzymes) or other bioprocessing, such as pulping or thermochemical processing. A crucial role of an efficient pretreatment is to extract hemicelluloses, affect the cellulosic fraction's structure, and alter the chemical bonds between hemicellulose, cellulose, and lignin for the effective enzymatic hydrolysis of cellulose (Mosier et al. 2005). In biorefining, pretreatment cost has a significant impact on whole process economics. Some features are needed for optimal, advanced, and cost efficient pretreatment processes: minimizing chemicals needed for pretreatment and following neutralization; high yields of hemicellulosic sugars after pretreatment; liquid prehydrolysate should be fermentable with a low-cost conditioning step without harmful byproducts; lignin and solid materials after pretreatment should be convertible to valuable co-products; and energy production for the pretreatment should be integrated within the whole process (Yang and Wyman 2008; Alvira et al. 2010; Chen et al. 2017).

Pretreatment processes could be divided into physical, chemical, physico-chemical, and biological treatments or a combination of these (Chen et al. 2017; Bhutto et al. 2017). Physical pretreatment methods include mechanical treatments such as chipping, crushing, or milling, as well as microwave and ultrasonic treatments. An advantage of physical pretreatment methods is relatively simple construction but in turn, the energy demand for physical processes is relatively high and affects production costs. Biological pretreatments consist of using micro-organisms or their enzymes for lignocellulose degradation. Without adding chemicals and with mild environmental conditions, biological methods are considered low energy and low pollution pretreatments. However, long treatment times are typical for biological pretreatments, and enzyme activities in the degradation of lignocellulose may be low. White-rot fungi, which produce lignin degrading enzymes such as laccases and peroxidases, are considered the most effective on lignocellulosic material (Kumar et al. 2009; Sánchez 2009).

Chemical pretreatment methods include ionic liquids, organosolv and oxidative delignification methods, and acid and alkaline hydrolysis. Ionic liquids (ILs) consist of salts composed of organic cations and inorganic or organic anions, with melting points below the boiling point of water (Chen et al. 2017). During ILs pretreatment, lignin and carbohydrates can dissolve when the structure of non-covalent interactions of lignocellulose is destroyed. At the same time, an advantage of ILs is minimizing the formation of degradation products, and the major disadvantage is the cost of the ILs' pretreatment process. It is also said that using ILs in pretreatment is not very cost efficient for utilizing the production of bulk chemicals but could be more suitable for refining fine and high-value chemicals from lignocellulose (Galbe and Zacchi 2012).

In organosolv pretreatment (OS), organic solvents such as methanol, ethanol, acetone, glycols, or phenols are used for lignin solubilization (Blanch and Simmons 2011). Three fractions occur after OS: lignin, pure cellulose, and an aqueous hemicellulose stream (Pan et al. 2006; Hu et al. 2008). Most of hemicellulose and lignin are solubilized to aqueous liquid, but the cellulose remains as solid. For environmental and process economy reasons, recovery of organic solvents is needed by distillation, for example (Galbe and Zacchi 2012). Separation of the solvent from the pretreated material is also necessary to avoid potential inhibitory effects in the following fermentation or enzyme hydrolysis. Oxidative delignification methods, such as wet-oxidation pretreatment, can be performed with only water and air or by adding alkali functioning as the oxidizing reaction catalyst to improve lignin solubilization.

Pretreatments are usually performed at low or high pH. Chemicals used in low-pH pretreatments are either organic or inorganic acids to enhance lignocellulose hydrolysis reactions. NaOH, $Ca(OH)_2$, and ammonia are most often used to achieve a higher pH. When alkaline liquids are used, they cause lignin and hemicellulose dissolution and de-esterification and change the crystallinity of lignocellulose mainly by removing amorphous material (hemicelluloses and lignin) (Kim et al. 2016). An advantage of alkaline conditions is improved hydrolysis of hemicellulosic polymers without serious degradation but one disadvantage is the volatility of alkaline chemicals which requires recycling to reduce costs and environmental damage (Wyman 1996; Taherzadeh and Karimi 2008; Chiaramonti et al. 2012; Prasad Maurya et al. 2015). Alkaline pretreatment methods are most effective with materials containing low lignin content (e.g., agricultural wastes, herbaceous crops), whereas they are less effective on softwood (Belkacemi et al. 1998; Galbe and Zacchi 2012). Hydrolysis with concentrated acids, usually sulfuric acid, is very effective in hydrolyzing lignocellulosic materials (Chiaramonti et al. 2012). With concentrated acids, pretreatment or

hydrolysis of lignocellulose can be performed at low temperatures but the drawback is the concentrated acid liquids' corrosiveness and need for recycling the acid. For these reasons, dilute acids are more often used, and they are suitable for many kinds of lignocellulosic materials. With dilute acids, however, higher reaction temperatures and reducing the size of lignocellulosic material are needed for optimal results. Like alkaline pretreatments, dilute acid pretreatments may be performed alone (e.g., concentrated acid and dilute acid hydrolysis), but they are usually assisted by physical pretreatment features like milling or microwave irridation, for example, and are termed physico-chemical pretreatments (Galbe and Zacchi 2012). A combination of physical and chemical pretreatments, like dilute acid pretreatment, steam pretreatment or steam explosion, ammonia fiber explosion (AFEX), and microwave-assisted dilute acid hydrolysis is usually favored for effective pretreatment (Teymouri et al. 2005; Lu et al. 2011).

When lignocellulosic biomasses are pretreated with only steam or hot water, a wide array of terms is used. In the literature, many terms such as autohydrolysis, hydrothermolysis, aqueous liquefaction, hot water treatment, pressurized liquid water extraction, water prehydrolysis or hydrothermal pretreatment, hydrothermal treatment, and steam treatment or steam extraction refer to pretreatments based on the same reactions of water autohydrolysis (henceforward water prehydrolysis) (Nitsos et al. 2013; Borrega and Sixta 2015). Basically, the difference between steam pretreatment and water prehydrolysis is the latter utilizes liquid hot water instead of steam and formally, they could also be considered rather physical or thermophysical pretreatments and not chemical pretreatments (Mosier et al. 2005). Steam pretreatment or water prehydrolysis are both pretreatment methods beginning at an almost neutral pH and ending at a pH of approximately 3.5-4 due to the water autohydrolysis reaction (Ragauskas 2014; Yan et al. 2016). A decrease of pH during autohydrolysis is caused by the auto-ionization of water, which generates hydronium ions, and thus significantly reduces pH (Garrote et al. 1999). This pH drop induces hemicellulose solubilization by hydrolyzing glycosidic linkages and liberates the acetyl groups (Wyman et al. 2005; Borrega et al. 2011; Ragauskas 2014). Acetic acid and uronic acids are byproducts of these reactions, which increase biomass hydrolysis by further lowering pH (Fengel and Wegener 1983).

Using water prehydrolysis for lignocellulose pretreatment in mild conditions produces mainly oligomeric sugars from hemicellulose, and in this sense, it only partially hydrolysizes hemicelluloses. Increasing water prehydrolysis intensity by increasing temperature and time not only enhances monosaccharide liberation but also increases the formation of monosaccharide degradation products (Nabarlatz et al. 2004). Several acids have been proposed to increase the release of monomeric sugars from mainly hemicelluloses during pretreatment. Dilute-acid pretreatment is performed using either organic acids or mineral acids such as sulfuric acid, which randomly cleaves the constituents of lignocellulose to smaller molecules. Adding a mineral acid such as H_2SO_4, HCl, or H_3PO_4 reduces the initial pH considerably to below 2, which results in more efficient hemicellulose hydrolysis and contributes to improving subsequent enzymatic hydrolysis. Dilute-acid catalyzed pretreatments can be performed either at high (over 160°C) or lower (120°C) temperatures, modifying the residence time according to temperature (i.e. high temperature-shorter time) (Taherzadeh and Karimi 2008; Alvira et al. 2010; Prasad Maurya et al. 2015).

2.2.2 Effects of acid catalyzed pretreatments on hemicellulose, cellulose, and lignin

Lignocellulose polysaccharides are hydrolyzed in acidic conditions via three main steps: 1) acidic protons interacting with the glycosidic linkages between sugar molecules, 2) conjugated acid formation, and 3) cleaving C-O bonds and forming cyclic carbonium cations (Fengel and Wegener 1983). The other reactions of polysaccharides in wood include dehydration, which is typical at acidic pH levels and usually unavoidable, causing sugars formed in polysaccharide hydrolysis to degrade (Figure 2) (Fengel and Wegener 1983). Acidic dehydration forms anhydro sugars, such as levoglucosan, by intramolecular glycosidic linkages. These glycosidic linkages can be easily hydrolyzed, and as further degradation products are formed (Figure 2). Most important of these degradation products are furfural (2-furaldehyde) and hydroxymethylfurfural (5-[hydroxymethyl]-2-furaldehyde, HMF). Furfural is formed from pentose sugars, and HMF is formed from hexose sugars. More severe conditions lead to the formation of levulinic acid and formic acid from the abovementioned compounds (Girisuta et al. 2006, Pedersen and Meyer 2010).

The polysaccharides' kinetic parameters with dilute mineral acids, organic acids, and water seem to depend on differences in the material together with reaction conditions and optimized pretreatment conditions; acids should enable nearly complete hydrolysis of hemicellulose to monomeric sugars (Wyman et al. 2005; Chundawat et al. 2011; Liu et al. 2012). Cellulose, however, is more stable than hemicellulose due to its crystalline and linear structure, as well as high degree of polymerization, enabling high resistance to degradation. Temperatures over 200°C are usually used for cellulose hydrolysis treatments to produce glucooligomers and glucose monomers. In the pretreatment reaction medium, many factors affect polysaccharide hydrolysis efficiency: type of acid used and its concentration, the medium's pH, and the temperature and pressure used in the treatment (Fengel and Wegener 1983). From the sample materials side, there are also several factors affecting the hydrolysis reaction, such as the acid catalyst's physical structure and accessibility, ring structures, and substituents of the treated material.

In acidic pretreatment media, the presence of protons induces intermediate carbonium ions formation, which causes lignin depolymerization (Fengel and Wegener 1983; Yan et al. 2016). Carbonium ions can also cause lignin recondensation reactions due to their nucleophilic nature. Higher pretreatment temperatures in acidic conditions enhance lignin depolymerization and thereby remove lignin from the lignocellulose (Yan et al. 2016). Lignin degradation products in the liquid prehydrolysates after acid-catalyzed pretreatments are partly low molar mass compounds and their soluble forms resemble in liquid prehydrolysate such as vanillin, coniferyl alcohol, syringaldehyde, and syringic acid (Larsson et al. 1999; Larsson et al. 2000; Borrega et al. 2013; Yan et al. 2016). The low molecular weight phenolics originating from lignin have been shown to be inhibitory to fermentative microorganisms, and in this sense, affects the utilization and downstream processing of liquid prehydrolysate by microbial fermentation processing (Gütch et al. 2012).

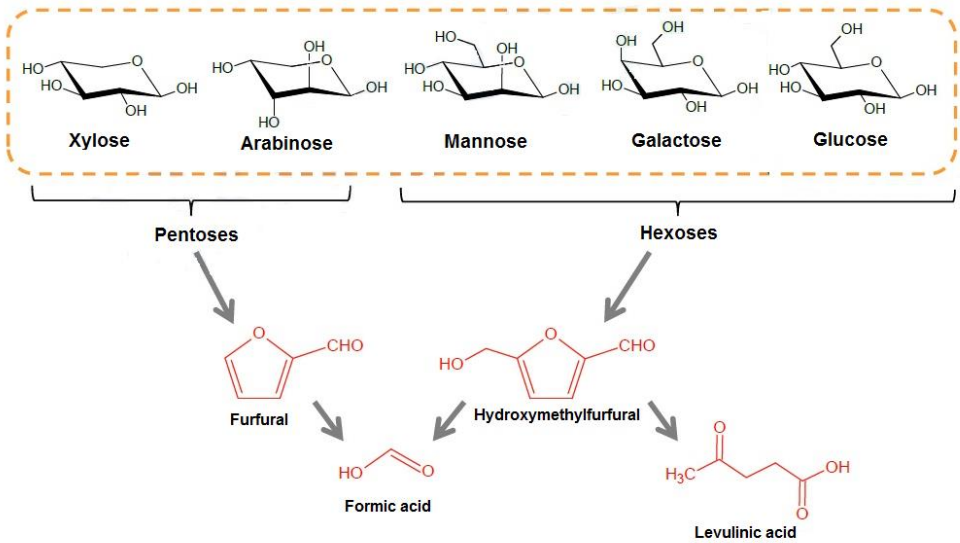

Figure 2. Monosaccharides from lignocellulosic polysaccharides and their further degradation (According to Mussatto 2016).

2.3 Acetone-butanol-ethanol fermentation of hemicelluloses

Solvent-producing *Clostridium* species can produce n-butanol by acetone–butanol–ethanol (ABE) fermentation (Jones and Woods 1986; Jurgens et al. 2012). The most known and well-characterized butanol producing *Clostridia* are *C. acetobutylicum*, *C. beijerinckii*, *C. saccharoperbutylacetonicum*, *C. saccharobutylicum*, and *C. aurantibutyricum*; of these species, *C. acetobutylicum* is probably most researched (Keis et al. 2001; Dürre 2007; Patakova et al. 2011; Procentese et al. 2015). In ABE fermentation, two distinct phases can be discerned: acidogenesis and solventogenesis. During acidogenesis, microbial cells grow exponentially, and the main products are butyric and acetic acids, as well as hydrogen and carbon dioxide (Procentese et al. 2014). In solventogenesis, the growth phase ends, bacteria sporulation is initiated, and the bacteria's metabolism switches to the stage where acids in the medium and the carbon source are metabolized to mainly butanol, acetone, ethanol (with a molar ratio of 6:3:1), and carbon hydroxide (Jones and Woods 1986). Finally, fermentation ends when the solvent concentration exceeds the limit where cell membranes are solubilized and cell death occurs. Although the butanol metabolic pathway has now been transferred into better understood, faster growing, more butanol tolerant and aerobic microorganisms like *Escherichia coli*, *Lactobacillus*, or *Saccharomyces cerevisiae*, and butanol production by these gene-modified organisms has been demonstrated, *Clostridia* still have potential use for efficient butanol production (Peng et al. 2012). However, although considerable knowledge and necessary information about ABE-producing *Clostridium* metabolism has been accumulated, results may not be automatically adapted to all *Clostridium* species or strains (Patakova et al. 2013).

Biobutanol production via microbial fermentation has serious production economy-limiting factors, such as the high price of feedstock, substrate inhibition, butanol's toxicity

to fermentative microbes, the low productivity of fermentation, and the high cost of butanol recovery (Jones and Woods 1986; Jurgens et al. 2012). In fact, the feedstock price is estimated to cover 60% of process costs when conventional starch or sugar-based substrates are used (Jones and Woods 1986; Green 2011; Procentese et al. 2015). Therefore, lignocellulosic feedstocks, especially process residues, offer attractive raw material for biobutanol production. Generally, *Clostridium acetobutylicum* strains are well-known in biochemical butanol production as strictly anaerobic bacteria capable of fermenting various hexoses and pentoses. However, glucose is their preferred fermentable sugar, most likely via a catabolite repression mechanism (Grimmler et al. 2010). Carbon catabolite repression denotes situations where the utilization of xylose, for example, is inhibited when the preferred carbon source exists in the growth medium (Ounine et al. 1985). Although *Clostridia* capable of ABE fermentation can utilize various mono-, di-, oligo-, and polysaccharides like glucose, fructose, xylose, arabinose, lactose, saccharose, starch, pectin and inulin, each specific strain is usually unable to utilize them all efficiently. There are major differences in the use of hexoses and pentoses in the solventogenic *Clostridium* metabolic pathway leading to solvent production in Embden-Mayerhof-Parnas (EMP) glycolysis. Unlike hexoses, pentoses are first converted to fructose-6-phosphate and glyceraldehyde-3-phosphate before they enter the EMP metabolic pathway (Cynkin and Delwiche 1958; Cynkin and Gibbs 1958).

As previously mentioned, dilute acid pretreatments are known for effectively removing and solubilizing hemicellulose but one of the main drawbacks is the further degradation of monosaccharidic sugars (Mosier et al. 2005; Alvira et al. 2010; Brodeur et al. 2011). Besides lowering the fermentable sugar yields, the formation of sugar degradation products (furfural, HMF, formic acid and levulinic acid) and aromatic compounds from lignin act as microbial inhibitors when liquid hemicellulosic fractions are used in biotechnology processes (Ezeji et al. 2007). The inhibitory compounds affect microbial cell growth and glycolytic and fermentative enzymes in the central metabolic pathways (Ezeji et al. 2007; Jönsson et al. 2013). They also affect the microorganism's energy metabolism. Contrary to ethanol-producing microorganisms, furfural, HMF and levulinic acid with concentrations below 1.0 g/L did not inhibit *Clostridium beijerinckii* strains (Ezeji et al. 2007, Lu et al. 2013). With furfural and HMF concentrations of 1-2 g/L in the medium, ABE fermentation with *C. acetobutylicum* ATCC 824 was enhanced and even better yields of solvents were achieved after an extended lag phase of bacteria growth (Zhang et al. 2012). It was shown that furfural and HMF were transformed to less inhibitory compounds by bacteria. Formic acid, dissolved lignin, and degradation products from lignin and hemicellulose (e.g., ferulic acid, syringaldehyde) in turn have inhibition effect on ABE fermentation (Ezeji et al. 2007; Wang and Chen 2011). A high concentration of inhibitory products affects whole process' cost efficiency by both lowering the yields of fermentable monosaccharides and fermentation efficiency. To remove these microbe inhibiting compounds, a costly detoxification step is needed to improve the fermentation process. Therefore, it is desirable to find optimal pretreatment conditions for each individual feedstock type to minimize the formation of these inhibitors.

2.4 Hemicelluloses from industrial residues

A main goal in the modern biorefinery concept is the flexible utilization of all lignocellulosic fractions to obtain a variety of value-added products. In a biobased economy, biomass should

be used cost-efficiently and innovatively to attain both biobased products and bioenergy (de Jong and Jungmeier 2015). This should be done in well-organized integrated systems. In both product-driven and energy-driven systems, process residues are traditionally used for minor value products, like animal feed or energy production (Cherubini et al. 2009). As an example of product-driven lignocellulosic biorefinery, conventional Kraft pulping dissolves hemicelluloses (oligomers and monomers) to a black liquor together with lignin and pulping chemicals for energy purposes (steam and electricity). Hemicelluloses, however, have a lower heating value (13.6 MJ/kg) compared to lignin (27 MJ/kg), and in this sense, combustion does not seem to be an economical way to utilize this resource (Peng et al. 2012; Farhat et al. 2017). Instead of combustion, extracted hemicelluloses can be used in manufacturing value-added products like films, fuels, or food additives (Borrega and Sixta 2015; Xu et al. 2016; Farhat et al. 2017). For example, spruce-derived oligomeric hemicelluloses have a function in many products, varying from the food industry to cosmetics, fine chemicals, and composites (Willför et al. 2008; Mikkonen et al. 2009). This would be the target in future well-organized biorefinery systems: economics and sustainability should be optimized, and materials such as hemicelluloses upgraded to added-value biobased products.

When lignocellulosic biomass is used for biorefining, there are many processes in which the selective fractionation of hemicelluloses would give more value for end-products from cellulosic and lignin fractions (Table 1). In the pelletizing process of wood material, for example, the process aims to densify and increase the pellets' calorific value with preprocessing (e.g., steam pretreatment) (Shahrukh et al. 2015). Pretreatment reactions can depolymerize and redistribute part of the lignin and cause more resistant binding between particles in the pelletizing process (Zandersons et al. 2004). Also, bio-oil production with a fast pyrolysis process has limitations with corrosion problems caused by the high acid content of bio-oil, unstableness due to the oxygen-rich compounds, and challenges with phase separation and bio-oil viscosity. There have been research studies on possible ways to modify the structure of lignocellulosic biomass prior pyrolysis, and thermochemical pretreatment with the ability to remove hemicellulose and some part of lignin is presented as one option (Hao et al. 2017). In the research of Stephanidis et al. (2011), water prehydrolysis at 190°C reduced carboxylic acids, phenols, and ketones in the bio-oil produced (Table 1). The liquid fraction after water prehydrolsis contained pentoses and hexoses, acetic acid, furfural, HMF, and some phenolic compounds which could be used for further upgrading with catalytic or biochemical processes.

Table 1. Some examples of research studies on biorefining processes aiming to add more value to biomass by hemicellulose removal with acidic conditions.

Feedstock and biorefining process	Aim for process development	Pretreatment	Result of hemicelluloses removal on process	Reference
Short rotation willow pelletizing	to investigate steam explosion's effect on wood pellet quality	steam explosion	improved physical properties: higher density and higher impact resistance	Biswas et al. 2011
Forest residues pelletizing	to evaluate energy and mass balance in pellet production from steam pretreated forest residues	steam pretreatment	improved heating value of the processed fuel increased process energy demand	Shahrukh et al. 2015
Poplar pelletizing	to investigate the effect of steam pretreatment on pellet production	steam pretreatment at different conditions	improvement of pellet durability at the expense of more energy consumption	Tang et al. 2018
Pine wood fast pyrolysis	to investigate pretreatments' effect on bio-oil properties	different alkaline/acid	molecular weights of bio-oils after acid pretreatment higher than from untreated or alkaline pretreated	Hassan et al. 2009
Beech wood flash pyrolysis	to study the flash pyrolysis of untreated and hydrothermally treated wood	hydrothermal pretreatment,	reduction of ketones, carboxylic acids, and phenols in bio-oil	Stephanidis et al. 2011
Pine wood sawdust fast pyrolysis	to remove hemicelluloses for better quality biooil	water prehydrolysis	increased heavy fraction of bio-oil reduction of acids from bio-oil	Hao et al. 2017
Hardwood dissolving pulp production	to remove hemicelluloses before Kraft based dissolving pulping	water prehydrolysis	both monomeric and oligomeric sugar were found in the prehydrolysate, some furfural	Saeed et al. 2012
Pine wood dissolving pulp production	to integrate water prehydrolysis to the dissolving pulp pilot scale process for hemicelluloses recovery	water prehydrolysis	high level of hemicellulose removal in laboratory scale experiments	Xu et al. 2016

3. AIM OF THE STUDY AND EXPERIMENTAL DESIGN

The overall aim of this study was to efficiently solubilize hemicellulosic sugars from different lignocellulosic materials through acid catalyzed pretreatment and test the usability of liquid prehydrolysate via acetone-butanol-ethanol (ABE) fermentation to butanol (Figure 3). The aim of the pretreatment studies with different materials was to clarify the conditions which liberate hemicellulosic sugars into a fermentable monomeric form without being seriously degraded and leave cellulosic fractions as intact as possible.

The lignocellulosic material utilized in this work was barley straw (*Hordeum vulgare*) (Article I), which is a harvesting residue from a common Finnish agricultural crop and hardwood willow species (*Salix schwerinii*) (Article II), which is utilized as a bioenergy crop, and softwood Norway spruce (*Picea abies*) (Article III), which is commonly utilized as a pulp and dissolving pulp raw material. The study was divided into two separate but closely related parts. The first part focused on the pretreatment and hemicellulose extraction of different Finnish lignocellulosic materials, and the second part focused on biochemical refining of hemicellulose-containing liquid prehydrolysate.

In the first part materials, were subjected to an acid-catalyzed pretreatment, followed by enzymatic hydrolysis to illustrate how pretreatment affects enzymatic hydrolysability.

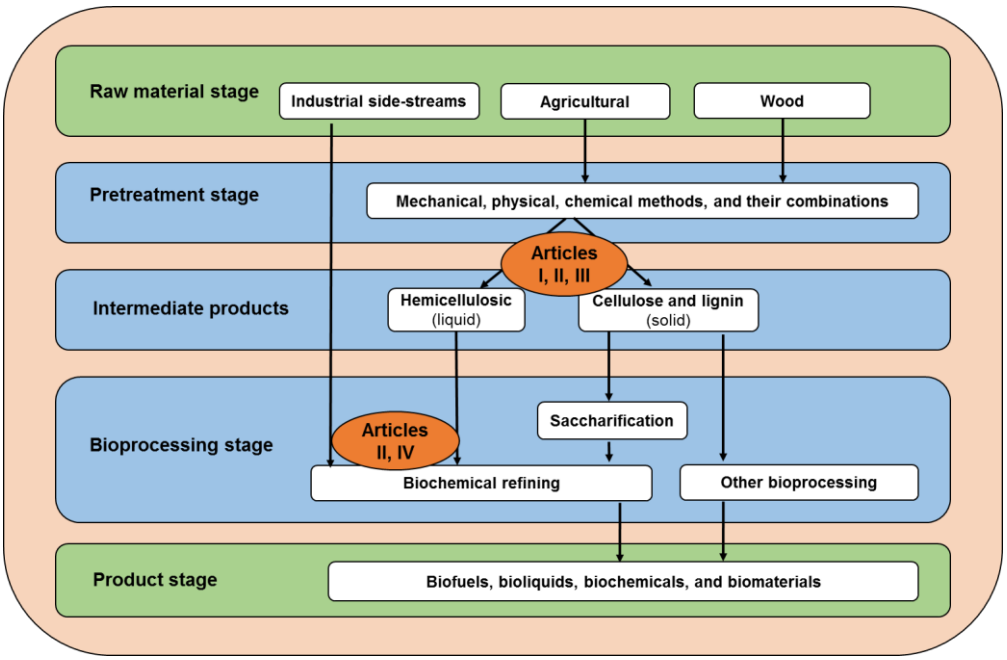

Figure 3. Overall framework for the present study on lignocellulose pretreatment, hemicelluloses extraction, and their biochemical refining. Articles I, II, and III focus on pretreating lignocellulosic materials to liberate monomeric sugars from hemicellulose, and Articles II and IV are related to pretreatment liquid fermentation.

Dilute sulfuric acid catalyzed pretreatment was assisted with different heating methods (cooking, pressurized heating, and microwave heating) with the conditions tested suitable for each material and according to properties of the lignocellulose feedstock used. Several pretreatment conditions (e.g., concentration of acid catalyst, temperature, time) were tested and presented in the means of combined severity factor (CS) to evaluate the pretreatment effect on lignocellulosic biomass, especially on hemicellulosic monosaccharide extraction for fermentation.

The second part focused on biochemical refining of extracted hemicellulosic sugars: the fermentability of hemicellulosic liquid prehydrolysate to butanol via anaerobic ABE fermentation utilizing *Clostridium acetobutylicum* (Articles II and IV). A fermentation medium made of liquid prehydrolyate was supplemented with starchy slurry (simulating starch waste) to observe its effect on butanol yield.

4. MATERIALS AND METHODS

4.1 Lignocellulosic materials used in this study

The barley (*Hordeum vulgare*) straw utilized in this study was grown in a field in North Karelia, Finland. Before conducting experiments in the laboratory, the whole biomass of barley (without straw and grain separation) was harvested, and the ratios of separate grains and straw fractions were measured. For pretreatment experiments, barley straw was dried at 60°C for 7 days, milled to a 0.25 mm particle size, and stored in paper bags at room temperature. Willow (*Salix schwerinii*) plants were harvested from a short rotation willow experimental plot in North Karelia at the age of 6 years. After harvesting, the material was debarked and chipped. Before the subsequent analysis, the chipped willow was air-dried (30°C), milled to a particle size of 1 mm, and stored in paper bags for further use. The softwood material used in this study was harvested at the age of 19 years from a clonal Norway spruce (*Picea abies*) trial established in Karkkila, Southern Finland. After harvesting, the material was debarked, cut into disc-shaped pieces, and air-dried at room temperature for storing and size reduction. After air-drying, the material was cut into smaller particles, milled to 1-mm size, and stored in paper bags at room temperature for microwave pretreatment.

4.2 Acid catalyzed pretreatment of lignocellulosic materials

The milled barley straw was dispersed in 20 mL dilute H_2SO_4 in 50 mL plastic tubes and heat-treated at 121°C (1.1 bar). Four concentrations of H_2SO_4 were applied in the pretreatment liquid: 0.5, 1.0, 1.5, and 2.0% (w/v) with a dry matter loading of 4% (w/v) for barley straw. To elucidate the effect of time in the dilute acid pretreatment process, four different pretreatment time lengths (5, 30, 60 and 120 min) with 1.0% H_2SO_4 were used. After pretreatment, the slurry was cooled to room temperature and filtered to separate liquid prehydrolysates from the pretreated solid materials. The pH of the liquid prehydrolysate was measured, and the monosaccharide concentration was directly analyzed with gas chromatography–mass spectrometry (GC–MS).

For the pretreatment of willow, air-dried material (10g dry weight) was mixed with the liquids using a ratio of 1:10 (w/v) in a steel cylinder and heated to a temperature of 170 or 200°C under corresponding pressure. Four different liquids for pretreatment were used: water (milli-Q, Millipore Corporation) and 0.05%, 0.1%, and 0.15% (w/v) H_2SO_4. After heating the sample to the desired temperature, the cylinder containing the sample was left to cool to room temperature. The cooled sample was filtered through a paper filter (Whatman® 589/1, Schleicher and Schuell) to separate the liquid prehydrolysate from the pretreated solid material. Then the separated liquid prehydrolysate was stored at -18°C for further analysis of carbohydrates and degradation products.

For microwave pretreatments with air-dried Norway spruce, the material (0.25 g dry weight, particle size 1 mm) was mixed with the pretreatment liquid with a dry/liquid material ratio of 1:28 (w/v) and treated with a microwave accelerated reaction system (MARS) (MARS, CEM Corporation, NC, USA) equipped with Teflon sample tubes (HP-500 Plus, Teflon PFA). Four different kinds of pretreatment liquids were used: water (milli-Q, Millipore Corporation) and three different H_2SO_4 concentrations (0.05, 0.1, and 0.15% w/v). Additionally, two microwave intensities (1200 and 600W), two pretreatment times (5 and 10 min), and two temperatures (170 and 200°C) were used. Each microwave experiment consisted of three replicate sample tubes and one control tube. The pretreatment temperature and pressure during the microwave pretreatment of Norway spruce were detected via a control tube thermometer and a control tube pressure probe. The temperature in the microwave test tubes was first increased to the desired temperature, and the following pretreatment time was either 5 or 10 minutes. Maximum pressures of approximately 8 and 16 bars were achieved at the temperatures of 170 and 200°C, respectively. The cooled samples after microwave pretreatment were then filtered through a paper filter (Whatman® 589/1, Schleicher and Schuell) to separate liquid prehydrolysate from the pretreated solid material, and the separated liquid prehydrolysate was stored at -18°C for further analysis of the carbohydrates and degradation products.

After the pretreatment experiments were completed, the pretreated solid materials were washed with water (100 mL) and stored in the freezer (-18°C) for enzymatic hydrolysis and analyses of residual carbohydrates, acid-insoluble lignin, and acid-soluble lignin.

4.3 Enzymatic hydrolysis

Enzymatic hydrolyses for washed pretreated barley straw solid materials were carried out in 10 mL centrifuge tubes. Dried solid materials (0.60 g) were mixed with 3 mL of a 0.05 M sodium citrate buffer (pH 5.0), 10 FPU/g biomass of Celluclast 1.5 L, and 200 nkat/g biomass of Novozyme 188. Enzyme hydrolysis was performed at 50°C for 48 h with shaking at 200 rpm/min. For the enzymatic hydrolysis of pretreated solid willow, 1% of the dry matter was mixed with a 50 mM sodium citrate buffer (pH 5.0), Celluclast 1.5L (Sigma-Aldrich) (10 FPU/g of dry matter), and β-glucosidase Novozyme 188 (Sigma-Aldrich) (200 nkat/g of dry matter). Enzyme hydrolysis was performed in a shaker (200 rpm/min) at 45°C for 48 h.

In the enzymatic hydrolysability tests for spruce, the washed microwave pretreated solid material (1% of dry matter) was mixed with a 50 mM sodium citrate buffer (pH 5.0), a commercial cellulase mixture Celluclast 1.5L (Sigma-Aldrich) (10 FPU/g of dry matter or 20 FPU/g of dry matter), and β-glucosidase Novozyme 188 (Sigma-Aldrich) (200 nkat/g or 400 nkat/g of dry matter). Like with willow, enzyme hydrolysis was performed in a shaker

(200 rpm/min) at 45°C for 48 h. Enzymatic hydrolysis assisted with BSA or PEG 4000 was performed by adding BSA (0.3 g/g of pretreated solid material) or PEG 4000 (0.3 g/g of pretreated solid material) 24 h prior to the hydrolytic enzymes (10 FPU/g dry matter Celluclast 1.5 L and 200 nkat/g of dry matter of Novozyme 188).

All the samples were prepared in triplicate and together with substrate blanks consisting of only the substrate and sodium citrate buffer without the enzymes. After the enzymatic hydrolysis, the samples were centrifuged for 10 min at 12 000 rpm and the supernatant was collected for reducing sugar analysis performed using the 3,5-dinitrosalicylic acid (DNS) method (Miller 1959). All the solid residual fractions were frozen (-18°C) for further analysis.

4.4 Butanol fermentation from hemicelluloses by *Clostridium acetobutylicum*

Freeze-stored *Clostridium acetobutylicum* DSM 1731 (DSMZ, Braunschweig, Germany) was activated in RCM media (Hirsch and Grinsted 1954) for 14–16 h. After activation, 1 mL of actively growing cells was inoculated into 50 mL of sterilized pre-fermentation medium (P2 medium; 30 g/L glucose and 1 g/L yeast extract) in a 125 mL screw-capped bottle. Before inoculating the bacteria, filter-sterilized P2 stock buffer solutions were added: KH_2PO_4 (50 g/L), K_2HPO_4 (50 g/L), ammonium acetate (220 g/L). Also, minerals and vitamins were added: $MgSO_4 \cdot 7H_2O$ (20 g/L), $MnSO_4 \cdot H_2O$ (1 g/L), $FeSO_4 \cdot 7H_2O$ (1 g/L), NaCl (1 g/L), para-aminobenzoic acid (0.1 g/L), thiamin (0.1 g/L), biotin (0.001 g/L). The culture grew for 16 h at 37°C before inoculation into the ABE production medium.

In Article II, the ABE production medium was prepared by mixing 30 mL liquid prehydrolysates with 20 mL heat-treated (121°C, 20 min) barley grain slurry containing starch in 125 mL screw-capped bottles. For this ABE medium, the pH was adjusted to 6.5 with 10 M NaOH prior to fermentation. As control fermentations, pure heat-treated starch (from barley) slurry and glucose media were used. Before the inoculation of bacteria, the medium was purged with N_2 for 10 min to maintain anaerobic conditions and sterilized at 121°C for 20 min. Fermentation began at 37 °C when the *C. acetobutylicum* DSM 1731 culture (10%, v/v) was inoculated. The fermentation samples were taken at 0, 24, 48, 72, 96, 120 and 144 h fermentation times. ABE fermentations were conducted in duplicate.

In Article IV, 50 g/L xylose and the hemicellulosic hydrolysates of willow were used as carbon sources of the fermentation media in which 1 g/L yeast extract was added. For the fermentations using P2 solutions as nutrients, each P2 stock solution (buffer, mineral and vitamin) was added into the media prior to the inoculation of *C. acetobutylicum*. For the fermentations without P2 solutions, different volumes of starch slurry were mixed into the media prior to sterilization to provide essential nutrients for bacteria. The pure xylose solution was mixed with starchy slurry with volume ratios of 1:4, 2:3, and 3:2, respectively. The hemicellulosic hydrolysate was mixed with the starchy slurry at a ratio of 3:2. The ABE fermentation was otherwise conducted similar to the ABE fermentation in Article II.

4.5 Analyses of compositional sugars and fermentation products

The carbohydrate composition of the liquid prehydrolysates in Articles I and III were analyzed using GC-MS after filtration through a 0.2-μm sterile syringe filter. The samples for gas chromatography-mass spectrometry (GC–MS) sugar analysis were centrifuged at

5000 g for 10 min, and the supernatant was filtered through a 0.2 μm sterilized syringe filter. The samples were spiked with internal standard glucose-^{13}C (0.2 mg/mL in methanol/water, 1/1) and evaporated to dryness. The samples were then treated with 80 μL of methoxyamine hydrochloride solution (20 mg/mL) in pyridine for 90 min at 37°C. Additionally, 80 μL MSTFA was added and samples were incubated during silylation for another 60 min at the same temperature. The carbohydrates were analyzed by GC–MS (Agilent 6890 N with 5973 MS, Agilent Technologies, Palo Alto, CA, USA) with split injection (20:1) onto a Rxi-5Sil MS column (30 m x 0.25 mm x 0.25 μm, Restek, USA). Injection port and transfer line temperatures were 260°C and 280°C, respectively. The helium flow rate was 1 mL/min. The oven's temperature was held at 70°C for 1 min and then increased at a rate of 5°C/min until 320°C was achieved; after which, the temperature was maintained for 3 min. The MS data were recorded in the mass range of 83–500 m/z. The analyses were identified by comparison with authentic standards. The carbohydrate composition of the raw material and the solid pretreated materials in Article I were determined using a two-step acid hydrolysis protocol (Sluiter et al. 2010). Oven-dried solid pretreated materials (0.03 g) were treated with 72% H_2SO_4 for 1 h at 30°C in 10 mL centrifuge tubes and then diluted to 4% H_2SO_4 with deionized water and autoclaved for 1 h at 121°C. The slurry was neutralized with solid $CaCO_3$ to pH 4-5 and centrifuged for 10 min at 12 000 rpm. The supernatant was collected for the GC–MS sugar analysis described above.

In Articles II and IV, a ^1H nuclear magnetic resonance (NMR) spectroscopy (Bruker AVANCE 500 or 600 DRX NMR spectrometer equipped with a 5 mm QNP SB or cryoprobe, respectively) was used for quantifying carbohydrates and their derivatives from liquid prehydrolysate, the fermentation products of ethanol, acetone, butanol, acetic acid, and butyric acid, and the residual sugars glucose and xylose in the fermentation media. The ^1H NMR spectra were collected with water presaturation (zgcppr) using a 90° pulse angle, 48 dB presaturation power, 20 s relaxation delay, and 16 scans at 300 K. Prior to the NMR measurements, 200 μl of the sample liquid was transferred to a 5 mm NMR tube, followed by the addition of D_2O (275 μL) and 3-(trimethylsilyl)-propionic-d_4 acid (25 μL, 20 mM) in D_2O as an internal standard of known concentration.

For Article I, original materials and dried pretreated solid materials (30 mg) were treated with 72% H_2SO_4 for 1 h at 30°C in 10 mL centrifuge tubes and then diluted to 4% H_2SO_4 with deionized water and autoclaved for 1 h at 121°C. Autoclaved samples were neutralized with solid $CaCO_3$ to pH 4–5 and centrifuged for 10 min at 12 000 rpm. The supernatant was collected for GC–MS sugar analysis.

In Articles II, III, and IV, the ash, extractives, and carbohydrates from solid materials (original materials and pretreated solid materials) were determined according to Hayes (2012). Klason acid-insoluble lignin (AIL) and acid-soluble lignin (ASL) were determined according to TAPPI (1991) and Sluiter et al. (2010). In the total acid hydrolysis of the original materials and solid prehydrolysate, 3 mL of 72% H_2SO_4 was added to a 300 mg sample, followed by incubation for 1 h at 30°C. The mixture was stirred every 5 min for 1 hour. After incubating for 1 h, the mixture was diluted to 4% H_2SO_4 by adding water and autoclaved at 121 °C for 60 min. Standard samples with 10 mL of a known sugar solution and 348 μL 72% H_2SO_4 were prepared and autoclaved to determine sugar loss during autoclaving. The autoclaved samples and standard mixtures were vacuum filtered through filter crucibles of known weight. From the filtrate, sugar composition and acid soluble lignin were analyzed on a DIONEX ICS-3000 ion chromatography system consisting of an electrochemical detector (using pulsed amperometric detection), a gradient pump, a temperature-controlled column and detector enclosure, and an AS50 autosampler with an

injection volume of 10 µL (Hayes 2012).

The efficiency of the pretreated solid materials' enzymatic hydrolysis in Articles I and II was estimated by the reducing sugar yield (RSY) measured by the DNS method using a spectrophotometer with a wavelength of 540 nm (Miller 1959). For glucose analysis after enzymatic hydrolysis in Article III, the supernatants of samples boiled and centrifuged were passed through a 0.2-µm filter and glucose content was measured using high performance liquid chromatography (HPLC) comprising a Micro-Guard De-Ash pre-column (Bio-Rad, USA), a SPO810 chromatography column (Shodex, Germany), and a type RID-10A refractive index detector (Shimadzu, Japan). The samples were passed through 0.45-µm PTFE filters, and 10 µL were injected into a SIL-20A autosampler (Shimadzu, Japan). Elution at the rate of 0.6 mL/min was performed in a column containing degassed deionized water at a temperature of 60 °C. Calibration was performed using the external standards of glucose. In Articles II and IV, glucose concentrations after enzymatic hydrolysability tests were analyzed using NMR with the method described together with other carbohydrate analyses.

4.6 Calculations

Hemicellulosic monosaccharide yields from the liquid prehydrolysates after the H_2SO_4 pretreatments were calculated as their contents (mg/g dry original material) and as percentages (%) of their individual contents in the original material. The factor for combined severity (CS) was used for comparing the various pretreatment conditions with each other (Chum et al. 1990). The combined severity factor relates the temperature, residence time, and pH of the pretreatment solution together in the equation:

$$CS = log\{t \times exp[(T_H - T_R)/14.75]\} - pH$$

where

t is the reaction time (min), T_H is the reaction temperature in °C, and T_R is the reference temperature (100 °C).

5. RESULTS AND DISCUSSION

5.1 Chemical compositions of studied materials

Lignocellulosic materials originating from different types of plants (herbaceous crops or woody plants) have typical properties. From the plant cell wall side, there are differences in the branching of hemicellulose structures, amount and composition of lignin, and with cellulose crystallinity and the degree of polymerization (de Costa Sousa et al. 2009). In the original barley straw and willow materials, the main carbohydrates were xylan and glucan, originating from xylan in hemicellulose and glucan originating mainly from cellulose (Table 2). The amount of xylan was 26.9% and 18.0% for barley straw and willow, respectively. These values are in accordance with other published information (Linde et al. 2006; Sassner et al. 2008; García-Aparicio et al. 2011; Han et al. 2013). In herbaceous plants and

Table 2. Chemical compositions of different lignocellulosic feedstocks used in this research (% of dry biomass).

	Barley straw		Willow		Spruce	
	Article I	Linde et al. 2006 García-Aparicio et al. 2011	**Article II**	Han et al. 2013 Sassner et al. 2008	**Article III**	Söderström et al. 2002 Shuai et al. 2010 Shafiei et al. 2013 Frankó et al. 2015
Xylan	26.9	16-24	18	15.0-18.6	6.5	5.3-8.4
Glucan	38.1	37-40	44	41.4-49.6	44.1	42.4-49.9
Arabinan	2.6	2.6-3.4	0.29	1.2	1.2	0.7-1.7
Galactan	-	0.4-1.1	0.68	0.3-2.3	2.0	1.3-2.6
Mannan	-	-	1.56	1.1-3.2	12.3	9.9-12.3
Rhamnan	-	nd	0.39	nd	nd	nd
AIL[a]	nd	19.5-23.9	21.26	24.2	26.7	26.2-29
ASL[b]	nd	nd	2.48	2.2	0.6	1.1-7.6
Ash	nd	2.6-7.2	0.52	0.9	0.4	0.2
Extractives	nd	nd	3.42	nd	3.8	3.3

nd=not determined

- = not detected

hardwoods, the amount of xylan in the cell wall constitutes 20-30% of the biomass (Girio et al. 2010). Due to the high content of hemicellulosic xylan, they both possess a remarkable potential as feedstocks for biochemical butanol production. Differences between herbaceous and hardwood xylans are that in herbaceous biomass, xylans mainly present as glucurunoarabinoxylans (Scheller and Ulvskov 2010). In hardwoods, however, glucurunoxylans predominate. Barley straw contained 2.6% of arabinan in its hemicellulose, but in willow material, only 0.29% was detected (Table 2). This could present the difference between the hemicellulosic polysaccharides' composition.

Contrary to the barley straw and willow, xylan content in spruce material was remarkably lower, at 6.5% of the dry matter (Table 2). That was expected, as the main hemicellulosic polysaccharide in softwoods is either glucomannan or galactoglucomannan (Girio et al. 2010, Scheller and Ulvskov 2010). This could be seen from the results as both galactose and mannose exist in the spruce material together with glucose. Glucan content in spruce material was 44.1%, originating from cellulose and hemicellulosic galactoglucomannan. Also, a minor percentage of arabinan exists in the spruce material. Glucan contents were close to each other, especially for willow and spruce (Table 2). It is typical for wood materials that cellulose for both hardwood and softwood remains the same (40-45%), but there are differences in the percentages of hemicellulose and lignin (Alén 2011). The total lignin content (Klason lignin and acid soluble lignin) of spruce was higher than for willow (Table 2). This is expected for softwood and hardwood materials, as lignin content in softwood material usually varies between 25-30% and 20-25% in hardwoods, and the higher percentage of lignin contributes to the softwood structure's higher recalcitrance compared to hardwoods. The general opinion about lignin in plant biomass is that as the lignin content

decreases, the bioavailability of material for enzymes increases (Pu et al. 2013). However, lignin composition, chemical structure, and lignin-carbohydrate complex (LCC) linkages in biomass also impact the material's digestibility.

According to the structure compositions of different lignocellulosic materials and preliminary tests, the acid catalyzed pretreatments were designed with different heating methods for every material studied. For barley straw, heating at 121°C, for willow, pressurized heating at temperatures 170 and 200°C, and for Norway spruce, microwave heating at temperatures of 170 and 200°C with equivalent pressures were chosen. With all heating modes, several H_2SO_4 concentrations and pretreatment times were used.

5.2 Yield of hemicellulosic fermentable sugars from the acid catalyzed pretreatment

5.2.1 Yield of monosaccharidic xylose from barley straw during acid catalyzed pretreatment

During pretreatment of lignocellulosic materials, both physical and chemical properties of cell structure are modified. Due to differences in plant cell wall structure, effects of the same pretreatment method on two different types of feedstock materials could vary considerably. When acid catalyzed pretreatment methods are used, the pretreatment effect is easily seen as amounts of solubilized hemicellulosic carbohydrates, their degradation products, and as the enhanced enzymatic hydrolysability of pretreated solid materials. For barley straw, a pretreatment time of 15 min at a temperature of 120°C with 1% (w/v) H_2SO_4 concentration was enough to release most of the hemicellulosic xylan, as the pretreated solid material's xylan content was as low as 0.12 g/g dry matter. At the same time, however, the xylose concentration in liquid prehydrolysate was low (4.61 g/L) (Article I, Table 2.). Elongating the pretreatment time from 15 min to 60 min and 120 min increased xylose liberation to liquid prehydrolysate and decreased the xylan content in pretreated solid materials to 0.09 and 0.04 g/g after 30 and 120 min, respectively. Generally, increased yield of xylose in the liquid prehydrolysate of barley straw was achieved with increasing combined severity (CS) values between 0.7-1.66 (considering H_2SO_4 concentration, pretreatment time, and pH) (Figure 4). At the highest severity, nearly all xylan from the original material was liberated to the liquid prehydrolysate as xylose. However, acid pretreatment efficiently removed hemicellulose from the solid material within 15 min but not the monosaccharidic form.

In the case of CS 1.51, an exception was found in the relationship between xylose yield and CS, as a drastic drop in xylose recovery was detected with CS 1.51, contrary to the higher CS 1.66 (Figure 4, with circle and square). CS 1.51 was calculated from pretreatment conditions with 2.0% H_2SO_4 and 60 min pretreatment time and CS 1.66 from 1.0 H_2SO_4 and 120 min pretreatment time.

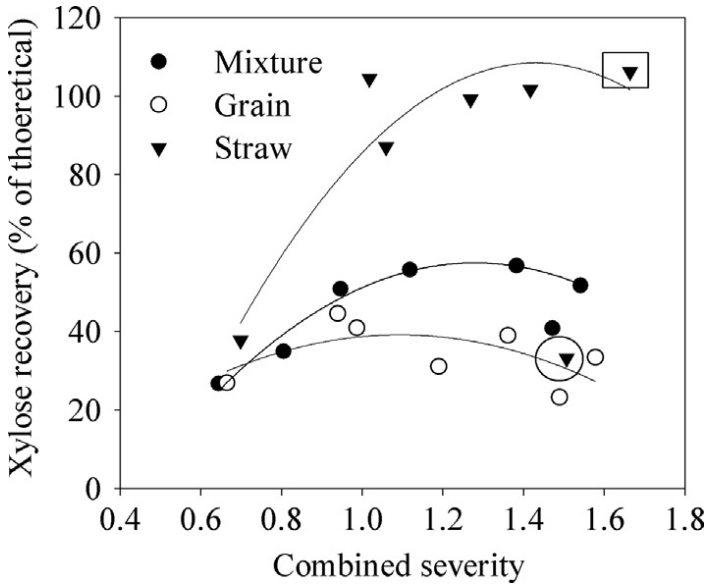

Figure 4. Correlation between xylose recovery in pretreated hydrolysates with CS. (○) CS of 1.51, corresponding to a 2.0% sulfuric acid treatment for 60 min, (□) CS of 1.66, corresponding to a 1.0% sulfuric acid treatment for 120 min (Adapted from Article I).

The observation revealed a stronger effect of the H_2SO_4 concentration compared to pretreatment time on the pretreatment's efficiency on barley straw hemicellulose. Especially when acid catalysts are used for pretreatment, increasing the severity not only accelerates monosaccharide liberation but also increases the degradation of these monosaccharides (Larsson et al. 1999). Here, the CS caused by higher concentration of H_2SO_4 most likely resulted in a larger proportion of xylose degradation products and was observed as a decrease in the concentration of xylose. Kabel et al. (2007) also came to this conclusion with pretreatment of wheat straw.

5.2.2 Willow hemicellulosic sugars yield from acid catalyzed pretreatment

With dilute acid pretreatment, acid catalyzes hemicellulosic polymers' degradation to shorter polymers and eventually to monosaccharides. In hydrothermal pretreatments, the same chemical reactions are present, only to a lesser extent due to the milder conditions induced by the organic acids released (Pu et al. 2013). This phenomenon could be seen with willow material when the highest yield of monosaccahridic xylose (65% of original xylan content) was achieved with a H_2SO_4 concentration of 0.1% and pretreatment temperature of 200°C (Article II, Table 2). Also, pretreatment conditions with 0.15% H_2SO_4 concentration and 170°C temperature liberated 62% of the original xylan as xylose. However, there was no xylose in the liquid prehydrolysate after pretreatment at 170°C with H_2O. However, a xylan content of 74.2% was found from the pretreated solid material, showing the same phenomenon as barley straw (120°C, 1 % sulfuric acid, 15 min). Pretreatment of the willow with H_2O at 170°C degraded the hemicellulosic xylan, not to the monosaccharidic but to the

oligosaccharidic form. During hot water extraction, a remarkable portion of xylan extracted from hardwood material has been reported to remain in a higher molecular form, either as a short polysaccharides or as an oligosaccharides (Borrega et al. 2011). However, by increasing time and temperature in water prehydrolysis, hemicellulose and cellulose could be directed more from oligomers towards monomers and a larger proportion of lignin removal occurs (Gallina et al. 2017).

In general, H_2SO_4 (0-0.15 %) pretreatment of willow (*Salix schwerinii*) at two temperatures (170 and 200°C), liberated increased concentrations of monosaccharidic xylose to liquid prehydrolysate in the CS 0.37-2.29 condition (Article II, Table 2) (Figure 5 A). Pretreatment temperature showed a strong effect, stronger than H_2SO_4 concentration, on hemicellulosic sugar degradation as concentrations of furfural and HMF were higher with the pretreatment at the 200°C temperature (Article II, Table 2). Even CS values of the pretreatment at the two different temperatures were at the same level, 1.77 and 1.87. There were great difference in furfural concentrations: 0.26 and 1.15 g/l, respectively (Figure 5 C, D). In the CS 2.29 condition, the furfural concentration was 1.75 g/l, at least 6 times higher than after pretreatment at CS 1.77. Xylose concentrations in the liquid prehydrolysates in these two different pretreatment conditions were close to each other; however, in the solid prehydrolysates, xylan contents were 7.42 and 33.48 mg/g for CS 2.29 and CS 1.77, respectively (Article II, Table 2). Additionally, after pretreatment with 200°C and without H_2SO_4 (CS 1.23), the furfural concentration was higher than with a 170°C pretreatment temperature but with H_2SO_4 concentrations of 0.1 and 0.15 % (CS 1.59 and 1.77, respectively). The ionization constant (K_W) is strongly correlated with temperature and refers to the concentration of hydronium ions in the water (Yan et al. 2016). The maximal ionization of water is achieved at temperatures of 250-300°C. The increased concentration of hydronium ions at the 200°C pretreatment temperature would in this sense be responsible for more severe pretreatment effects on willow material. Also, in water prehydrolysis, temperature has been shown to be the most critical parameter for hemicellulose extraction and the prehydrolysis temperature should be chosen for the purpose of extracting hemicelluloses (Li et al. 2010; Leppänen et al. 2011; Li et al. 2017).

Acetic acid, a common result of cleaving hemicellulose polymers during pretreatment, increased constantly along with increased CS, thereby illustrating the cleavage of hemicellulosic polysaccharides' chemical bonds during pretreatment (Figure 5 E). Amounts of another organic acid, formic acid, remained rather low in all experiments (Figure 5 F). At elevated temperatures under acidic conditions, formic acid is formed from furfurals (Larsson et al. 1999). Formic acid concentrations in willow liquid prehydrolysate was 0.09 g/L at the lowest and 0.29 g/L at the highest. The concentration of formic acid is considerable, as it has shown a detrimental effect on fermentation processes with *C. acetobutylicum* at a concentration of 0.4 g/l in previous studies (Cho et al. 2012). The highest concentrations of overall sugar degradation products were detected at CS 2.51, with concentrations of furfural and HMF 2.87 mg/mL and 0.42 mg/mL, respectively. At that point, the decrease in liquid prehydrolysate xylose concentration was also observed, together with xylan in solid residual fractions being the lowest, 2.43 mg/g (Article II, Table 2.).

5.2.3 Effect of microwave assisted acid catalyzed pretreatment on Norway spruce hemicelluloses extraction

Softwood material has been classified the most recalcitrant lignocellulosic biomass material with a need for more severe pretreatment conditions (Shuai et al. 2010). For fermentable

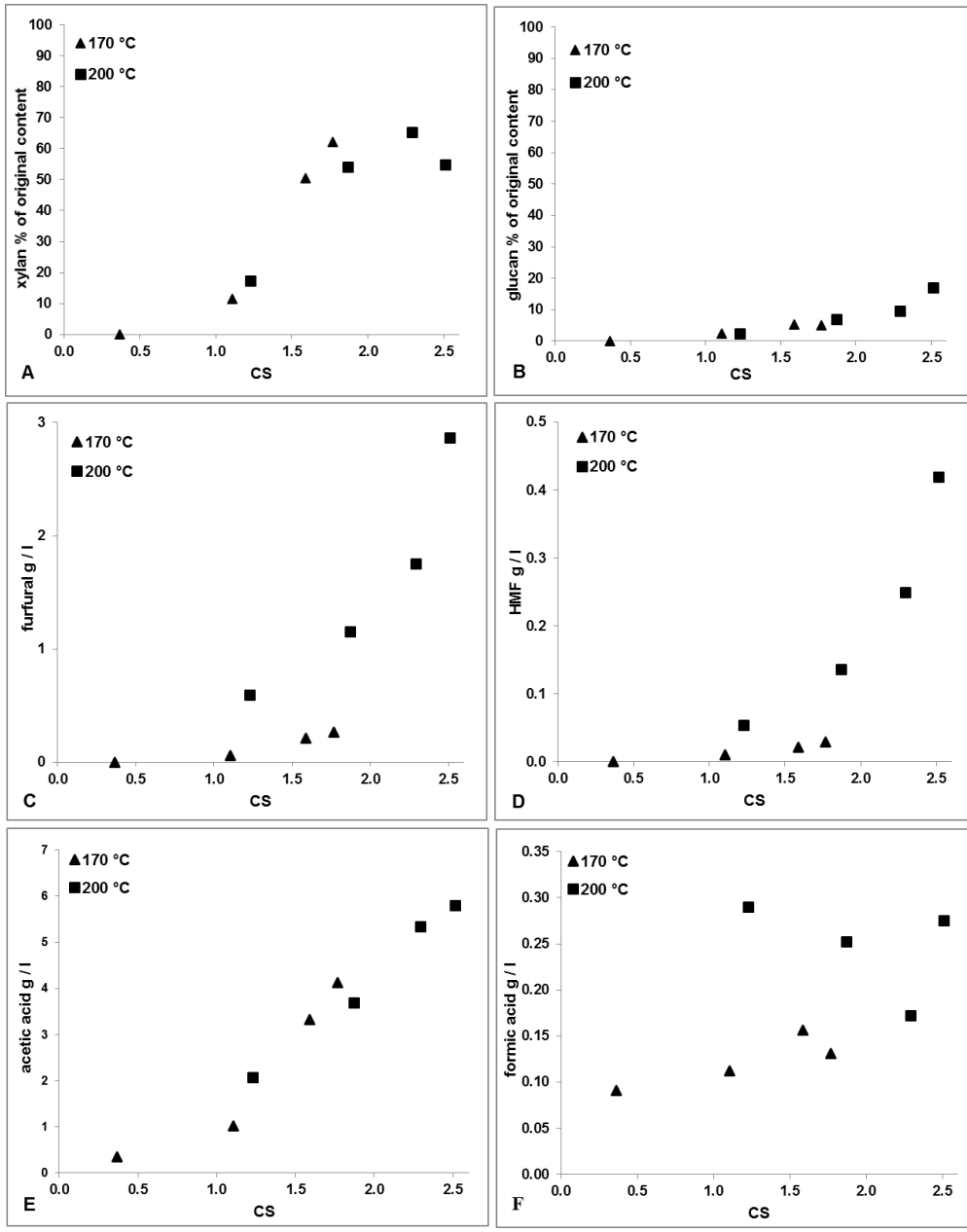

Figure 5. Concentrations of **A)** xylan (% of original xylan content), **B)** glucan (% of original xylan content), **C)** furfural (mg/l), **D)** HMF (mg/l), **E)** acetic acid (mg/l) and **F)** formic acid (mg/l) in liquid prehydrolysate with different pretreatment combined severities (CS). (According to Article II).

carbohydrates production with acid catalysts, this usually means utilizing higher temperatures and longer pretreatment times to degrade polymeric hemicelluloses and alter cellulosic and lignin fractions (Yang et al. 2011; Yan et al. 2016). With increased severity, the result is also the decreased yield of fermentable sugars due to the increased degradation of hemicellulosic monosaccharides. The utilization of microwaves has been introduced with the advantage of internal heating which increases the local temperature of organic molecules and results in rapid and energy-efficient heating of biomasses (Azuma et al. 1984; Ooshima et al. 1984; Chen et al. 2011; Mihiretu et al. 2017). Palm and Zacchi (2003), for example, used a pressurized microwave treatment of Norway spruce with water (200°C, 5 min) to extract hemicellulosic polysaccharides. They reported that 70% of mannan was released during microwave pretreatment with water.

With Norway spruce pretreated with microwave pretreatment (1200 or 600W), nearly all mannose, galactose, and xylose from hemicellulose were released to the liquid prehydrolysate with 0.05% H_2SO_4 at 200°C and a 5 min pretreatment time, a corresponding CS 1.46 and 1.51 for 1200W and 600W, respectively (Article III, Table 1) (Figure 6 A). Conversely, microwave assisted pretreatment with water at 600W, 200°C and 5 min conditions resulted in 30%, 46% and 44% release of mannose, galactose, and xylose, respectively. Interestingly, with CS 1.54 and 1.60 but a microwave pretreatment with 0.15% H_2SO_4 and microwave intensities 1200W and 600W at 170°C, the mannose yield in liquid prehydrolysate was only 63% and 69% of the original mannose material for 1200W and 600W, respectively (Article III, Table 1). This demonstrated the effect of higher H_2SO_4 concentration in microwave pretreatment; even the CS values were close to each other. In all this study's microwave pretreatment conditions, there were only minor mannan contents or none left in solid pretreated materials, representing hemicellulosic polysaccharide liberation to shorter polysaccharides or oligosaccharides during pretreatment (Article III, Table 2). So, the decrease in mannose yield from liquid prehydrolysates with 0.15% H_2SO_4 was due to the further degradation of hemicellulosic sugars. With 0.15% H_2SO_4 and CS 1.54, the amount of HMF produced to the liquid prehydrolysate was 0.29 mg/g. For comparison, with 0.05% H_2SO_4 and CS 1.46 and 1.51, the amounts of HMF produced to the liquid prehydrolysate were 0.09 and 0.15 mg/g, respectively. In general, amounts of HMF and levulinic acid in the liquid prehydrolysates increased along with pretreatment severity, and the HMF concentration was 0.45 mg/g at the highest (Figure 6 B, C). For example, compared to steam pretreated (200°C, 5 min impregnation with SO_2) softwood (particle size 1-2 mm) liquid prehydrolysate containing HMF 6.7 mg/g original dry wood (Monavari et al. 2009), the amounts of HMF and levulinic acid produced during microwave pretreatment of Norway spruce were quite low (Figure 6 B and C).

5.3 Acid catalyzed pretreatment effects on the cellulosic fraction of studied materials

5.3.1 Monosaccharidic glucose extraction from cellulose during acid catalyzed pretreatment

In contrast to hemicellulosic xylan or mannan, glucan extraction to the liquid prehydrolysate was minor with all the lignocellulosic materials studied (Articles I, II and III). As previously mentioned, cellulose has crystalline regions and a higher polymerization stage, causing greater thermal stability compared to hemicellulose (Borrega et al. 2011).

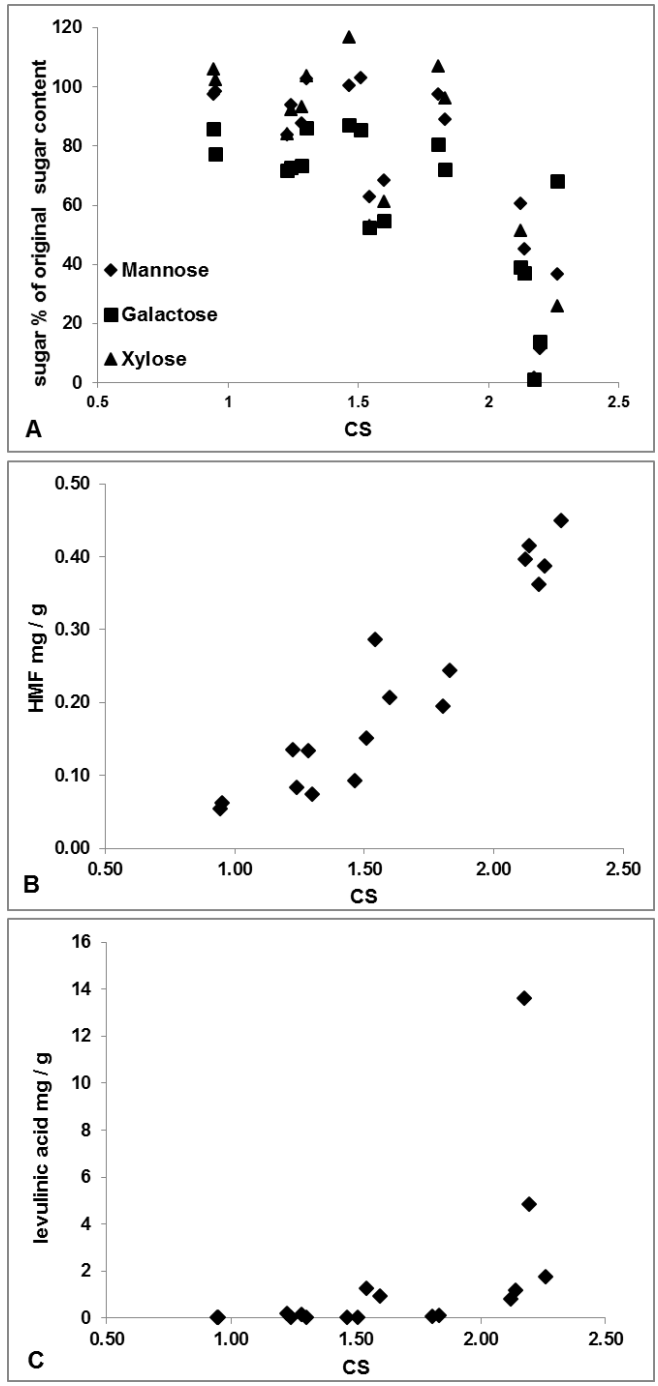

Figure 6. Release of **A**) monosaccharidic mannose, galactose, and xylose (% of original monosaccharide content), **B**) HMF (mg/g original dry material), and **C**) levulinic acid (mg/g original dry material) as a function of combined severity (CS) during microwave pretreatment (Adapted from Article III).

Therefore, higher temperatures and more severe pretreatment conditions would be necessary for cellulose degradation; however, in our case this was not the intention. With barley straw, the concentration of monosaccharidic glucose in the liquid prehydrolysate was increased slightly with increasing severity (Article I, Figure 4). At the highest, H_2SO_4 catalyzed pretreatment released approximately 10% glucose of the original glucan content when the highest concentration of xylose was extracted to the liquid prehydrolysate.

With the H_2SO_4 pretreated willow, when the highest yields of xylose in liquid prehydrolysate were measured, the liberation of glucan to liquid prehydrolysate was 5.13% and 9.45% of the original glucan content, for 170°C and 200°C, respectively (Article II, Table 2). The highest glucose concentration, achieved after pretreatment at 200°C with H_2SO_4 (CS 2.51), was 75.83 mg/g original material (17.12% of original glucan content) (Figure 5 B). In the hot water extraction research of Borrega et al. (2011) on silver birch, glucan remained mostly stable up to a temperature of 180°C. With temperatures above 180°C, glucan started to degrade to liquid prehydrolysate due to the increased pretreatment severity. With hardwood and softwood materials pretreated with water prehydrolysis, the degradation of cellulosic glucan is reported to begin at a temperature of 230°C (Ando et al. 2000). With the willow, pretreatment with water at 170°C did not result in monosaccharidic glucose extraction to the liquid prehydrolysate at all, contrary to the 200°C temperature which resulted in 10.98 mg/g (2.50% of original glucan content).

When the most effective extraction of hemicellulosic sugars from Norway spruce with microwave pretreatment was achieved, it resulted in the release of 10–15% of the original material's glucose to the pretreatment liquid (Article III, Table 2). The hemicellulose fraction of softwood material contains acelylated galactoglucomannan and arabinoglucurunoxylan (Sjöström 1981; Alén 2011) The major part of galactoglucomannan is galactose-poor, accounting for about 10–15% of dry wood in a galactose:glucose:mannose molar ratio of 0.1:1:3. This would mean that the hemicellulose fraction of glucose would represent at least 10% of the wood's total glucose content. Therefore, it can be estimated that almost all monosaccharide glucose from hemicellulosic galactoglucomannans of Norway spruce were released into the liquid prehydrolysate at the same pretreatment conditions with the highest mannose extraction (0.05% H_2SO_4 with 200°C temperature and 0.1% H_2SO_4 with 170°C temperature) (Article III, Table 2). A pretreatment study of Norway spruce using steam explosion with SO_2 impregnation showed that about 13% of glucose was released into the liquid prehydrolysate (Monavari et al. 2009). Also, in various steam pretreated softwood materials, 12–25% of theoretical glucan content was released into the pretreatment liquid (Kumar et al. 2010). With pressurized hot water extraction with Norway spruce, the degradation of cellulose was recognized at 240°C temperature but not lower (Leppänen et al. 2011). At the highest the glucose yield was 30% of the original glucose content after microwave assisted 0.1% H_2SO_4 acid pretreatment at 200°C temperature. This indicates the release of a significant amount of cellulosic glucose into the pretreatment liquid from Norway spruce material.

5.3.2 Enzymatic hydrolysability of pretreated solid materials

In lignocellulosic biomass, the accessibility of cellulose for enzymes is highly prohibited by a shield or network composed of hemicellulose and lignin. Cellulose itself as a structure is compact, and without pretreatment, it is challenging target for effective enzyme hydrolysis. So, if the biorefining process is aiming for the total saccharification of hemicellulosic and cellulosic fractions to the fermentable sugars, it is important that the chosen pretreatment

sufficiently alters cellulosic fraction for enzymatic hydrolysis. With an efficient pretreatment method for cellulose, however, there is a risk that solubilized hemicellulosic monosaccharides will degrade further and are not in a fermentable form. It is said that to biochemically refine lignocellulose, process parameters for pretreatment should be carefully chosen to obtain cellulose susceptible to enzyme hydrolysis as well as liquid prehydrolysate which could be upgraded to biochemicals (Nitsos et al. 2013). Here, the meaning of enzymatic hydrolyses was only to test the hydrolysability of pretreated solid materials rather than to get maximum enzymatic hydrolysis yields of cellulosic glucose.

In pretreated barley straw, enzymatic hydrolysis yield (70% of the original glucose) was highest with CS 1.49 (Article I, Figure 5). When the results were compared to other results reported in the literature, the yield was low. In the research of Saha and Cotta (2010), for example, 82% of the theoretical glucose yield was gained from dilute acid pretreated barley straw. One reason for low enzymatic hydrolysis yield could be the residual xylan in the pretreated solid barley straw which inhibits enzyme hydrolysis and hinders the fermentable sugars' release from the pretreated material. Contrary to Saha and Cotta (2010), we did not use xylose degrading hemicellulases in our enzyme hydrolysis experiments. Residual xylan has also been shown to have a serious negative effect on the enzymatic hydrolysis efficiency of pretreated hybrid poplar (Bura et al. 2009).

Differences in enzymatic hydrolysis efficiency on the solid materials pretreated at different temperatures was clearly seen with the pretreated willow (Figure 7). After pretreatment at 200°C, the willow material became more accessible to the enzymes used, and this effect of pretreatment temperature was observed in the enzymatic hydrolysis' efficiency regardless of the overall CS value. Most likely, the effect was caused by the residual xylan in the pretreated solid materials and by the reduced degree of cellulose polymerization after pretreatment at the 200°C temperature. However, residual xylan was not the only factor hindering the enzyme hydrolysis yield, because the amounts of residual xylan in pretreated solid materials after experiments with 4 and 5 were close to each other (33.48 mg/g and 29.65 mg/g, respectively). Despite this, enzymatic hydrolysis was more efficient after experiment 5 (CS 1.23, temperature 200°C) (yield 55.9% of the pretreated material glucan) than after experiment 4 (33.3% of pretreated material glucan). Residual lignin in the pretreated wood biomass material has also been shown to retard enzymatic hydrolysis with steam-pretreated poplar wood chips (Panagiotopoulos et al. 2013). Lignin most likely also influenced the enzymatic hydrolysis of dilute acid pretreated willow. At the highest, yield from enzymatic hydrolysis of H_2SO_4 pretreated willow was 70% of pretreated solid material glucan.

The lignin in solid prehydrolysate most likely also hindered the enzymatic hydrolysis of Norway spruce. At the highest, 54% and 39% of pretreated solid material glucose was hydrolyzed after pretreatment with 0.1% H_2SO_4 at 200°C for 5 min and with microwave intensities of 1200W and 600W, respectively (Article III, Table 2). In contrast, enzymatic hydrolysis of the microwave-pretreated Norway spruce with water resulted in 12%, 14%, and 23% of pretreated material glucose at 170°C/600W/5 min, 170°C/600W/10 min and 200°C/600W/5 min, respectively. These enzymatic hydrolysis yields were low, as research conducted by Shuai et al. (2010) on dilute acid and SPORL pretreated spruce yielded 49% and 71% cellulose-to-glucose conversion, respectively. Also, with lodgepole pine and Douglas fir, 60-72% of cellulose was converted to glucose during enzymatic hydrolysis (Kumar et al. 2010).

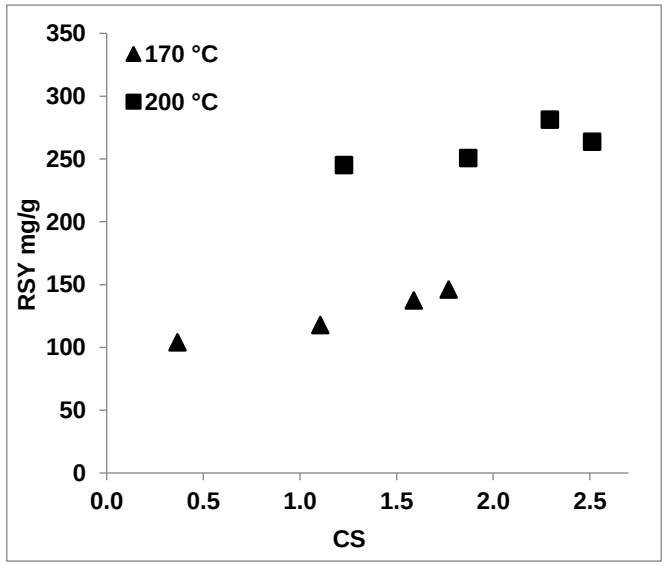

Figure 7. Enzymatic hydrolysis yield of H_2SO_4 pretreated willow materials (mg reducing sugars/g of the original *S. schwerinii* biomass) at different combined severities. (▲) Enzymatic hydrolysis with pretreated material at 170°C; (■) Enzymatic hydrolysis with pretreated material at 200°C. (Adapted from Article II).

Regarding the reasons why lignin restricts enzymatic hydrolysis, it has been suggested that lignin binds and inactivates enzymes, thereby decreasing the accessibility of cellulose (Pan et al. 2005; Rahikainen et al. 2011; Kumar et al. 2012). This could be seen with microwave-pretreated Norway spruce, where increased enzyme loading (20 FPU/g Celluclast 1.5L and 400 nkat/g substrate of Novozyme 188) had an enhancing effect on enzymatic hydrolysis pretreated with 0.05% H_2SO_4 (Figure 8). As result, the release of 30% for original material glucose was achieved at 600W/200°C/5 min and 29% at 1200W/200°C/5 min. Bovine serum albumin (BSA) and polyethylene glycol (PEG), which are used as additives in enzymatic hydrolysis, prevent the unproductive binding of enzymes on the surface of lignin (Yang and Wyman 2008). The addition of BSA (0.3 g/g) to the microwave pretreated Norway spruce increased glucose yields in enzymatic hydrolysis in all pretreatment conditions, and the effect of PEG (0.3 g/g) was even more clearly seen (Figure 8). In the work of Kumar et al. (2012), the rate of enzymatic hydrolysis was also remarkably increased with the addition of BSA, as 16% to 66% of cellulose was hydrolyzed with low cellulase loading (5 FPU/g cellulose).

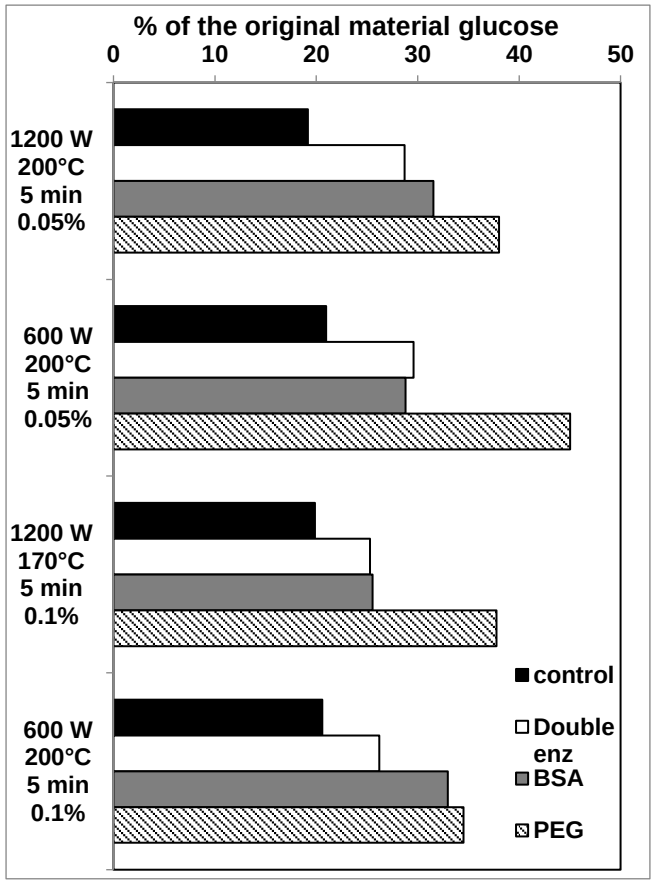

Figure 8. Glucose recovery (% of original material glucose) during enzymatic hydrolysis of microwave pretreated Norway spruce with 10 FPU Celluclast 1.5L and 200 nkat of Novozyme 188 at a temperature of 45°C **(control),** with the enzyme loading increased to 20 FPU Celluclast 1.5L and 400 nkat of Novozyme 188 **(double enz)**, with bovine serum albumin (0.3 g/g of pretreated material) added to the hydrolysis solution prior to enzymes (**BSA**) and with polyethyleneglycol 4000 (0.3 g/g of pretreated material) added to the hydrolysis solution prior to enzymes (**PEG**) (adapted from Article III).

5.4 Fermentability of hemicellulose-rich liquid prehydrolysates of willow

Species of genus *Clostridium* are common and important soil bacteria utilizing lignocellulosic biomass and plant detritus for their metabolism (Aristilde et al. 2015). *Clostridium acetobutylicum* can metabolize hemicellulosic sugars, both hexoses (mannose, galactose, glucose) and pentoses (xylose, arabinose), but when presented, glucose is preferred over others (Ezeji and Blaschek 2008; Grimmler et al. 2010; Xiao et al. 2012). Moreover, Aristilde et al. (2015) found that xylose is not one of the preferred pentose sugars for *C. acetobutylicum* metabolism. In their work, when a combination of pure arabinose and

xylose was used for the fermentation medium, arabinose was metabolized with minimal consumption of xylose. In this work, the same phenomenon was seen with the fermentation of pure xylose with and without the addition of nutrients (P2 solutions), as the productivity of butanol was 0.04 g/g sugars from both fermentation media (Article IV, Table 2). However, with the addition of nutrients-containing P2 solutions, *C. acetobutylicum* produced 1.73 g/L and 2.63 g/L acetic and butyric acid into the solutions, respectively. These were higher concentrations than in fermentation medium without P2 solutions, and most likely indicate the fermentation process had already begun but not shifted to the solventogenic phase. The consumption of xylose was 25% and 29% in fermentation media without and with P2 solutions, respectively.

After pretreatment with 0.1% H_2SO_4 at 200°C, liquid prehydrolysate from willow consisted of 16.3 g/L xylose and 3.6 g/L glucose (Article IV). When this liquid prehydrolysate was used as the fermentation media combined with the P2 solutions, *C. acetobutylicum* could not grow at all (Article IV, Table 4). However, when this liquid prehydrolysate was mixed with 20 mL of starch slurry and fermented for 120 h, 6.7 g/L butanol, 3.4 g/L acetone, and 0.6 g/L ethanol were produced (total 10.6 g/L ABE) (Article IV, Figure 3). This represents butanol and ABE yields of 0.22 and 0.35 g/g sugars, respectively. During fermentation, most of the sugars (77% glucose, 91% xylose, and 99% starch) were consumed (Article IV, Table 4). A 30 mL starch supplement (xylose:starch 2:3) increased the yields relatively. Also, when liquid prehydrolysate after acid catalyzed pretreatment (H_2SO_4 0.15%) at 170°C was combined with starch, 7.9 g/L xylose, 6.6 g/L glucose of wood origin, and 17.6 g/L glucose from starch (barley grain) origin were in the fermentation medium (Article II, Table 3). During the fermentation, 10.1 g/L ABE was produced; of which, 6.3 g/L was butanol (Article II, Figure 3). After fermentation, 98% of the starch, 67% from xylose, and 95% of the glucose were utilized by *C. acetobutylicum* (Article II, Table 3). This also corresponds to the ABE and butanol yields of 0.35 g/g and 0.22 g/g monosaccharidic sugar, respectively.

During fermentation, starch was added to increase the level of fermentable sugars in the media. It was also noticed earlier that grain starch accelerated the xylose utilization of *C. acetobutylicum* in the ABE fermentation of barley straw liquid prehydrolysate after acid-catalyzed pretreatment (Yang et al. 2015). Starch from edible biomass (e.g., corn) as such has traditionally been used for butanol production (Madihah et al. 2001; Ezeji et al. 2007; Li et al. 2014), but the food versus fuel discussion and increased price of substrates inhibit the utilization of edible biomasses in the future. However, utilizing starch-containing waste materials, for example, food waste, organic household waste, brewery wastes, and potato peeling waste have received vast attention for producing butanol via ABE fermentation (Jesse et al. 2002; Kheyrandish et al. 2015; Heinz Stein et al. 2017; Maiti et al. 2017).

Here, it was seen that starch supplement provided nutrients essential for fermentation of liquid prehydrolysates from acid catalyzed pretreatment of willow and P2 solutions were not needed for nutrient increment (Article IV, Table 4). However, Kheyrandish et al. (2015) found that with the ABE fermentation of potato peel waste by *C. acetobutylicum* NRRL B-591 without P2 solutions, there was no shift of bacteria from the acetogenic to the solventogenic phase, although the bacteria's growth rate was higher than with P2 solutions. Growth factors of *C. acetobutylicum*, such as pH, minerals, vitamins, buffers and their impact on carbon metabolism and solvent production are widely researched and reported (Oxford et al. 1940; Monot et al. 1982; Bahl et al. 1986). With this information, there is no clear reason why starchy slurry from barley grain induced solvent production by *C. acetobutylicum* in our work. One possible reason is that grain starch contains enough

vitamins, nutrients, and minerals to shift the bacteria into the solvent producing phase.

One primary reason for starch accelerating the hemicellulosic liquid prehydrolysate is most likely the dilution effect of added starch on inhibitory chemicals. Qureshi et al. (2010), for example, diluted hemicellulosic hydrolysate of wheat straw with water, and ABE production was probably improved by diluting the concentration of inhibitory chemicals in the medium. Han et al. (2013) assumed that potential butanol fermentation inhibitors in the liquid prehydrolysates from willow could be the reason *C. beijerinckii* did not switch from the acetogenic to the solventogenic phase. In their work, furfural, HMF, and formic acid were presented in the willow liquid prehydrolysates with concentrations of 0.09, 0.02, and 0.4 g/L, respectively, and a butanol yield of 0.12 g/g sugar with 72% xylose consumption was achieved. The liquid prehydrolysate of willow in this research study (Article IV) contained 1.45 g/l furfural, 0.36 g/L HMF, 0.3 g/L formic acid, and 0.4 g/L levulinic acid. Formic acid has shown to be an especially critical inhibitor for *C. acetobutylicum* at levels of 0.4 g/L, but there are also opinions that for *C. beijerinckii*, formic acid concentrations of 0.2-0.3 g/L do not inhibit the fermentation process (Cho et al. 2012).

The concentration of sugar degradation products is an important factor in butanol fermentation from the hemicellulosic prehydrolysates, and as here, it could be avoided with optimized acid catalyzed pretreatment. When only water is used for pretreatment at different temperatures, it quite often results in more oligosaccharidic sugars to the liquid prehydrolysate, and prior to the fermentation stage, secondary hydrolysis with H_2SO_4 is necessary (Sun and Liu 2012; Mechmech et al. 2016) (Table 3). Secondary hydrolysis, in addition to increasing process costs, also often leads to increased concentrations of degradation products and therefore costly detoxification processes (Sun and Liu 2012; Kudahettige-Nilsson et al. 2015; Mechmech et al. 2016; Khedkar et al. 2017) or the development and utilization of gene-modified microorganisms tolerant to inhibitory products from sugar degradation (Guo et al. 2013).

Currently, there is also much research work combining hemicellulosic hydrolysates and different sugar or starch- containing waste-derived fractions or industrial side-streams for butanol production (Table 3). It would be an interesting point from the practical biorefining side if the hemicellulosic liquid prehydrolysates and starch-containing wastes as such could be used for butanol production. Substrate price is an essential factor affecting the profitability of ABE fermentation, but the price for nutrients also denotes a remarkable share of the fermentation process' costs. Additionally, both the substrate and nutrients have a large effect on the process' sustainability, which cannot be forgotten even when designing the use of waste-derived resources, and it certainly offers interesting and important opportunities for research.

Table 3. Recent examples of research studies on ABE fermentation of hemicellulosic liquid prehydrolysates with and without sugar or starchy amendments.

Feedstock	Pretreatment and detoxification	Micro-organism	Yield g/g[a]	Reference
With supplement:				
Barley straw prehydrolysate and starch	Dilute acid pretreatment, no detoxification	*Clostridium acetobutylicum* DSM 1731	0.29	Yang et al. 2015
The roots of *Coleus forskohlii* after the extraction of forskolin	Overliming and passing through hydrophobic polymeric resin	*Clostridium acetobutylicum* NCIM 2877	0.24	Harde et al. 2016
Spruce liquid prehydrolysate and market refused vegetables (5% w/v)	SO_2-ethanol-water for spruce, cooking (121°C) for vegetables, anion exchange resin and filtration	*Clostridium acetobutylicum* DSM 792	0.27	Survase et al. 2013
Aspen (60%) and maple (40%) hemicellulosic hydrolysate and alfalfa juice	Dissolving pulp prehydrolysis and secondary hydrolysis with acid, flocculation with ferric sulfate	*Clostridium acetobutylicum* ATTC 824	0.17	Mechmech et al. 2016
Willow prehydrolysate and starch	Dilute acid hydrolysis, no detoxification	*Clostridium acetobutylicum* DSM 1731	0.22	This work, Article IV
Without supplement:				
Corn fiber hydrolysate (+P2)	Dilute acid pretreatment, overliming	*Clostridium beijerinckii* IB4	0.26	Du et al. 2013
Pine apple peel waste + nutrients	Acid and alkaline hydrolysis, activated carbon	*Clostridium acetobutylicum* B527	0.12[b]	Khedkar et al. 2017
Xylan from Kraft pulp black liqueur	Dilute acid hydrolysis, activated carbon	*Clostridium acetobutylicum* ATCC 824	0.1	Kudahettige-Nilsson et al. 2015
Corn fiber hemicellulosic hydrolysate (+P2)	Dilute acid pretreatment, no detoxification	*Clostridium beijerinckii* IB4, inhibitor tolerant strain	0.32	Guo et al. 2013
Maple hemicellulosic hydrolysate + nutrients	Hot water extraction + secondary hydrolysis with sulfuric acid, nanofiltration	*Clostridium acetobutylicum* ACT 824	0.20	Sun and Liu 2012

[a] g BuOH/g monosaccharides

[b] total solvents yield (g ABE/g sugars)

6. CONCLUSIONS

This study aims to clarify utilizable conditions to liberate hemicellulosic sugars for further downstream processing. Three commonly available lignocellulosic materials were used: barley straw (*Hordeum vulgare*), willow (*Salix schwerinii*), and Norway spruce (*Picea abies*). These were pretreated with three different acid catalyzed methods, and the hemicellulosic liquid prehydrolysate of *S. schwerinii* was utilized for butanol production via acetone-butanol-ethanol (ABE) fermentation. The lignocellulosic materials were pretreated with dilute sulfuric acid to achieve fermentable hemicellulosic monosaccharides without sugar degradation compounds inhibitory to the fermentative bacteria. Residual carbohydrates and lignin in solid pretreated materials were analyzed to evaluate the pretreatment outcomes. Enzymatic hydrolysis of the solid pretreated materials was also tested to observe the effect of pretreatment on cellulosic fractions.

We showed that by adjusting pretreatment severity by changing the dilute sulfuric acid concentration, reaction time and temperature, it was possible to efficiently release hemicellulosic sugars from different lignocellulosic materials as monosaccharides into the liquid prehydrolysates with low concentrations of degradation products including furfural, HMF, formic acid, and levulinic acid. The barley straw hemicellulosic xylan was completely extracted into the liquid prehydrolysate with the combined severity (CS) 1.27 (120°C, 1% H_2SO_4 and 60 min) and from willow, about 65% of hemicellulosic xylan was extracted as monosaccharidic xylose to liquid prehydrolysate with CS 2.29 (0.1% H_2SO_4, 200°C, 30 min). Microwave pretreatment was shown to be effective with a recalcitrant softwood material, Norway spruce (*Picea abies*), as hemicellulosic mannan, galactan, and xylan were almost totally extracted to the liquid prehydrolysate in their monosaccharide forms. Results also showed that the dilute acid catalyzed pretreatment with the tested moderate pretreatment temperatures gave incomplete enzymatic saccharification of the pretreated solid materials of willow (*S. schwerinii*) and Norway spruce (*P. abies*) with only a cellulase and cellobiase enzyme mixture. However, as nearly intact cellulosic and lignin fractions were detected, this kind of treatment could offer a basis for further refining pretreated solid materials for biomaterials, for example.

It was also noticeable that with the different acid catalyzed pretreatments, we were able to liberate fermentable sugars from hemicellulose fractions in utilizable amounts. Hemicellulosic liquid prehydrolysate without detoxification and amended with starch was successfully fermented to butanol using *Clostridium acetobutylicum*, with butanol and ABE yields of 0.22 g/g and 0.35 g/g of monosaccharides, respectively. By supplementing with starch, ABE production from the hemicellulosic liquid prehydrolysate of willow was maintained and the utilization of xylose by *C. acetobutylicum* was promoted by the dilution of concentrations of inhibitory compounds in the fermentation medium. Additionally, the starch from barley grain ensured the essential nutrients for ABE fermentations without the need to add other nutrient solutions to the fermentation medium.

For the efficient utilization of hemicellulosic fractions from lignocellulosic materials for butanol production, combining industrial starch-containing side-streams to the hemicellulosic side-streams would offer an attractive option. With the optimization of pretreatment of lignocellulosic biomass, hemicelluloses could be extracted more selectively for conserving fermentable sugars and cellulose could be preserved for material use and therefore obtain the desired products from biorefining. This, of course, must be subjected to further research on technoeconomical observations and analyses of value chains.

REFERENCES

Alén R. (ed.) (2011). Biorefining of forest resources. Papermaking Science and Technology. Book 20. 381 p. Bookwell. ISBN 952-5216-39-4

Alén R. (2000). Structure and chemical composition of wood. In: Stenius P. (ed.) Forest products chemistry. Paper Making Science and Technology. Book 3. p. 12-55. ISBN 952-5216-03-9.

Algayyim S.J.M., Wandel A.P., Yusaf T., Hamawand I. (2018). Production and application of ABE as a biofuel. Renewable and Sustainable Energy Reviews 82: 1195-1214. http://dx.doi.org/10.1016/j.rser.2017.09.082

Alvira P., Tomás-Pejó E., Ballesteros M., Negro M. (2010). Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. Bioresource Technology 101: 4851-4861. https://doi.org/10.1016/j.biortech.2009.11.093

Ando S., Arai I., Kiyoto K., Hanai, S. (1986). Identification of aromatic monomers in steam-exploded poplar and their influences on ethanol fermentation by *Saccharomyces cerevisiae.* Journal of Fermentation Technology 64(6): 567-570. https://doi.org/10.1016/0385-6380(86)90084-1

Anugwom I., Mäki-Arvela P., Virtanen P., Willför S., Sjöholm R., Mikkola, J.P. (2012). Selective extraction of hemicelluloses from spruce using switchable ionic liquids. Carbohydrate Polymers 87(3): 2005-2011. https://doi.org/10.1016/j.carbpol.2011.10.006

Aristilde L., Lewis I.A., Park J.O., Rabinowitz J.D. (2015). Hierarchy in pentose sugar metabolism in *Clostridium acetobutylicum*. Applied and Environmental Microbiology 81(4): 1452-1462. https://doi.org/10.1128/AEM.03199-14

Azuma J., Tanaka F., Koshijima T. (1984). Enhancement of enzymatic susceptibility of lignocellulosic wastes by microwave irradiation. Journal of Fermentation Technology 62(4): 377-384.

Bahl H., Gottwald M., Kuhn A., Rale V., Andersch W., Gottschalk G. (1986). Nutritional factors affecting the ratio of solvents produced by Clostridium acetobutylicum. Applied Environmental Microbiology 52: 169-172.

Belkacemi K., Turcotte G., De Halleux D., Savoie P. (1998). Ethanol Production from AFEX-Treated Forages and Agricultural Residues. Applied Biochemistry and Biotechnology 70-72: 441-462. https://doi.org/10.1007/BF02920159

Bhutto A.W., Qureshi K., Harijan K., Abro R., Abbas T., Bazmi A.A., Karim S., Yu G. (2017). Insight into progress in pre-treatment of lignocellulosic biomass. Energy 122: 724-745. https://doi.org/10.1016/j.energy.2017.01.005

Biswas A.K., Yang W., Blasiak W. (2011). Steam pretreatment of *Salix* to upgrade biomass fuel for wood pellet production. Fuel Processing Technology 92: 1711-1717. https://doi.org/10.1016/j.fuproc.2011.04.017

Blanch H.W., Simmons B.A., Klein-Marcuschamer D. (2011). Biomass deconstruction to sugars. Biotechnology Journal 6(9): 1086-1102. https://doi.org/10.1002/biot.201000180

Borrega M. & Sixta H. (2015). Water prehydrolysis of birch wood chips and meal in batch and flow-through systems: A comparative evaluation. Industrial and Engineering Chemistry Research 54: 6075-6084. https://doi.org/10.1021/acs.iecr.5b00908

Borrega M., Tolonen L.K., Bardot F., Testova L., Sixta H. (2013). Potential of hot water extraction of birch wood to produce high-purity dissolcing pulp after alkaline pulping. Bioresource Technology 135: 665-671. https://doi.org/10.1016/j.biortech.2012.11.107

Borrega M., Nieminen K., Sixta H. (2011). Degradation kinetics of the main carbohydrates in birch wood during hot water extraction in a batch reactor at elevated temperatures. Bioresource Technology 102: 10724-10732. https://doi.org/10.1016/j.biortech.2011.09.027

Bozell J.J. (2010). An evolution from pretreatment to fractionation will enable successful development of the integrated biorefinery. BioResources 5(3): 1326-1327.

Brodeur G., Yau E., Badal K., Collier J., Ramachandran K.B., Ramakrishnan S. (2011). Chemical and physicochemical pretreatment of lignocellulosic biomass: A review. Enzyme Research. ID 787532. https://doi.org/10.4061/2011/787532

Bura R., Chandra R., Saddler J. (2009). Influence of xylan on the enzymatic hydrolysis of steam pretreated corn stover and hybrid poplar. Biotechnology Progress 25: 315-322. https://doi.org/10.1002/btpr.98

Chen H., Liu J., Chang X., Chen D., Xue Y., Liu P., Lin H., Han S. (2017). A review on the pretreatment of lignocellulose for high-value chemicals. Fuel Processing Technology 160: 196-206. https://doi.org/10.1016/j.fuproc.2016.12.007

Chen W.H., Tu Y.J., Herng-Kuang S. (2011). Disruption of sugarcane bagasse lignocellulosic structure by means of dilute sulfuric acid pretreatment with microwave-assisted heating. Applied Energy 88: 2726-2734. https://doi.org/10.1016/j.apenergy.2011.02.027

Cherubini F., Jungmeier G., Wellisch M., Willke T., Skiadas J., Van Ree R., De Jong E. (2009). Toward a common classification approach for biorefinery systems. Biofuels, Bioproducts, & Biorefining 3:534-546. https://doi.org/10.1002/bbb.172

Chiaramonti D., Prussi M., Ferrero S., Oriani L., Ottonello P., Torre P., Cherchi F. (2012). Review of pretreatment processes for lignocellulosic ethanol production, and development of an innovative method. Biomass and Bioenergy 46: 25-35. https://doi.org/10.1016/j.biombioe.2012.04.020

Cho D.H., Shin S.J., Kim Y.H. (2012). Effects of acetic and formic acid on ABE production by *Clostridium acetobutylicum* and *Clostridium beijerinckii*. Biotechnology and Bioprocess Engineering 17: 270-275. https://doi.org/10.1007/s12257-011-0498-4

Chum H.L., Johnson D.K., Black S.K., Overend R.P. (1990). Pretreatment-catalyst effects of the combined severity parameter. Applied Biochemistry and Biotechnology 24(25):1-14. https://doi.org/10.1007/BF02920229

Chundawat P.S., Beckham G.T., Himmel M.E., Dale B.E. (2011). Deconstruction of lignocellulosic biomass to fuels and chemicals. Annual Review of Chemical and Biomolecular Engineering 2: 121-145. https://doi.org/10.1146/annurev-chembioeng-061010-114205

Cynkin M.A. & Delwiche E.A. (1958). Metabolism of pentoses by *Clostridia*. I. Enzymes of ribose dissimilation in extracts of *Clostridia perfringens*. Journal of Bacteriology 75(3): 331-334.

Cynkin M.A. & Gibbs M. (1958). Metabolism of pentoses by clostridia. II. The fermentation of C14-labeled pentoses by *Clostridium perfringens*, *Clostridium beijerinckii*, and *Clostridium butylicum*. Journal of Bacteriology 75(3): 335-338.

de Costa Sousa L., Chundawat S.P.S., Balan V., Dale B.E. (2009). 'Cradle-to-grave' assessment of existing lignocellulose pretreatment technologies. Current Opinion in Biotechnology 20: 339-347. https://doi.org/10.1016/j.copbio.2009.05.003

de Jong E. & Jungmeier G. (2015). Biorefinery concepts in comparison to petrochemical refineries. In: Pandey et al. (eds.) Industrial biorefineries and white biotechnology. Elsevier, Amsterdam, Netherlands. https://doi.org/10.1016/B978-0-444-63453-5.12001-4

Du T., He A., Wu H., Chen J., Kong X., Liu J., Jiang M., Ouyang P. (2013). Butanol production from acid hydrolyzed corn fiber with *Clostridium beijerinckii* mutant. Bioresource Technology 135: 254-261. https://doi.org/10.1016/j.biortech.2012.11.033

Dürre P. (2007). Biobutanol: An attractive biofuel. Biotechnology Journal 2: 1525-1534. https://doi.org/10.1002/biot.200700168

Ezeji T. & Blaschek H.P. (2008). Fermentation of dried distiller's grains and solubles (DDGD) hydrolysates to solvents and value-added products by solventogenic *Clostridia*. Bioresource Technology 99: 5232-5242. https://doi.org/10.1016/j.biortech.2007.09.032

Ezeji T., Qureshi N., Blaschek H.P. (2007). Butanol production from agricultural residues: impact of degradation products on *Clostridium beijerinckii* growth and butanol fermentation. Biotechnology and Bioengineering 97: 1460-1469. https://doi.org/10.1002/bit.21373

Farhat W., Venditti R., Quick A., Taha M., Mignard N., Becquart F., Ayoub A. (2017). Hemicellulose extraction and characterization for applications in paper coatings and

adhesives. Industrial Crops and Products 107: 370-377. https://doi.org/10.1016/j.indcrop.2017.05.055

Fengel D. & Wegener G. (1983). Wood: Chemistry, ultrastructure, reactions. Walter de Gruyter, Berlin, Germany.

Frankó B., Galbe M., Wallberg O. (2015). Influence of bark on fuel ethanol production from steam-pretreated spruce. Biotechnology for Biofuels 8: 15. https://doi.org/10.1186/s13068-015-0199-x

Galbe M. & Zacchi G. (2012). Pretreatment: The key to efficient utilization of lignocellulosic materials. Biomass and Bioenergy 46: 70-78. https://doi.org/10.1016/j.biombioe.2012.03.026

Gallina G., Cabeza Á., Grénman H., Biasi P., Carcía-Serna J., Salmi T. (2017). Hemicellulose extraction by hot pressurized water pretreatment at 160°C for 10 different woods: Yield and molecular weight. The Journal of Supercritical Fluids. In press. https://doi.org/10.1016/j.supflu.2017.10.001

García-Aparicio M.P., Oliva J.M., Manzanares P., Ballesteros M., Ballesteros I., González A., Negro M.J. (2011). Second-generation ethanol production from steam exploded barley straw by *Kluyveromyces marxianus* CECT 10875. Fuel 90: 1624-1630. https://doi.org/10.1016/j.fuel.2010.10.052

Garrote G., Domínguez H., Parajó J.C. (1999). Hydrothermal processing of lignocellulosic materials. Holz als Roh- und Werkstoff 57: 191-202. https://doi.org/10.1007/s001070050039

Girio F.M., Fonseca C., Carvalheiro F., Duarte L.C., Marques S., Bogel-Łukasik R. (2010). Hemicelluloses for fuel ethanol: A review. Bioresource Technology 101: 4775-4800. https://doi.org/10.1016/j.biortech.2010.01.088

Girisuta B., Janssen L.P.B.M., Heeres H.J. (2006). A kinetic study on the conversion of glucose to levulinic acid. Chemical Engineering Research and Design 84(5): 339-349. https://doi.org/10.1205/cherd05038

Goring D.A.I. & Timell T.E. (1962). Molecular weight of native celluloses. Tappi 63(2): 453-460.

Green E.M. (2011). Fermentative production of butanol-the industrial perspective. Current Opinion in Biotechnology 22 (3): 337-343. https://doi.org/10.1016/j.copbio.2011.02.004

Grimmler C., Held C., Libl W., Ehrenreich A. (2010). Transcriptional analysis of catabolite repression in *Clostridium acetobutylicum* growing on mixtures of D-glucose and D-xylose. Journal of Biotechnology 150: 315-323. https://doi.org/10.1016/j.jbiotec.2010.09.938

Guo T., He A., Du T., Zhu D., Liang D., Jiang M., Wei P., Ouyang P. (2013). Butanol production from hemicellulosic hydrolysate of corn fiber by a *Clostridium beijerinckii* mutant with high inhibitor-tolerance. Bioresource Technology 135: 379-385. https://doi.org/10.1016/j.biortech.2012.08.029

Gütch J.S., Nousiainen T., Sixta H. (2012). Comparative evaluation of autohydrolysis and acid-catalyzed hydrolysis of *Eucalyptus globulus* wood. Bioresource Technology 109: 77-85. https://doi.org/10.1016/j.biortech.2012.01.018

Han S.H., Cho D.H., Kim Y.H., Shin S.J. (2013). Biobutanol production from 2-year-old willow biomass by acid hydrolysis and acetone-butanol-ethanol fermentation. Energy 61: 13-17. https://doi.org/10.1016/j.energy.2013.04.069

Harde S.M., Jadhay S.B., Bankar S.B., Ojamo H., Granström T., Singhal R.S., Survase S.A. (2016). Acetone-butanol-ethanol (ABE) fermentation using the root hydrolysate after extraction of forskolin from *Coleus forskohlii*. Renewable Energy 86: 594-601. http://dx.doi.org/10.1016/j.renene.2015.08.042

Hassan E.M., Steele P.H., Ingram L. (2009). Characterization of fast pyrolysis bio-oils produced from pretreated pine wood. Applied Biochemistry and Biotechnology 154: 182-192. https://doi.org/10.1007/s12010-008-8445-3

Hao N., Bezerra T.L., Wu O., Ben H., Sun O., Adhikari S., Ragauskas A.J. (2017). Effect of autohydrolysis pretreatment on biomass structure and the resulting bio-oil from a pyrolysis process. Fuel 206: 494-503. https://doi.org/10.1016/j.fuel.2017.06.013

Hayes D.J.M. (2012). Development of near infrared spectroscopy models for the quantitative prediction of the lignocellulose components of wet *Miscanthus* samples. Bioresource Technology 119: 393-405. https://doi.org/10.1016/j.biortech.2012.05.137

Heinz Stein U., Wimmer B., Ortner M., Fuchs W., Bochmann G. (2017). Maximizing the production of butyric acid fromfood waste as a precursor for ABE-fermentation. Science of the Total Environment 598: 993-1000. http://dx.doi.org/10.1016/j.scitotenv.2017.04.139

Hirsch A. & Grinsted E. (1954). Methods for the growth and enumeration of anaerobic spore-formers from cheese, with observations on the effect of nisin. Journal of Dairy Research 21: 101-110. https://doi.org/10.1017/S0022029900007196

Hu G., Heimann J., Rojas O. (2008). Feedstock pretreatment strategies for producing ethanol from wood, bark, and forest residues. BioResources. 3: 270-294.

Isikgor F.H. & Remzi Becer C. (2015). Lignocellulosic biomass: a sustainable platform for the production of bio-based chemicals and polymers. Polymer Chemistry 6: 4497-4559. https://doi.org/10.1039/C5PY00263J

Jesse T.W., Ezeji T.C., Qureshi N., Blaschek H.P. (2002). Production of butanol from starch-based waste packing peanuts and agricultural waste. Journal of Industrial Microbiology and Biotechnology 29: 117-123. https://doi.org/10.1038/sj.jim.7000285

Jones D.T. & Woods D.R. (1986). Acetone-butanol fermentation revisited. Microbiological Reviews 50(4): 484-524.

Jönsson L.J., Alriksson B., Nilvebrant N. (2013). Bioconversion of lignocellulose: Inhibitors and detoxification. Biotechnology for Biofuels 68(16): 1-10. https://doi.org/10.1186/1754-6834-6-16

Jurgens G., Survase S., Berezina O., Sklavounos E., Linnekoski J., Kurkijärvi A., Väkevä M., van Heiningen A., Granström T. (2012). Butanol production from lignocellulosics. Review. Biotechnology Letters 34: 1415-1434. https://doi.org/10.1007/s10529-012-0926-3

Kabel M.A., Bos G., Zeevalking J., Voragen A.G.J., Schols H.A. (2007). Effect of pretreatment severity on xylan solubility and enzymatic breakdown of the remaining cellulose from wheat straw. Bioresource Technology 98: 2034‑2042. https://doi.org/10.1016/j.biortech.2006.08.006

Kamm B. & Kamm M. (2004). Principles of biorefineries. Applied Microbiology and Biotechnology 64: 137-145. https://doi.org/10.1007/s00253-003-1537-7

Keis S., Shaheen R., Jones D.T. (2001). Emended description of *Clostridium acetobutylicum* and *Clostridium beijerinckii*, and description of *Clostridium saccharoperbutylacetonicum* sp. nov. and *Clostridium saccharobutylicum* sp. nov. International Journal of Systematic and Evolutionary Microbiology 51: 2095-2103. https://doi.org/10.1099/00207713-51-6-2095

Khedkar M.A., Nimbalkar P.R., Gaikwad S.G., Chavan P.V., Bankar S.B. (2017). Sustainable biobutanol production from pineapple waste by using *Clostridium acetobutylicum* B 527: Drying kinetics study. Bioresource Technology 225: 359-366. https://doi.org/10.1016/j.biortech.2016.11.058

Kheyrandish M., Asadollahi M.A., Jeihanipour A., Doostmohammadi M., Rismani-Wazdi H., Karimi K. (2015). Direct production of acetone-butanol-ethanol from waste starch by free and immobilized *Clostridium acetobutylicum*. Fuel 142: 129-133. https://doi.org/10.1016/j.fuel.2014.11.017

Kim J.S., Lee Y.Y., Kim T.H. (2016). A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. Bioresource Technology 199: 42-48. https://doi.org/10.1016/j.biortech.2015.08.085

Ko J.K., Um Y., Woo H.M., Kim K.H., Lee S. (2016). Ethanol production from lignocellulosic hydrolysates using engineered Saccharomyces cerevisiae harboring xylose isomerase-based pathway. Bioresource Technology 209: 290-296. https://doi.org/10.1016/j.biortech.2016.02.124

Kudahettige-Nilsson R.L., Helmerius J., Nilsson R.T., Sjöblom M., Hodge D.B., Rova U. (2015). Biobutanol production by *Clostridium acetobutylicum* using xylose recovered from birch Kraft black liquor. Bioresource Technology 176: 71-79. https://doi.org/10.1016/j.biortech.2014.11.012

Kumar L., Arantes V., Chandra R., Saddler J. (2012). The lignin present in steam pretreated softwood binds enzymes and limits cellulose accessibility. Bioresource Technology 103: 201–208. https://doi.org/10.1016/j.biortech.2011.09.091

Kumar L., Chandra R., Chung P.A., Saddler J. (2010). Can the same steam pretreatment conditions be used for most softwoods to achieve good, enzymatic hydrolysis and sugar yields? Bioresource Technology 101: 7827–7833. https://doi.org/10.1016/j.biortech.2010.05.023

Kumar P., Barrett D.M., Delwiche M.J., Stroeve P. (2009). Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Industrial & Engineering Chemistry Research 48: 3713–3729. https://doi.org/10.1021/ie801542g

Lai Y. (2001). Chemical degradation. In: Hon, D. N.-S. and Shiraishi, N. (eds.) Wood and cellulosic chemistry. Marcel Dekker, Inc., New York, New York. ISBN 0-8247-0024-4.

Larsen J., Östergaard Haven M., Thirup L. (2012). Inbicon makes lignocellulosic ethanol a commercial reality. Biomass and Bioenergy 46: 36-45. https://doi.org/10.1016/j.biombioe.2012.03.033

Larsen J., Östergaad Petersen M., Thiruo L., Wen Li H., Krogh Iversen F. (2008). The IBUS process – lignocellulosic bioethanol close to a commercial reality. Chemical Engineering & Technology 31: 765-772. https://doi.org/10.1002/ceat.200800048

Larsson S., Quintana-Sáinz A., Reimann A., Nilvebrant N.-O., Jönsson L.J. (2000). Influence of lignocellulose-derived aromatic compounds on oxygen-limited growth and ethanolic fermentation by *Saccharomyces cerevisiae*. Applied Biochemistry and Biotechnology 84: 617–632. https://doi.org/10.1385/ABAB:84-86:1-9:617

Larsson S., Palmqvist E., Hahn-Hägerdahl B., Tengborg C., Stenberg K., Zacchi G., Nilvebrant N.O. (1999). The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. Enzyme and Microbial Technology 24:151–159. https://doi.org/10.1016/S0141-0229(98)00101-X

Leppänen K., Spetz P., Pranovich A., Hartonen K., Kitune V., Ilvesniemi, H. (2011). Pressurized hot water extraction of Norway spruce hemicelluloses using a flow-through system. Wood Science and Technology 45: 223-236. https://doi.org/10.1007/s00226-010-0320-z

Li Z., Jiang J., Fu Y., Wang Z., Qin M. (2017). Recycling of pre-hydrolysis liquor to improve the concentrations of hemicellulosic saccharides during water pre-hydrolysis of aspen woodchips. Carbohydrate Polymers 174: 385-391. https://doi.org/10.1016/j.carbpol.2017.06.046

Li H., Luo W., Wang Q., Yu X. (2014). Direct fermentation of gelatinized cassava starch to acetone, butanol, and ethanol using *Clostridium acetobutylicum* mutant obtained by

atmospheric and room temperature plasma. Applied Biochemistry and Biotechnology 172(7): 3330-3341. https://doi.org/10.1007/s12010-014-0765-x

Li H., Saeed A., Jahan M.S., Ni Y., van Heiningen A. (2010). Hemicellulose removal from Hardwood chips in the pre-hydrolysis step of the Kraft-based dissolving pulp production process. Journal of Wood Chemistry and Technology 30(1): 48-60. https://doi.org/10.1080/02773810903419227

Linde M., Galb M., Zacchi G. (2006). Steam pretreatment of acid-sprayed and acid soaked barley straw for production of ethanol. Applied Biochemistry and Biotechnology 6: 546–562. https://doi.org/10.1385/ABAB:130:1:546

Liu W., Yuan Z., Mao C., Hou Q., Li K. (2012). Extracting hemicelluloses prior to aspen chemi-thermochemical pulping: Effects of pre-extraction on pulp properties. Carbohydrate Polymers 87: 322-327. https://doi.org/10.1016/j.carbpol.2011.07.050

Lu C., Dong J. Yang S. (2013). Butanol production from wood pulping hydrolysate in an integrated fermentation-gas stripping process. Bioresource Technology 143: 467-475. http://dx.doi.org/10.1016/j.biortec.2013.06.012

Lu X., Xi B., Zhang Y., Angelidaki I. (2011). Microwave pretreatment of rape straw for bioethanol production: focus on energy efficiency. Bioresource Technology 102: 7937–7940. https://doi.org/10.1016/j.biortech.2011.06.065

Madidah M.S., Ariff A.B., Sahaid K.M., Suraini A.A., Karim M.IA. (2001). Direct fermentation of gelatinized sago starch to acetone-butanol-ethanol by Clostridium acetobutylicum. World Journal of Microbiology and Biotechnology 17: 567-576. https://doi.org/10.1023/A:1012351112351

Maiti S., Gallastegui G., Suresh G., Brar S.K., LeBihan Y., Drogui P., Buelna G., Ramirez A.A., Verma M., Soccol C.R. (2017). Two-phase partitioning detoxification to improve biobutanol production from brewery wastes. Chemical Engineering Journal 330: 1100-1108. https://doi.org/10.1016/j.cej.2017.08.035

Mechmech F., Marinova M., Chadjaa H., Rahni M., Ben Akacha N., Gargouri M. (2016). Co-fermentation of alfalfa juice and hardwood hydrolysate for butanol production in combined biorefinery systems. Industrial Crops and Products 89: 29-33. https://doi.org/10.1016/j.indcrop.2016.04.057

Mikkonen K. S., Tenkanen M., Cooke P., Xu C., Rita H., Willför S., Holmbom B., Hicks K. B., Yadav M. P. (2009). Mannans as stabilizers of oil-in-water beverage emulsions. LWT - Food Science and Technology 42(4): 849-855. https://doi.org/10.1016/j.lwt.2008.11.010

Miller G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry 31: 426. https://doi.org/10.1021/ac60147a030

Mihiretu G.T., Brodin M., Chimphango A.F., Öyaas K., Hoff B.H., Görgens J.F. (2017).

Single-step microwave-assisted hot water extraction of hemicelluloses from selected lignocellulosic materials – A biorefinery approach. Bioresource Technology 241: 669-680. https://doi.org/10.1016/j.biortech.2017.05.159

Monavari S., Galbe M., Zacchi G. (2009). Impact of impregnation time and chip size on sugar yield in pretreatment of softwood for ethanol production. Bioresource Technology 100: 6312–6316. https://doi.org/10.1016/j.biortech.2009.06.097

Monot F., Martin J.R., Petitdemange H., Gay R. (1982). Acetone and butanol production by Clostridium acetobutylicum in a synthetic medium. Applied Environmental Microbiology 44: 1318-1324.

Mori T., Tsuboi Y., Ishida N., Nishikubo N., Demura T., Kikuchi J. (2015). Multidimensional highresolution magic angle spinning and solution-state NMR characterization of 13C-labeled plant metabolites and lignocellulose, Scientific Reports 5: 1-12. https://doi.org/10.1038/srep11848

Mosier N., Wyman C., Dale B., Elander R., Lee Y.Y., Holtzapple M., Ladisch M. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource Technology 96: 673-686. https://doi.org/10.1016/j.biortech.2004.06.025

Mussatto S.I. (2016). Biomass pretreatment with acids. In: Mussatto S.I. Biomass fractionation technologies for a lignocellulosic feedstock based biorefinery. Chapter 8. Elsevier Inc., Amsterdam. ISBN 9780128025611. https://doi.org/10.1016/B978-0-12-802323-5.00008-6

Nabarlatz D., Farriol X., Montané D. (2004). Kinetic modeling of the autohydrolysis of lignocellulosic biomas for the production of hemicellulose-derived oligosaccharides. Industrial & Engineering Chemistry Research 43: 4124-4131. https://doi.org/10.1021/ie034238i

Nitsos C.K., Matis K.A., Triantafyllidis K.S. (2013). Optimization of hydrothermal pretreatment of lignocellulosic biomass in the bioethanol production process. ChemSusChem 6: 110-122. https://doi.org/10.1002/cssc.201200546

Ooshima H., Aso K., Harano Y. (1984). Microwave treatment of cellulosic materials for their enzymatic hydrolysis. Biotechnology Letters 6(5): 289-294. https://doi.org/10.1007/BF00129056

Ounine K., Petitdemange H., Raval G., Gay R. (1985). Regulation and butanol inhibition of d-xylose and D-glucose uptake in *Clostridium acetobutylicum*. Applied and Environmental Microbiology 49(4): 874-878.

Oxford A.E., Lampen J.O., Peterson W.H. (1940). Growth factor and other nutritional requirements of the acetone-butanol organism *Cl. acetobutylicum*. Biochemical Journal 34: 1588-1597. https://doi.org/10.1042/bj0341588

Palm M. & Zacchi G. (2003). Extraction of hemicellulosic oligosaccharides from Norway

spruce using microwave oven or steam treatment. Biomacromolecules 4:617–623. https://doi.org/10.1021/bm020112d

Pan X., Gilkes N., Kadla J., Pye K., Saka S., Gregg D. (2006). Bioconversion of hybrid poplar to ethanol and co-products using an organosolv fractionation process: optimization of process yields. Biotechnology and Bioengineering 94(5):851-861. https://doi.org/10.1002/bit.20905

Pan X.J., Xie D., Gilkes N., Gregg D.J., Saddler J.N. (2005). Strategies to enhance the enzymatic hydrolysis of pretreated softwood with high residual lignin content. Applied Biochemistry and Biotechnology 121-124: 1069-1079. https://doi.org/10.1385/ABAB:124:1-3:1069

Panagiotopoulos I.A., Chandra R.P., Saddler J.N. (2013). A two-stage pretreatment approach to maximize sugar yield and enhance reactive lignin recovery from poplar wood chips. Bioresource Technology 130: 570-577. https://doi.org/10.1016/j.biortech.2012.12.093

Patakova P., Linhova M., Rychtera M., Paulova L., Melzoch K. (2013). Novel and neglected issues of acetone-butanol-ethanol (ABE) fermentation by clostridia: *Clostridium* metabolic diversity, tool for process mapping and continuous fermentation systems. Biotechnology Advances 31: 58-67. https://doi.org/10.1016/j.biotechadv.2012.01.010

Patakova P., Maxa D., Rychtera M., Linhova M., Fribert P., Muzikova Z., Lipovsky J., Paulova L., Pospisil M., Sebor G., Melzoch K. (2011). Perspectives of biobutanol production and use. In: Dos Santos Bernardes, M.A. (Ed.). Biofuel's Engineering Process Technology. pp. 243-266. ISBN 978-953-307-480. https://doi.org/10.5772/16464

Pedersen M. & Meyer A.S. (2010). Lignocellulose pretreatment severity – relating pH to biomatrix opening. New Biotechnology 27(6): 739-750. https://doi.org/10.1016/j.nbt.2010.05.003

Peng F., Peng P., Xu F., Sun R. (2012). Fractional purification and bioconversion of hemicelluloses. Biotechnology Advances 30: 879-903. https://doi.org/10.1016/j.biotechadv.2012.01.018

Prasad Maurya D., Singla A., Negi S. (2015). An overview of key pretreatment processes for biological conversion of lignocellulosic biomass to bioethanol. 3 Biotech 5: 597-609. https://doi.org/10.1007/s13205-015-0279-4

Procentese A., Raganati F., Olivieri G., Russo M.E., Salatino P., Marzocchella A. (2015). Continuous lactose fermentation by *Clostridium acetobutylicum* – Assessment of solventogenic kinetics. Bioresource Technology 180: 330-337. https://doi.org/10.1016/j.biortech.2015.01.008
Procentese A., Raganati F., Olivieri G., Russo M.E., Salatino P., Marzocchella A. (2014). Continuous xylose fermentation by *Clostridium acetobutylicum* – Kinetics and energetics issues under acidogenesis conditions. Bioresource Technology 164: 155-161. https://doi.org/10.1016/j.biortech.2014.04.054

Pu Y., Hu F., Huang F., Davison B.H., Ragauskas A.J. (2013). Assessing the molecular structure basis for biomass recalcitrance during dilute acid and hydrothermal pretreatments. Biotechnology for Biofuels 6:15. https://doi.org/10.1186/1754-6834-6-15

Qureshi N., Saha B.C., Dien B., Hector R.E., Cotta M.A. (2010). Production of butanol (a biofuel) from agricultural residues: Part I – Use of barley straw hydrolysate. Biomass and Bioenergy 34: 559-565. https://doi.org/10.1016/j.biombioe.2009.12.024

Ragauskas A.J. (2014). Materials for biofuels. World Scientific Series in Materials and Energy. 4. Stallion Press, Singapore. ISBN 978-981-4513272. https://doi.org/10.1142/8835

Rahikainen J., Mikander S., Marjamaa K., Tamminen T., Lappas A., Viikari L., Kruus K. (2011). Inhibition of enzymatic hydrolysis by residual lignins from softwood - study of enzyme binding and inactivation on lignin-rich surface. Biotechnology and Bioengineering 108(12): 2823–2834. https://doi.org/10.1002/bit.23242

Rissanen J.V., Grénman H., Willför S., Murzin D.Y., Salmi T. (2014). Spruce hemicellulose for chemicals using aqueous extraction: Kinetics, mass transfer, and modeling. Industrial and Engineering Chemistry Research 53: 6341-6350. https://doi.org/10.1021/ie500234t

Rowell R.M, Pettersen R., Tshabalala M.A. (2013). Cell wall chemistry. In: Rowell, R.M (ed.). Handbook of wood chemistry and wood composites. p. 33-72. CRC Press, Boca Raton, Florida. ISBN 978-1-4398-5380-1.

Saeed A., Sarwar Jahan M., Li H., Liu Z., Ni Y., van Heiningen A. (2012). Mass balances of components dissolved in the pre-hydrolysis liquor of kraft-based dissolving pulp production process from Canadian hardwoods. Biomass and Bioenergy 39: 14-19. https://doi.org/10.1016/j.biombioe.2010.08.039

Saha B.C. & Cotta M.A. (2010). Comparison of pretreatment strategies for enzymatic saccharification and fermentation of barley straw to ethanol. New Biotechnology 1: 10-16. https://doi.org/10.1016/j.nbt.2009.10.005

Sánchez C. (2009). Lignocellulosic residues: Biodegradation and bioconversion by fungi. Biotechnology Advances 27: 185–194. https://doi.org/10.1016/j.biotechadv.2008.11.001

Sassner P., Mårtensson C., Galbe M., Zacchi G. (2008). Steam pretreatment of H_2SO_4-impregnated *Salix* for the production of bioethanol. Bioresource Technology 99: 137-145. https://doi.org/10.1016/j.biortech.2006.11.039

Scheller H.V., Ulvskov P. (2010). Hemicelluloses. Ann Rev Plant Biol 61: 263-289. https://doi.org/10.1146/annurev-arplant-042809-112315
Shafiei M., Zilouei H., Zamani A., Taherzadeh M.J., Karimi K. (2013). Enhancement of ethanol production from spruce wood chips by ionic liquid pretreatment. Applied Energy 102: 163-169. https://doi.org/10.1016/j.apenergy.2012.05.060

Shahrukh H., Oyedun A.O., Kumar A., Ghiasi B., Kumar L., Sokhansanj S. (2015). Net energy ratio for the production of steam pretreated biomass-based pellets. Biomass and Bioenergy 80: 286-297. https://doi.org/10.1016/j.biombioe.2015.06.006

Shuai L., Yang Q., Zhu J.Y., Lu F.C., Weimer P.J., Ralph J., Pan X.J. (2010). Comparative study of SPORL and dilute-acid pretreatments of spruce for cellulosic ethanol production. Bioresource Technology 101: 3106-3114. https://doi.org/10.1016/j.biortech.2009.12.044

Sluiter A., Hames B., Ruiz R., Scarlata C., Sluiter J., Templeton D., Crocker D. (2010). Determination of structural carbohydrates and lignin in biomass. Technical Report NREL/TP-510-42618. National Renewal Energy Laboratory, Golden, Colorado.

Sjöström E. (1981). Wood chemistry: Fundamentals and applications. Academic Press, New York, New York.

Söderström J., Pilcher L., Galbe M., Zacchi G. (2002). Teo-step steam pretreatment of softwood with SO_2 impregnation for ethanol production. Applied Biochemistry and Biotechnology 98-100: 5-21. https://doi.org/10.1385/ABAB:98-100:1-9:5

Stephanidis S., Nitsos C., Kalogiannis K., Iliopoulou E.F., Lappas A.A., Triantafyllidis K.S. (2011). Catalytic upgrading of lignocellulosic biomass pyrolysis vapours: Effect of hydrothermal pre-treatment of biomass. Catalysis Today 167: 37-45. https://doi.org/10.1016/j.cattod.2010.12.049

Sun Z. & Liu S. (2012). Production of n-butanol from concentrated sugar maple hemicellulosic hydrolysate by *Clostridia acetobutylicum* ATCC 824. Biomass and Bioenergy 39: 39-47. https://doi.org/10.1016/j.biombioe.2010.07.026

Sun R.C., Sun X.F., Tomkinson J. (2004). Hemicellulose and their derivatives. In: Gatenholm I. and Tenkanen M. (eds.) Hemicelluloses: science and technology. ACS Symposium Series 864. American Chemical Society. Oxford University Press. ISBN 0-8412-1.

Survase S.A., Sklavounos E., van Heiningen A., Granström T. (2013). Market refused vegetables as a supplement for improved acetone-butanol-ethanol production by *Clostridium acetobutylicum* DSM 792. Industrial Crops and Products 45: 349-354. https://doi.org/10.1016/j.indcrop.2012.12.049

Taherzadeh M.J. & Karimi K. (2008). Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review. Int. J. Mol. Sci. 9: 1621-1651. https://doi.org/10.3390/ijms9091621

Tang Y., Chandra R.P., Sokhansanj S., Saddler J.N. (2018). Influence of steam explosion processes on the durability and enzymatic digestibility of wood pellets. Fuel 211: 87-94. https://doi.org/10.1016/j.fuel.2017.09.053

TAPPI. (1991). TAPPI standard UM 250. Acid soluble lignin in wood and pulp. *Tappi J*, Atlanta GA.

Taylor R., Nattrass L., Alberts G., Robson P., Chudziak C., Bauen A., Marsili Libelli I., Lotti G., Prussi M., Nistri R., Chiaramonti D., López Contreras A., Bos H., Eggink G., Springer J., Bakker R., van Ree R. (2015). From the sugar platform to the biofuels and biochemical. Final report for the European Commission Dictorate-General Energy. N° ENER/C2/423-2012/SI2.673791.

Teymouri F., Laureano-Perez L., Alizadeh H., Dale B.E. (2005). Optimization of ammonia fiber explosion (AFEX) treatment parameters for enzymatic hydrolysis of corn stover. Bioresource Technology 96: 2014-2018. https://doi.org/10.1016/j.biortech.2005.01.016

Wang L. & Chen H. (2011). Increased fermentability of enzymatically hydrolyzed steam-exploded corn stover for butanol production by removal of fermentation inhibitors. Process Biochemistry 46: 604-607. https://doi.org/10.1016/j.procbio.2010.09.027

Willför S., Sundberg K., Tenkanen M., Holmbom B. (2008). Spruce derived mannans - A potential raw material for hydrocolloids and novel advanced natural materials. Carbohydrate Polymers 72(2): 197−210. https://doi.org/10.1016/j.carbpol.2007.08.006

Wyman C.E., Dale B.E., Elander R.T., Holtzapple M., Ladisch M.R., Lee Y.Y. (2005). Coordinated development of leading biomass pretreatment technologies. Bioresource Technology 96: 1959–1966. https://doi.org/10.1016/j.biortech.2005.01.010

Wyman C.E. (1996). Handbook on bioethanol: production and utilization. Taylor and Francis, Washington, DC. ISBN 1-56032-553-4.

Xiao H., Li Z., Jiang Y., Yang Y., Jiang W., Gu Y., Yang S. (2012). Metabolic engineering of D-xylose pathway in *Clostridium beijerinckii* to optimize solvent production from D-xylose mother liquid. Metabolic Engineering 14: 569-578. https://doi.org/10.1016/j.ymben.2012.05.003

Xu C., Nunez T., Willför S., Sundberg A. (2016). Feasibility of integrating hot water extraction into a dissolving pulp process to recover hemicelluloses from *Pinus radiate*. Cellulose Chemistry and Technology 50(5-6): 535-544.

Yan L., Ma R., Li L., Fu J. (2016). Hot water pretreatment of lignocellulosic biomass: an effective and environmentally friendly approach to enhance biofuel production. Chemical Engineering & Technology 39: 1759–1770. https://doi.org/10.1002/ceat.201600394

Yang B., Dai Z., Ding S.Y., Wyman C.E. (2011). Enzymatic hydrolysis of cellulosic biomass. Biofuels 2(4), 421–450. https://doi.org/10.4155/bfs.11.116
Yang B. & Wyman C.E. (2008). BSA Treatment to enhance enzymatic hydrolysis of cellulose in lignin containing substrates. Biotechnology and Bioengineering 94(4): 611-617. https://doi.org/10.1002/bit.20750

Yang M., Kuittinen S., Zhang J., Vepsäläinen J., Keinänen M., Pappinen A. (2015). Co-fermentation of hemicellulose and starch from barley straw and grain for efficient pentoses utilization in acetone-butanol-ethanol production. Bioresource Technology 179: 128-135.

https://doi.org/10.1016/j.biortech.2014.12.005

Zandersons J., Gravitis J., Zhurinsh A., Kokorevics A., Kallavus U., Suzuki C.K. (2004). Carbon materials obtained from self-binding sugar cane bagasse and deciduous wood residues plastics. Biomass and Bioenergy 26: 345-360. https://doi.org/10.1016/S0961-9534(03)00126-0

Zech K.M., Meisel K., Brosowski A., Villadsgaard Toft L., Müller-Langer F. (2016). Environmental and economic assessment of the Inbicon lignocellulosic ethanol technology. Applied Energy 171: 347-356. https://doi.org/10.1016/j.apenergy.2016.03.057

Zhang Y., Han B., Ezeji T.C. (2012). Biotransformation of furfural and 5-hydroximethyl furfural (HMF) by Clostridium acetobutylicum ATCC 824 during butanol fermentation. New Biotechnology 29(3): 345-351. https://doi.org/10.1016/j.nbt.2011.09.001

Zhao X., Xiong L., Zhang M., Bai F. (2016). Towards efficient bioethanol production from agricultural and forestry residues: exploration of unique natural microorganisms in combination with advanced strain engineering. Bioresource Technology 215: 84-91. https://doi.org/10.1016/j.biortech.2016.03.158