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Autohydrolysis Pretreatment of Mixed Lignocellulosic Biomass

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Abstract

Lignocellulosic biomass, such as agricultural and forestry residues, can be transformed into valuable fuels and chemicals. However, the high cost of feedstock handling and the complex nature of cellulosic biomass processing and conversion remain two major challenges in biorefineries. This thesis aims to tackle these challenges by assessing the transformation of multi-feedstock using autohydrolysis pretreatment.

Corn cobs and stover (residues from corn processing), poplar chips (forestry residue), bagasse and wheat straw (agricultural residues) are abundant and often under-utilized cellulosic residues. These feedstocks were used to investigate the effects of biomass characteristics on the composition of the autohydrolysis pretreatment products. Moreover, biomass mixtures were used to improve the feedstock characteristics and increase their conversion into value-added compounds.

It was found that the relative abundance of xylan, Klason lignin, and acetate correlate with a feedstock's susceptibility to hydrolysis, therefore affecting the autohydrolysis products. These structural compounds were used to develop the C5-Hydrolysis Index (C5HI) of each feedstock. The C5HI was then analyzed for the production of xylo-oligosaccharides (XOS), for solvents production by ABE (Acetone-Butanol-Ethanol) fermentation, and to assess the potential for ethanol production. Furthermore, the C5HI of individual feedstocks were used to select mixtures and study how the change in the feedstock C5HI affects the autohydrolysate.

Results show that feedstocks presenting high C5HI (poplar and corn cob) result in high xylan hydrolysis and high XOS production, while low C5HI (bagasse) leads to low XOS amounts and low total xylan recovery. Mixing feedstocks either improved or maintained a linear relationship for XOS production compared to the individual components. Additionally, higher solvents (acetone-butanol-ethanol) production by ABE fermentation were obtained when feedstocks with low C5HI were present in the mixture, attributed to the lower presence of sugar degradation products that inhibit fermentation. The pretreatment of mixed feedstocks also presented higher xylan solubilization and higher monomeric xylose, which increased enzymatic hydrolysis of the cellulose and could potentially decrease the need for oligosaccharides hydrolysis prior to ethanol fermentation. Therefore, the autohydrolysis of mixed biomass is presented as an alternative to improve feedstock logistics and pretreatment products.

Keywords

Lignocellulosic biomass, mixed feedstocks, autohydrolysis pretreatment, oligosaccharides, fermentation, C5HI

Co-Authorship Statement

Contents of chapters 4, 5 and 6 are based on manuscripts to be published in peer-reviewed journals and were co-authored by Dr. Lars Rehmann, who provided editorial and analytical advice.

Saverio Niglio assisted the ABE fermentation of diluted corn syrup. Dr. Yi (Ethan) Su assisted with enzymatic hydrolysis experiments.

Claudio Lira planned and developed the experimental setup; designed and conducted all experiments, composition analysis and data collection and analytics; Claudio Lira created diagrams and drafted the manuscript.

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List of Notations and Abbreviations

AHP - Autohydrolysis pretreatment

BA – Bagasse

BrIndx – Branching Index

C5HI – C5-Hydrolysis Index

CC – Corn cobs

CS – Corn stover

DP – Degree of polymerization

HAcF – Fraction of acetyl groups in plant cell wall structure

LCB - Lignocellulosic biomass

LCFBR - Lignocellulosic Feedstock Based Biorefinery

LSR – Liquid-to-Solid Ratio

MLB - Mixed Lignocellulosic Biomass

PCA – Principal Component Analysis

PO – Poplar

SLB - Single Lignocellulosic Biomass

WS – Wheat straw

XOS – Xylo-oligosaccharides

XRI – Xylan Removal Index

Section 1 – Introduction and Literature Review

1 Introduction

The increasing world population has raised concerns about our ability to meet our growing mobility and chemical needs (Tilman *et al.*, 2002). Most of the transportation fuels used at present are derived from oil reserves located in few countries around the world, but the oil crisis in the early 1970s and high prices of the early 2000s resulted in an increased interest in biofuels (Ragauskas *et al.*, 2006). Towards the end of the 20th century, key issues were faced by governments and policy makers around the world, such as energy security (due to the increasing demand, dwindling oil supplies and international conflicts and wars), economic development (creation and sustaining of jobs, especially in agriculture), and greenhouse gases emissions (affecting global climate change) (Demirbas, 2009; IPCC, 2007; Koh & Ghazoul, 2008). These concerns led countries to seek alternative and home-grown energy sources, advancing biofuels research and utilization. The US is now the world's largest ethanol producer. In 2017, it produced 15.8 billion liters of bioethanol (58% of world production), mostly derived from corn. Brazil follows in second place with 7.1 billion liters, representing 26% of world production in 2017, with ethanol mostly produced from sugarcane (RFA, 2017).

Nonetheless, as the industry grew, it was also blamed for the increased prices of corn and other grains by repurposing cereals from food uses. For example, the "tortilla riots" started in Mexico in late 2006 as a result of increased price of corn tortillas. This rise was attributed to the increased cost of yellow corn (imported from the US) to use as animal feed, which caused Mexicans to use white corn as animal feed instead; however, white corn is also a traditional ingredient to make tortilla, resulting in increased costs of both white corn and tortilla (Westhoff, 2010). Although biofuels production has been blamed for the rise in food prices, which affects the poor (Gallagher, 2008), the extent of this contribution still has little consensus, as other factors also have strong influence. Mitchel, (2008) mentions that up to 75% of the increase in food price from June 2002 to June 2008 was due to low grain stocks, land-use change, speculative activities and export bans. Other factors affecting food prices include high oil/energy prices (increasing fertilizer and transportation costs) and weak dollar values, poor harvests, changes in the structure in food demand in certain countries and general population growth (Gerber *et al.*, 2008). Other factors such as policies regarding agriculture and export and the politicization of food availability represent even greater determining factors. All these complex issues, some of which have been recognized since the early 90’s (Hall, 1991), also require careful examination.

Another contentious issue that emerged from evaluations of US corn bioethanol is indirect land use change (iLUC) (Searchinger *et al.*, 2008). The iLUC is based on the assumption that biofuels crops grown in agricultural land causes new cultivation of crops elsewhere in the world to compensate for the decline in their production, resulting in the potential release of substantial amounts of greenhouse gases. Nonetheless, calculating these emissions and the attribution of iLUC in extremely complex and burdened with uncertainty. For these reasons, some reports show that corn-based bioethanol produce more GHG emissions than fossil fuels, while other studies present more encouraging results (Baffes & Haniotis, 2010; Searchinger *et al.*, 2008). Aspects related to indirect LUC are recognized as relevant in principle, but the methods used for calculation and how iLUC should be dealt with are still unresolved matters of debate.

In Brazil, negative environmental effects caused by the increased production of sugar cane ethanol are related to water use, deforestation (requires clearing rainforests and other environmentally valuable lands) and field burning (Martinelli & Filoso, 2008; Sawyer, 2008). Similarly, agricultural developments and energy security brought by biodiesel production in Malaysia also come with concerns over biodiversity loss (Koh & Wilcove, 2008; Wich *et al.*, 2008) and environment degradation, as the conversion of forests to oil palm plantations may increase greenhouse gas emissions (Fargione *et al.*, 2008; Raman *et al.*, 2008). All these factors represent lessons learned from first generation biofuels production.

Replacement of fossil fuels with first generation biofuels has proceeded regardless of these concerns. As the biofuels industry matures, technology improvements and land use changes can decrease issues such as reducing greenhouse gas emissions. Changing the sugar source can also provide further economic and environmental benefits, as obtained with the utilization of cellulosic biomass as feedstock (S. Raman *et al.*, 2014; Wang *et al.*, 2011). Lignocellulosic feedstock based biorefineries (LCFBR) can process a wide variety of feedstocks, including agricultural, forestry and some types of industrial residues (Naik *et al.*, 2010). Agricultural and woody residues, such as wheat straw and poplar chips, are options that have been checked and certified to be free of conflicts with food (Mohr & Raman, 2013). Liang *et al.* (2012) demonstrated that second generation biofuels produced from cellulose-based feedstocks generally present positive economic, energy and environmental performance, while corn grain and wheat grain present negative impact on the same aspects. Therefore, lignocellulosic biomass residues are presented as a renewable, low-cost energy source able to provide biofuels while avoiding the competition with food (Lynd *et al.*, 2002; Tonini *et al.*, 2016), which are improvements to the lessons learned from first generation biofuels.

Lignocellulosic biomass (LCB) can be classified in 4 categories: agricultural (corn stover and cobs, sugarcane bagasse, wheat straw) and forestry (sawdust, mill wastes) residues, herbaceous (switchgrass) and woody (poplar trees) crops (Wyman *et al.*, 2004). Therefore, LCB are relatively cheap to obtain and sustainable, providing an economical and environmentally friendly resource for biofuels production (Hess *et al.*, 2007). These feedstocks are composed of cellulose (glucose), hemicellulose (mostly xylose), and lignin. Glucose and xylose are sugars that can be fermented to several types of fuels and chemicals. Together, cellulose and hemicellulose may represent up to 80% of lignocellulosic material, of which there is an estimated 10 to 50 billion tones globally (Claassen *et al.*, 1999). Additionally, some types of biomass can grow in relatively nutrient deficient soils (Ashraf *et al.*, 2017), providing a an additional advantage for the growth and utilization of lignocellulosic material.

Issues associated with utilization of LCB arise from the feedstock's variability in physical and chemical characteristics (Li *et al.*, 2016). The cellulose fibers in the cell wall are embedded in a matrix of hemicellulose held together by lignin, rendering a natural resistance to degradation commonly referred to as biomass recalcitrance (Zhao *et al.*, 2012). Therefore, a robust conversion technology (able to economically convert multiple biomass) is needed to decrease the biomass recalcitrance and allow the structural sugars to be released and converted by microorganisms (saccharification and fermentation).

Pretreatments are generally divided into four main categories: mechanical, physico-chemical, chemical and biological methods (Agbor *et al.*, 2011). Although all methods have their own advantages and disadvantages, the most promising methods seem to need the utilization of either acid or alkaline chemical catalysts, depending on the purpose of the pretreatment (Brodeur *et al.*, 2011). Acid pretreatments solubilize hemicelluloses, while alkaline methods act on lignin (Aboagye *et al.*, 2017). Regardless of the process used, the ultimate objective of a pretreatment is to economically recover as much sugar as possible from the feedstock while decreasing the formation of products that can inhibit enzymatic hydrolysis and fermentation.

Autohydrolysis is a type of chemical pretreatment method where no extraneous chemicals are added to the process (Carvalheiro *et al.*, 2004). Two distinct products are obtained from autohydrolysis pretreatment: a liquid phase containing easily hydrolysable hemicellulose sugars, and a solid fraction containing mostly cellulose and lignin.

The focus of autohydrolysis is to obtain high sugar recovery by solubilizing the hemicellulose in the feedstock and increasing the accessibility of the cellulose to enzymes. This total sugars recovery is challenged by the formation of sugar degradation products that inhibit bioconversion and factors that can decrease the accessible surface area; therefore, the optimum pretreatment parameters for hemicellulose hydrolysis may differ from the best conditions for enzymatic hydrolysis of cellulose (Cara *et al.*, 2008), resulting in a compromise between xylose degradation and glucose production.

Most of the literature on cellulosic feedstock conversion focuses on the effects of single biomass (SLB) on the pretreatment products. An economically feasible biorefinery will potentially need to process multiple feedstocks throughout the year (Maung *et al.*, 2013), so the utilization of different feedstocks may be imperative for the economic success of a biorefinery.

Multiple feedstocks could be processed separately, which would likely cause the process to operate at different parameters (temperature and time), as different biomass types may require different process conditions to achieve the highest sugar recovery (R. Kumar *et al.*, 2009), making studies of multi feedstocks complicated and time-consuming. Alternatively, mixing different feedstocks prior to processing could simplify the operation due to constant process parameters, can decrease seasonality of the feed material, increase overall sugars recovery and decrease production of inhibitor compounds (Vera *et al.*, 2015). Nonetheless, the utilization of mixed lignocellulosic biomass (MLB) for pretreatment and production of value-added products has not been widely explored (Oke *et al.*, 2016), representing a promising topic for further studies.

The pretreatment of mixed feedstocks by autohydrolysis is an alternative to improve the conversion efficiency (production of fermentable sugars) in biorefineries while using a relatively cheap and environmentally friendly pretreatment method. Nonetheless, the challenges posed by biomass recalcitrance and the intrinsic characteristic of lignocellulosic materials have to be overcome if we are to produce fuels and chemicals derived from this resource (Agbor *et al.*, 2011). Two important factors to be considered when processing multi lignocellulosic feedstocks are: (i) the effects of different process parameters on the pretreatment products; (ii) the effect of different biomass characteristics on the pretreatment products. This results in the need of a systematic and consecutive approach to study the effects of mixed feedstocks on the pretreatment products.

The objective of this work is to study both biomass characteristics and the interaction between structural compounds and the autohydrolysis pretreatment products. More specifically, this thesis presents i) an analysis of lignocellulosic biomass characteristics affecting xylo-oligosaccharides (XOS) production by autohydrolysis, ii) studies of the effects of various lignocellulosic biomass mixtures on autohydrolysate composition and bioconversion and iii) an analysis of the potential for ethanol production by autohydrolysis pretreatment of mixed lignocellulosic cellulosic biomass.

2 Literature Review of Autohydrolysis Pretreatment

2.1.1 Overview of biochemical biorefineries

Biorefinery is a facility where biomass feedstocks are transformed into several value-added products and energy, as defined by the International Energy Agency – IEA (Jong *et al.*, 2012). In biorefineries, all plant materials such as carbohydrates, oils, and lignin can be converted into chemicals, fuels and other value-added products (Carvalheiro *et al.*, 2008), as illustrated in Figure 2-1.

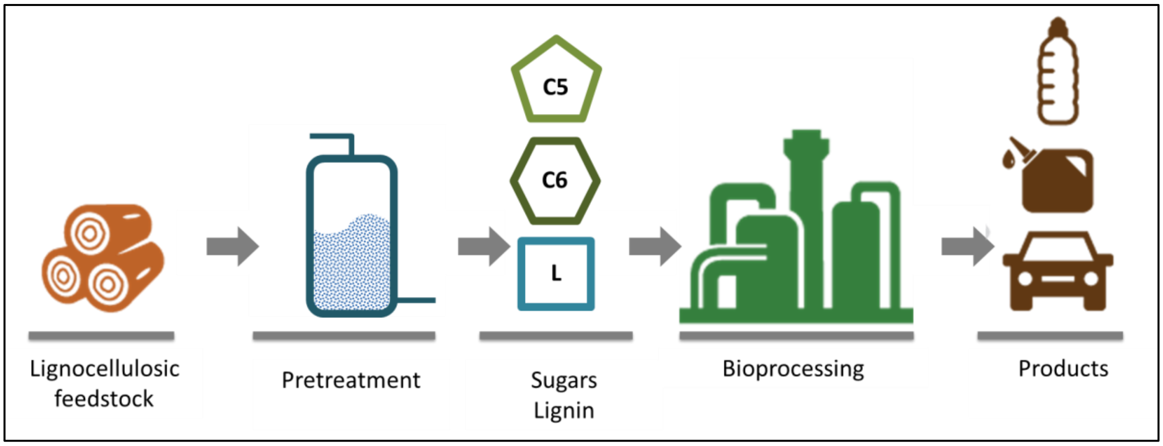

Figure 2-1 - Representation of processes in a cellulosic biorefinery

The types of biorefineries will depend on raw materials, technological process and products obtained. For example, biochemical biorefineries are sugar based, while thermochemical are generally based on the production of syngas (Carvalheiro *et al.*, 2008). Multiple platforms of otherwise unrelated industries can also be incorporated, generating an industrial symbiosis; for instance, the fermentation effluents can be utilized for anaerobic digestion (Realff & Abbas, 2003) as an strategic tool for economic development, resource efficiency and innovation.

The biochemical platform is seen as one of the most promising routes for commercial operations (Alex Marvin *et al.*, 2012). It is based on the fermentation of carbohydrates in the feedstock, as shown in Figure 2-2.

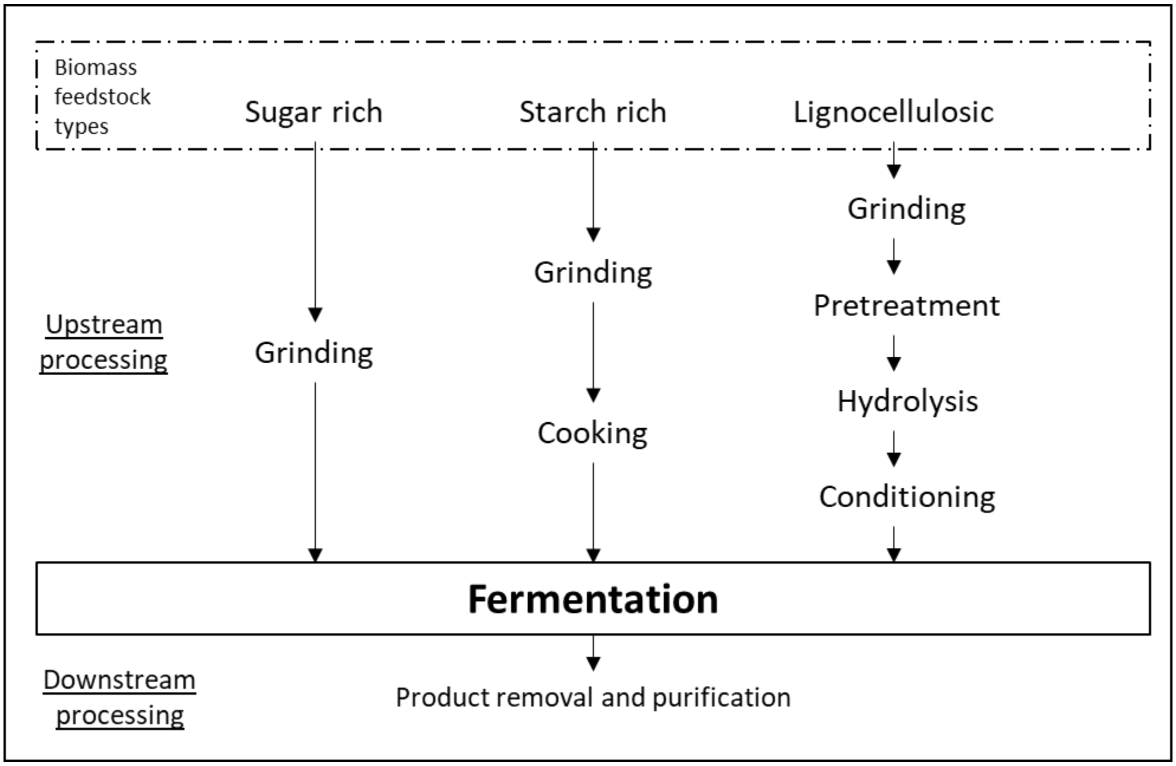

Figure 2-2 – Schematic diagram of common steps in biochemical biorefineries

Figure 2-2 shows the general steps in a biochemical biorefinery. Depending on the feedstock type, steps can include only size reduction or more extensive feedstock preparation, including conversion of biomass to sugars or other substrates that undergo conversion using biocatalysts (Kamm & Kamm, 2004). The final steps include processing the products to yield value-added chemicals, fuels, heat, etc.

2.1.1.1 Primary products in biochemical biorefinery

Fermentation of lignocellulosic sugars have been used for production of lactic acid (Bayitse, 2015), butanol (Barathiraja, *et al.*, 2017; Garcia *et al.*, 2011), ethanol (Verma *et al.*, 2011; Tian *et al.*, 2016), among other compounds.

Different types of bacteria, yeasts, and fungi can be used for fermentation in biorefineries (Badger, 2002). These organisms can produce various chemicals with high market value, such as ethanol, antibiotics, and lactic and acetic acids.

Platform intermediate chemicals are of special interest in biorefineries (Wisconsin

Biorefining Development Initiative, 2015), as they can be transformed into various products for consumers and industries, such as succinic acid and butanol.

2.1.1.2 Process byproducts in biorefineries

In a process where the hemicellulose fraction is removed in the pretreatment liquor, the cellulose and lignin remaining in the solids can be used as fuel for boilers to produce electricity and/or steam. The fermentation process produces carbon dioxide (Ghosh & Prelas, 2011) that can be captured and sold to the beverage industry or used in greenhouses.

Some purification processes result in high amounts of solid by-products, such as gypsum (calcium sulfate), which could be recycled, sold as a soil amendment (Norton, 2008), or simply disposed.

Other by-products, such as the volatile organic compounds, may be produced during fermentation and may require remediation prior to release (Muñoz *et al.*, 2007). Waste water from distillation and membrane separation processes may require retreatment (Valderrama *et al.*, 2002) due to the high biological oxygen demand which can be damaging to aquatic ecosystems upon release.

2.1.2 Sugars production from lignocellulosic biomass (LCB)

Lignocellulosic feedstocks (Figure 2-2) include wastes such as agricultural, agro-industrial and forestry by-products. These materials are often neglected, leading to billions of tons of valuable feedstock being discarded annually (Saha, 2003) and representing an economical and environmental source of fermentable sugars. Cleaning and milling may be required for lignocellulosic feedstock processing (Figure 2-2), to aid sugars release during pretreatment, followed by hydrolysis of celluloses (Limayem & Ricke, 2012). During the fermentation step, both five-carbon (C5) and six-carbon (C6) sugars will be converted to specific products, followed by a specialized products recovery step.

2.1.2.1 Lignocellulosic biomass (LCB) structure

Lignocellulosic biomass refers to plant material composed of the polymers: cellulose, hemicellulose and lignin, which are often the targeted compounds in biorefineries. These complex polymers are structural compounds found in plant trunks, branches, leaves, stalks ad husks (Anderson, 1958; Lee *et al.*, 2007). Theoretically, any type of plant matter containing cellulose and hemicellulose could potentially be used for cellulosic ethanol production. Figure 2-3 shows typical chemical composition of the three major components in LCB feedstocks.

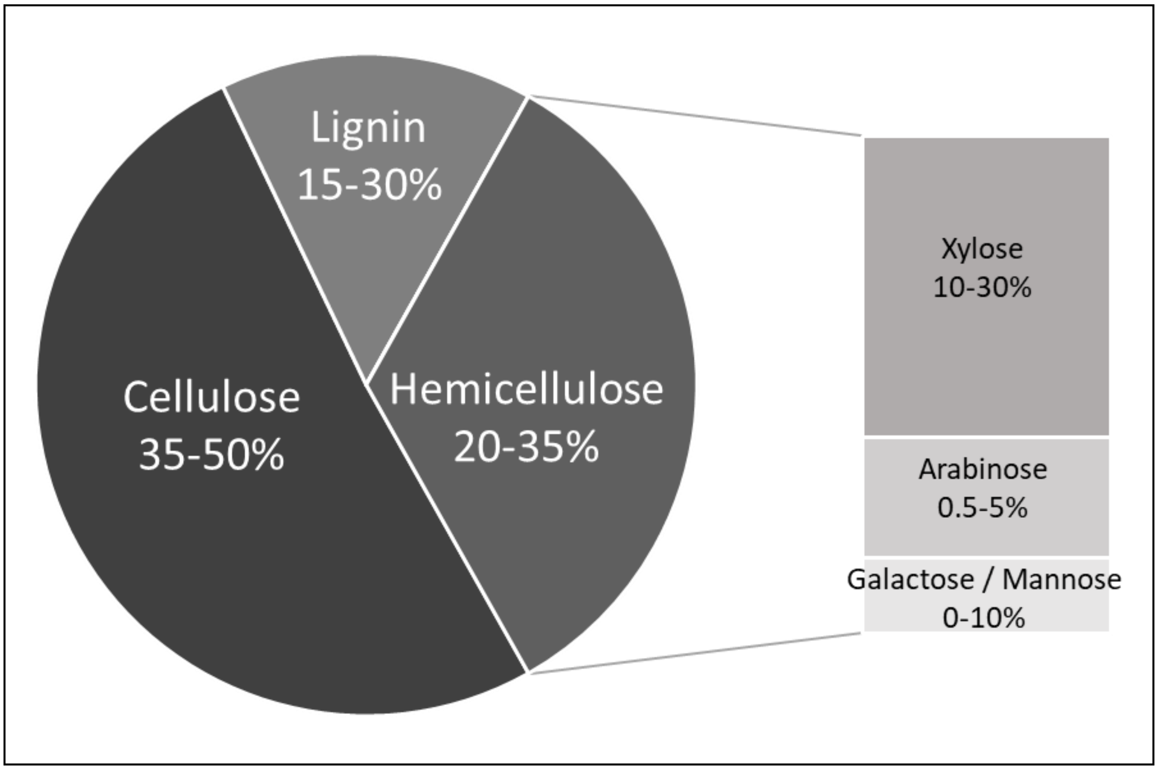

Figure 2-3 - General composition of lignocellulosic biomass (Adapted from Garrote *et al.*, 1999)

The exact composition can vary widely, changing in function of the feedstock type, plant family, species and part of the plant. In general, woody biomass contains higher amounts of cellulose and lignin, while agricultural residues and grass feedstock present higher content of hemicellulose (mostly xylan), ashes, and smaller fractions of various extractive compounds (Garrote *et al.*, 1999; Zhao *et al.*, 2012).

Cellulose

Cellulose is both the major structural polymer in the plant cell walland the most abundant polymer on earth. It is composed of a linear chain of up to ten thousand β (1→4) linked glucose monomers, which are arranged in long straight cellulose polymer chains where cellobiose is the repeating unit (Zhao *et al.*, 2012; Amarasekara, 2014). Hydrogen bonds are formed from the evenly distributed hydroxides in the glucose molecules in the chain, resulting in several closely-packed parallel chains attached to each other, forming a crystal structure that provides chemical stability and mechanical strength to the plants. The cellulose structure is shown in Figure 2-4

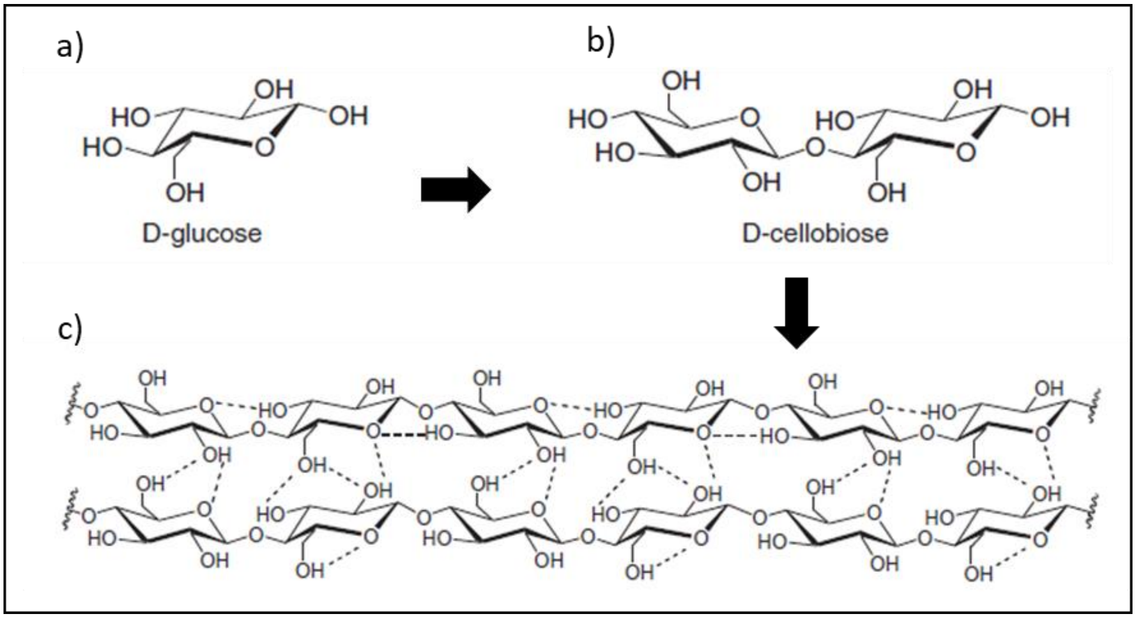

Figure 2-4 - a) Glucose, the basic monomer in cellulose; b) Cellobiose, the repeating disaccharide in cellulose; c) Linear polysaccharide chains forming cellulose microfibrils, dashed lines represent inter- and intramolecular hydrogen bonds. (adapted from Amarasekara, 2014)

Hemicellulose

The second major component in the plant cell wall is hemicellulose, which is a term used to represent a group of different sugar polymers such as arabinoxylans, and glucomannans, galactans (Ebringerová, 2005). Figure 2-5 presents some common hemicellulose found in lignocellulosic biomass.

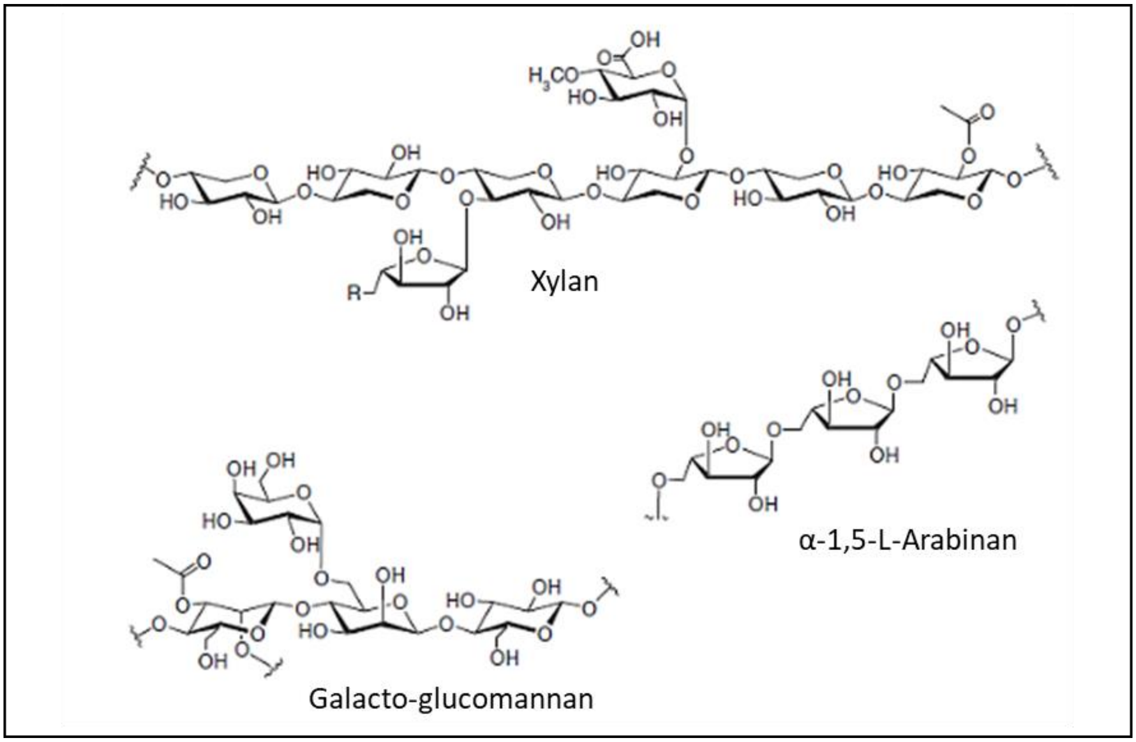

Figure 2-5 - Common polymers found in hemicellulose

These compounds present different structures and compositions in the plant cell wall, being composed of a combination of several heteropolymers of different C5 and C6 sugars. Hemicellulose presents an amorphous structure, in contrast with the crystalline structure of cellulose. Acetyl groups attached to the hemicellulose backbone causes a branched structure where xylose (C5-sugar) is the main compound, and glucose (C6-sugar) is present in lower amounts (Harmsen *et al.*, 2010). Generally, xylose is the main sugar monomer in agricultural residues and hardwoods, while mannose is often the most abundant sugar in softwoods (Harmsen *et al.*, 2010; Zhao *et al.* 2012; Amarasekara, 2014). As xylose is the second biggest sugar compound in cellulosic biomass, its conversion is essential for the economic aspects of ethanol production.

Lignin

Lignin is considered as the glue that holds the cell together, forming a protective layer for the plant walls. It is a polymer composed of aromatic compounds, making lignin the most complex natural polymer in LCB. It has been identified that hardwood (angiosperm) and softwood (gymnosperm) present different types of lignin, where over 90% of softwood is composed of coniferyl alcohol and minor amounts of p-coumaryl. Hardwoods, on the other hand, present varying fractions of coniferyl and sinapyl (Harmsen *et al.*, 2010), as shown in Figure 2-6.

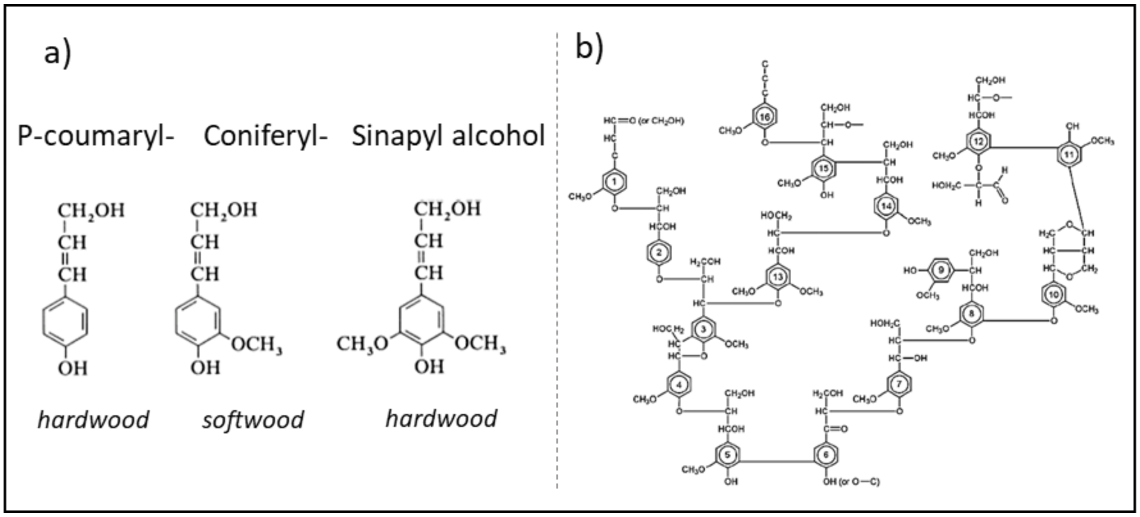

Figure 2-6 - a) Main components of lignin; b) Model structure of spruce lignin. Adapted from (Adapted from Harmsen *et al.*, 2010)

Other compounds

Other compounds in all plant materials include extractives (proteins, fats, oils), acetyl groups and minerals (ash) (Harmsen *et al.*, 2010; Wyman *et al.*, 2004). Although some authors recognize these minor compounds as being not important in biomass refining (Zhao *et al.*, 2012), this review further explores the influence of minor compounds in plant cell wall structure, such as acetic acid and ash, on the AHP processing of LCB.

2.1.2.2 Biomass recalcitrance and challenges in commercialization

Recalcitrance is an evolutionary trait of plants developed to protect their sugars from microbial and animal attack. Most of the glucose in the plant cell wall is present in the form of highly crystalline cellulose polymers. Hemicellulose and lignin surround the cellulose, providing structural integrity and protecting the plant. Therefore, the biomass recalcitrance impedes degradation of plant carbohydrates (structural sugars) by micro-organisms and enzymes (Zhao *et al.*, 2012). To change and/or remove these natural constraints (recalcitrance), physical and chemical disruption are used to pretreatlignocellulosic material, allowing enzymes and other micro-organisms to access the sugars in the biomass structure (Mosier *et al.*, 2005), resulting in improved hydrolysis rates and increased yields of sugars for further conversion.

2.1.3 Overview of lignocellulosic biomass pretreatment

2.1.3.1 Introduction

Pretreatment is an important step for the biochemical transformation of lignocellulosic sugars. It disrupts the carbohydrate-lignin structure and makes the sugars (cellulose and hemicellulose) more accessible to enzymes, increasing conversion of the polymers into fermentable sugar monomers (Mosier *et al.*, 2005). Figure 2-7 presents some effects of pretreatment that lead to sugars production from lignocellulosic biomass.

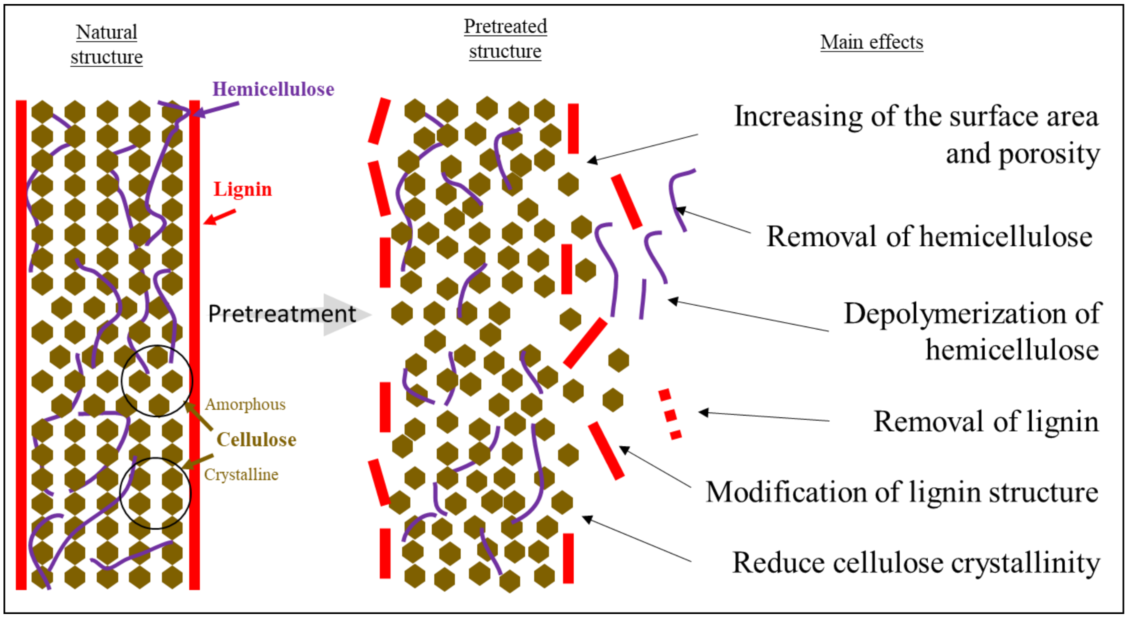

Figure 2-7 - Pretreatment effects on cell wall structure

2.1.3.2 Overview of pretreatment methods

Pretreatment technologies are constantly being developed, and many researchers have worked with different methods aiming to develop a process that can efficiently remove the sugars from LCB (Yang & Wyman, 2008). In general, pretreatments are classified into 4 main categories: biological, physical, physico-chemical and chemical.

Biological processes utilize enzymes secreted by some streams of fungi (e.g. brown, white, and soft rot-fungi) to remove mostly lignin and some hemicellulose. The advantages of this process include mild processing conditions (safe) and relatively low energy requisites (reduce costs). However, this method presents very low hydrolysis rate (Eliana *et al.*, 2014), requiring longer pretreatment times.

Physical pretreatments involve milling, grinding, and chipping. These processes of mechanical comminution decrease the particle size and cellulose crystallinity, resulting in increased particles surface area and reduced degree of polymerization. These processes increases the enzymatic hydrolysis of the LCB, but the high required power consumption exceeds the theoretical energy content in the biomass (Harmsen *et al.*, 2010), making this type of pretreatment unfeasible for full-scale production.

Physico-chemical pretreatments include CO_2 explosion, ammonia fiber explosion, steam explosion and oxidation delignification, among others. In general, the “explosion” processes increase feedstock’s surface area, while the chemical used helps to solubilize different fractions of the lignocellulosic structure. Drawbacks from the utilization of these methods include the formation of carbohydrates degradation products that inhibit fermentation and costs associated with chemicals recovery (e.g. ammonia) (Harmsen *et al.*, 2010) due to the need of extra detoxification equipment.

Chemical pretreatments are used to solubilize different cell wall compounds, depending on the chemical used, thus the pH of the system. In general, acid methods aim to remove hemicellulose, releasing sugars monomers and soluble oligomers into the hydrolysate, resulting in higher porosity of the solid biomass residues, and therefore increasing the enzymatic digestibility (Y. Sun & Cheng, 2002). Alkaline chemicals mostly remove lignin and increase cellulose digestibility. Figure 2-8 represents the effect of pH on the different cell wall structural polymers.

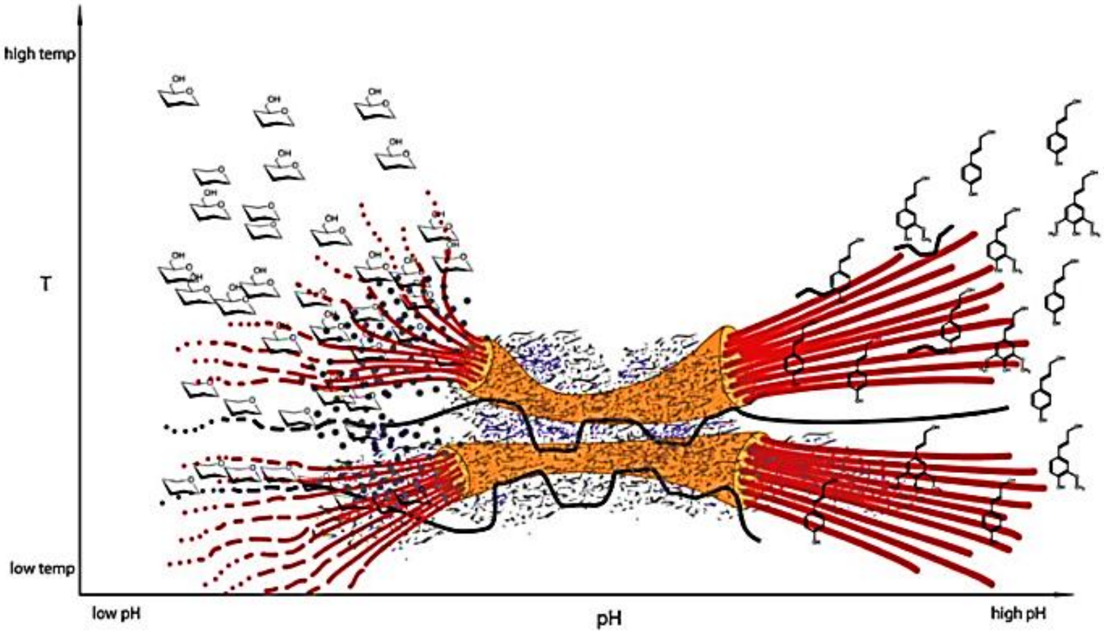

Figure 2-8 - Effect of pH during chemical pretreatment (Pedersen & Meyer, 2010)

The most promising pretreatment methods seem to require chemical process in order to efficiently disrupt the cell wall structure. Alkaline pretreatment uses chemical catalysts such as potassium, sodium, ammonium and calcium hydroxides. Acid pretreatment uses H_2SO_4 and HCl. Some chemical processes comprise ozone, organosolv and ionic liquids. The drawback of chemical pretreatments are the corrosives caused during reaction and the need of an extra step to recycle chemicals and to lower processing costs; furthermore, some chemicals are converted and become irrecoverable or are incorporated into the biomass (Harmsen *et al.*, 2010), resulting in higher consumption of these chemicals.

2.1.3.3 Pretreatment at low pH

In low pH processes, hydrogen ions (often provided by adding acid) catalyze the hydrolysis of hemicellulose chains (Kapu *et al.*, 2016), forming sugars oligomers and monomers that can be further degraded. These processes produce a liquid product rich in xylose and a solid fraction presenting mostly cellulose and lignin. Both concentrated and dilute acid can be used to pretreat biomass (Trajano & Wyman, 2013). Although concentrated acid has the advantages of flexibility in terms of feedstock choice and milder temperature conditions, dilute acid is more operationally attractive and widely used for LCB pretreatment. Up to 90% of the sugars in cellulosic biomass can be recovered through acid pretreatment (C. Liu & Wyman, 2005); however, the good performance is counteracted by the problems associated with the utilization of acid chemicals, such formation of sugar degradation, corrosiveness and the need for neutralization (Harmsen *et al.*, 2010), which increases the overall costs associated with the biorefinery operations. In some cases, steam used to heat up the biomass, and the organic acids in the biomass, e.g. acetic acid, can provide hydrogen ions to promote (auto) hydrolysis (Kapu *et al.*, 2016; Stelte, 2013). Autohydrolysis is seen as a potential solution for the issues associated with utilization of acid chemicals. Figure 2-9 shows the different pretreatment categories seen for low pH methods.

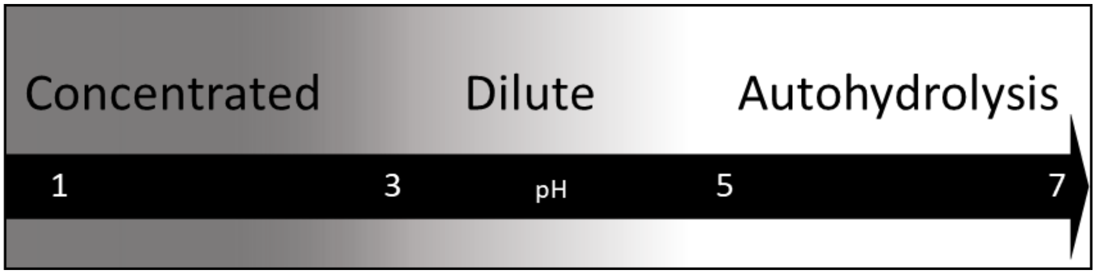

Figure 2-9 - Low pH pretreatment methods

Autohydrolysis can be considered as another type of chemical pretreatment, although no extraneous chemicals are added to the process. Autohydrolysis has been used to produce sugars in biorefineries (Sabiha-Hanim *et al.*, 2011), to convert agro-food byproducts into functional oligosaccharides useful in food ingredients (Carvalheiro *et al.*, 2004), and to improve biomass combustion (Runge *et al.*, 2013). Autohydrolysis techniques have been receiving increased attention due to their advantages, which include safer operation, cheaper materials, and lower degradation.

2.1.4 Autohydrolysis pretreatment (AHP) of single lignocellulosic biomass (SLB)

2.1.4.1 General background of autohydrolysis pretreatment

Autohydrolysis presents many benefits that include: (i) an environment-friendly process where no chemicals other than water are used; (ii) hemicelluloses are hydrolyzed into oligosaccharides at high yields and with low by-product generation, leading to a solution rich in sugar presenting various molecular weights and that can be used to produce several products; (iii) the mild pH avoids the need for expensive equipment; (iv) simplified process, as detoxification and recovery steps are avoided; and (v) the changes in the cellulose and lignin structures facilitate downstream separation and processing of the compounds in the solid fraction (Amiri & Karimi, 2015; Yoon, 1998). Autohydrolysis is a type of hydrothermal pretreatment. Other pretreatments present the same kind of reaction, such as hydrothermolysis (Suryawati *et al.*, 2009), aquasolv (Kubikova *et al.*, 2000), among others (Carvalheiro *et al.*, 2008). Regardless of the description used, all these processes present similar reaction mechanisms.

2.1.4.2 AHP effects on hemicellulose - Fundamentals

During autohydrolysis, the hydronium ions released due to autoionization of water selectively hydrolyses glycosidic linkages in hemicelluloses, causing them to depolymerize to dissolved xylo-oligomers and xylose. This process also liberates O-acetyl group attached to the hemicellulose backbone, forming acetic acid. Other acid moieties, such as uronic acids, can also form, increasing the concentration of hydronium ions in the reaction media, decreasing the pH of the autohydrolysate and further catalyzing xylan depolymerization (Garrote *et al.*, 2002; Sabiha-Hanim *et al.*, 2011; Pu *et al.*, 2013). The amorphous hemicellulose structure (Figure 2-5) is what makes it susceptible to autohydrolysis, as it enables ions to have easier access to bonds, opposite of what can be expected from the crystalline cellulose (Figure 2-4).

Models using stepwise kinetics were developed to describe hemicellulose hydrolysis, where it is assumed to be a first order reaction mechanism (Sidiras *et al.* 2011), as seen in Figure 2-10.

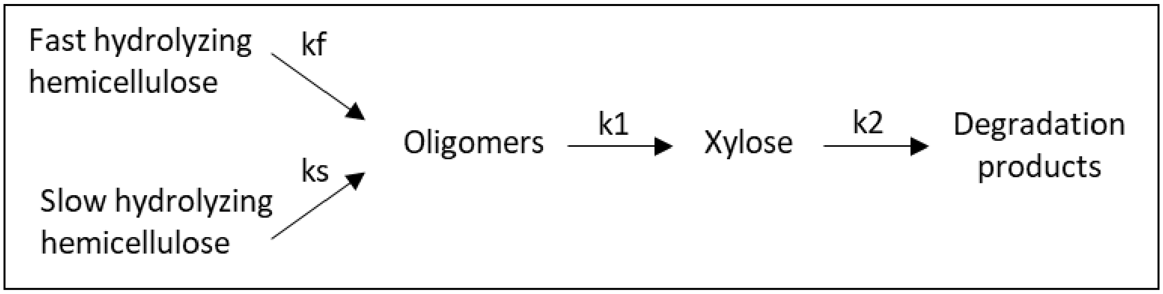

Figure 2-10 – Proposed mechanism of hemicellulose solubilization

In general, these models face real operational challenges. Process parameters usually involve determining the amount of water used for autohydrolysis, and this addition increases the yields of monomers and oligomers (Eliana *et al.*, 2014; Mussatto & Roberto, 2006). Flow through processes push liquid through biomass, increasing removal of hemicellulose with flow rate (Kilpeläinen *et al.*, 2014). Furthermore, analysis of chain length through liquid chromatography in uncatalyzed reactor shows the formation of oligosaccharides with high molecular weight, followed by their hydrolysis to shorter oligomers and posteriorly to monomers (Ramos, 2003). Once released into solution, pentoses can be degraded into furfural, while hexoses can be degraded into HMF (Ko *et al.*, 2015), these degradation products are also represented in Figure 2-10.

The process parameters often used in AHP makes the formation of hydronium ions from water autoionization important only in the initial stages of hydrolysis; posteriorly, acetic acid derived from hemicellulose hydrolysis presents higher effect on autohydrolysis (Carvalheiro *et al.*, 2008). Therefore, the amount of acetic acid in the feedstock may indicate that higher hydrolysis may take place during pretreatment.

Autohydrolysis is performed in temperatures between 120-220°C, where lower temperatures require longer processing time (up to 240min), as opposed to higher temperature where processing may take only few seconds (Archambault-Léger, 2014). Little hydrolysis takes place at temperatures below 100°C, and high cellulose degradation over 220°C (Ewanick & Bura, 2010). For these reasons, temperature and time are the most common process parameters used in autohydrolysis conversion research.

2.1.4.3 AHP effects on cellulose and lignin

Autohydrolysis decreases the biomass recalcitrance by solubilizing the hemicelluloses located on the cellulose fibers, resulting in structural changes that increase the access of cellulose fibers to hydrolytic enzymes (Pu *et al.*, 2013), which is a crucial step in the bioconversion of cellulosic residues.

The physicochemical characteristics of cellulose, such as linear configuration and high crystallinity and degree of polymerization, hinders cellulose from degradation during autohydrolysis (D. J. Gregg & Saddler, 1996), as compared to the easier hydrolysis of hemicellulose due to its amorphous structure and lower degree of polymerization (Figure 2-5).

Autohydrolysis of the lignin fraction releases a variety of compounds in the reaction media, such as aromatic, polyaromatic, phenolic and aldehydic compounds (Pielhop *et al.*, 2015). However, these phenolic compounds present high inhibitory effect and present higher inhibitory effect than furfural and HMF, even at low concentrations. In general, low molecular weight phenolics present the highest levels of toxicity. These lignin products reduce the cell growth and sugar assimilation by fermenting microorganisms (Harmsen *et al.*, 2010; Kang *et al.*, 2014), as they cause partition and loss of integrity of cell membranes.

Pretreatment time and temperature are important factors that influence lignin degradation; for example, negligible lignin degradation is found at temperatures lower than 180°C if no strong chemical (acid or alkaline) conditions are used. At higher pretreatment severities, lignin can undertake both degradation and repolymerization reactions (Hu *et al.*, 2014). The degradation of lignin occurs in two steps: a first fast stage where lignin is solubilized by breaking lignin-carbohydrates bonds, and a second where organic acids released yield insoluble condensation products (Li *et al.*, 2014). Pseudo-lignin is formed by condensation of lignin and results in high Klason lignin (acid-insoluble lignin) in the pretreated solid fraction. Delignification using alkaline chemicals can be performed after hemicellulose removal during pretreatment to increase enzyme accessibility to cellulose fibers (El Hage *et al.*, 2010). In general, lignin has negative effect on the bioconversion of cellulosic residues, so aspects of lignin deposition and the resulting decrease in the accessible surface area to the cellulose fibers should be avoided during pretreatment.

2.1.4.4 AHP effects on fermentation

Hemicellulose is hydrolyzed to sugars oligomers and monomers. Monomeric pentose sugars (xylose, arabinose) dehydrate to furfural, whereas monomeric hexose sugars (glucose, galactose, mannose) degrade to hydroxymethyl-furfural (HMF). Both degradation products inhibit the fermentation by affecting cell growth and respiration, although furfural is considered more toxic than HMF (Harmsen *et al.*, 2010). For these reasons, operating the pretreatment at conditions that reduces the formation of degradation products is essential in biochemical refineries.

Acetic acid lowers the cell pH and inhibits cell activity, and its formation in unavoidable in during hydrolysis, as it is derived from the acetyl groups attached to the hemicellulose backbone. Acetic acid (pKa=4.75) is in the undissociated form at low pH and diffuses into the cells in the fermentation medium. Inhibition is caused when the acid dissociates in the cell (pH=7.4), lowering of cell pH and inhibiting the cell activity (Palmqvist & Hahn-Hägerdal, 2000a, 2000b). This toxicity will depend on the fermentation conditions, and strategies such as increasing media pH (neutralization) prior to fermentation can decrease the inhibition effect.

Fermentation can also be influenced by other minor compounds in the feedstock, such as extractives and ash. Extractives are minor compounds in the lignocellulose structure which are less toxic than lignin derived phenolic compounds or acetic acid (Palmqvist, Hahn-Hägerdal, Galbe, & Zacchi, 1996). The corrosion of process equipment may cause the appearance of heavy metal ions (e.g. Fe, Cr, Ni and Cu) that are toxic and can cause inhibition of enzymes during the metabolism of fermenting organisms (Gadd & Griffiths, 1977), but this effect should be lower in autohydrolysis compared to acid/alkaline catalyzed pretreatment methods.

2.1.5 Main LCB feedstock characteristics affecting AHP

Some aspects of biomass characteristics affecting autohydrolysis pretreatment of LCB include: i) modifications caused by compounds such as tannins and ash in wood; ii) reactions of other compounds such as lignin occur simultaneously; iii) presence of acid compounds such as acetic and uronic acids; iv) recalcitrance due to the presence of lignin limits the accessibility of hydronium ions to the sugars bonds; v) variations in substrate accessibility (available surface area) during reactions; vi) and the time-dependence of the concentration of hydronium ions (Foston *et al.*, 2015; Garrote *et al.*, 1999). Desirable aspects may include formation of acetic acid, which helps solubilize hemicelluloses, while ash and extractives decrease the hydrogen ion concentration in the reaction media, decreasing pretreatment severity. This section describes with more details the effects of acetyl groups, ash, and buffering capacity characteristics of LCB.

2.1.5.1 Acetyl content

The acetyl groups present in the hemicellulose structure are released as acetic acid, providing increased catalyst concentration in the reaction media during autohydrolysis pretreatment; therefore, biomass with high acetyl content are often more favorable to autohydrolysis (Chen *et al.*, 2010). For this reason, hardwoods tend to be more favorable than softwoods as feedstocks.

During autohydrolysis, a fraction of the xylan (hemicellulose) esterified as xylo-oligomers present acetyl groups attached to them, remaining covalently linked to the xylan backbone. The effect of pretreatment severity on acetyl release is similar to the xylan release, as acetyl groups cause an increase in xylan solubility. The initially dissolved high DP xylo-oligosaccharides in the medium present a high degree of acetylation. Higher pretreatment severity, such as higher temperature or longer residence time, are required to cleave these acetyl groups attached to the dissolved xylo-oligomers (Yang and Wyman, 2009; Kumar *et al.*, 2011; Pu *et al.*, 2013); therefore, acetyl content in the feedstock will affect the extent of autohydrolysis reaction and consequently the pretreatment products and further bioconversion.

The release of acetic acid changes according to the pretreatment parameters. At low severity, only free acetic acid is released to the medium due to low hemicellulose hydrolysis. At higher severity, roughly 50% of the initial acetyl groups can remain linked to oligomers. Free acetic acid increases steadily with pretreatment severity, but for the temperature range used for autohydrolysis (around 180-210˚C), the concentration of free acetic acid is lower than that of acetyl groups in oligosaccharides (Garrote *et al.*, 2002). The presence of free or bound acetic acid affect the pretreatment yields and downstream processes, as described below.

Effect on recovery

The catalytic effects of acetic acid during autohydrolysis has been demonstrated by Zhao *et al.* (2014), who performed acetic-acid catalyzed hydrothermal pretreatment of corn stover. The results showed that maximum sugar recovery was obtained for acetic acid concentration of 0.05%, treatment time of 5 min and temperature of 195°C; the treatments at 210°C and 10min showed higher acetic acid and furfural concentration when the catalyst concentration was 0.05% and 0.25%, respectively, demonstrating the higher degradation caused by higher amount of catalyst.

Similar results were found by Xu *et al.* (2009), who also found a decrease in solids yields during acetic acid-catalyzed AHP. The solids yields were shown to depend on factors that include feedstock type, processing temperature and the type of catalyst used. Compared to the non-catalyzed autohydrolysis of corn stover, the HAc added experiments presented lower glucan and xylan contents in AHS. Glucan was higher in the autohydrolysate of HAc treated samples, but xylan in liquor was lower in comparison to biomass only sample, indicating higher sugar degradation.

Effect on pH

Y. Li *et al.* (2014) showed that higher pretreatment temperature and time increase the acetic acid release in the autohydrolysate. This higher acetic acid was significant in the carbohydrates solubilization and the final pH value. The authors noted that a fraction of O-acetyl groups after pretreatment are bound to the backbone of dissolved xylan oligomers. It was found that given a temperature, the release of acetic acid occurs mainly in early periods (up to 30min of AHP). The authors indicate that higher acetic acid released also presented higher solubilized hemicellulose, where temperature showed higher influence than time. Similar results were found by Chen *et al.* (2010).

Given a defined processing temperature, the media presenting lower pH will result in higher degradation of glucose and xylose, increasing the concentration of HMF and furfural in the AHL (Cara *et al.*, 2008; Kabel, Bos, Zeevalking, Voragen, & Schols, 2007; Xu *et al.*, 2009), resulting in less xylose in the liquor and lower total xylan recovery.

Xu *et al.* (2009) found that the degradation effect of sugars is confirmed through mass balance, where approximately 95% of glucan and 80% of xylan in original biomass was recovered when no acid was added, while the recovery for HAc added was 84% and 72% for glucan and xylan, respectively. This demonstrate how lower mass balance is obtained when more degradation occurs, and xylose yields in AHL does not necessarily relate with the xylan removed from the feedstock. Regarding the convertibility of AHS, pretreatment with HAc presented the highest ethanol production efficiency of 88.7% (31.5g/l).

The positive effect of the deacetylation, allowing higher solubilization and hemicellulose hydrolysis, is counteracted by the ash content in the biomass, due to its neutralizing capacity. Therefore, the pH response varies according to the rate of ash neutralization compared to the rate of hemicellulose deacetylation.

2.1.5.2 Ash content and buffering capacity

Hydronium ions released during autohydrolysis can undergo exchange reactions with inorganic cations in the feedstock, decreasing the hydronium ion concentration and neutralizing the solution, leading to higher pH. Pretreatment temperature and a feedstock’s neutralizing capacity significantly influences the pH of a system, as demonstrated by Lloyd and Wyman (2004).

Herbaceous biomass and agricultural residues present high mineral content, therefore higher neutralizing capacity than wood materials. For this reason, corn stover requires more severe treatment when compared to poplar. Substrates with the lower ash content present higher hydrolysis rates (McKenzie, 2012), exhibiting lower energy requirements.

The initial pH of the hydrolysate will depend on the feedstock used, and the pH and buffering capacity of the feedstock can affect the AHP products. A large buffering capacity maintains a nearly constant pH; therefore, impeding the reduction in pH which is necessary for the solubilization of hemicelluloses; therefore, more acidic feedstock may need a lower pretreatment temperature (Lloyd & Wyman, 2004; Springer & Harris, 1985). Similarly, the processing of wood with different acidities and buffering capacities may require the adjustment of process conditions to achieve higher hemicellulose solubilization while decreasing degradation products.

During AHP, the neutralizing effect depends mostly on feedstock particle size and reaction temperature (Ruiz *et al.*, 2011). The neutralizing effect of bigger particle size becomes less significant, as more ash is found in smaller particle size. Ash studies are important for industrial operations, which usually operate at higher solids loading and therefore higher ash content.

The buffering capacity of the biomass feedstock is not as important at high acid levels. However, as autohydrolysis does not require any acid addition, the effect of buffering capacity is relevant and should be further understood, especially in regards to the extent the feedstock buffering capacity is able to decrease the severity of the process (Król *et al.*, 2017). This factor may be taken advantage of if a biomass with low buffering capacity is used. At the same process conditions, low buffering capacity would likely degrade sugars.

Vera *et al.* (2015) mixed wheat straw (WS) and hybrid poplar (HP) and observed that when WS was added to the mixture, less sugar degradation was produced. This is due to the higher presence of ash, which could ‘buffer’ extreme acidic conditions. High buffering capacity prevents acid production, making the pretreatment appear less severe and resulting in less xylan solubilization and decreasing the generation of sugar degradation products.

Mixed feedstocks may improve pretreatment products, increasing the overall efficiency of the pretreatment process; furthermore, it may improve the feedstock costs, as more feedstocks from the same region are being used as feedstocks. The next sections describe current research on the synergistic effects of mixing different feedstock types.

2.1.6 Pretreatment of mixed feedstocks

The mixed biomass approach involves the simultaneous processing and conversion of different types of biomass feedstocks to produce digestible sugars. Figure 2-11 shows a schematic representation of the different approaches that can be used for feedstock processing.

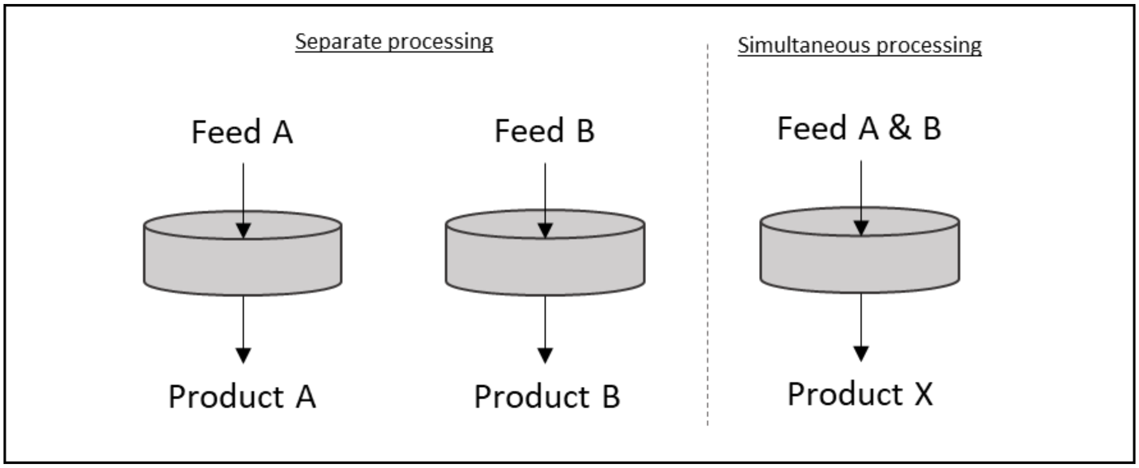

Figure 2-11 – Approaches used for feedstock processing.

The utilization of mixed feedstocks can provide improvements in both conversion technology and feedstock supply logistics (Ashraf *et al.*, 2017), resulting in cost reduction in the processing of cellulosic feedstock (Banerj*ee et al.*, 2010). Mixed lignocellulosic feedstocks have been studied, although to a limited level, for the production of biohydrogen, biogas, bioethanol, microbial enzymes, and for thermal applications (power, heat an heating processes) as reviewed in details elsewhere (Oke *et al.*, 2016).

This section discusses some of the main advantages and disadvantages of mixed lignocellulosic biomass (MLB), as well as operational challenges a plant would face if using mixed feedstocks and strategies to solve some of these issues.

Advantages

Stable supply, better economics and improved storage

Lignocellulosic plants at commercial scale present feedstock capacity ranging from 600 to 4500 MT/day (Balan *et al.*, 2013). In any region with limited feedstock production, such as arid and regions with poor soil nutrients, the availability of sustainable single feedstock supply would be limited, affecting the plant throughput. These situations where the year-round supply of a single feedstock is unsustainable could benefit from the utilization of multiple feedstocks (Ashraf *et al.*, 2017). The higher throughput from multi feedstocks processing improves the economy of scale and leads to lower plant cost per tonne of product, resulting in an optimum capacity (Ashraf & Schmidt, 2018; Huang *et al.*, 2009). Therefore, using multiple feedstocks may help to overcome the issues of plants operating at suboptimal capacity when processing single feed, allowing plants to operate more economically.

Mixed feedstocks also decreases the seasonality effect, allowing a stable feedstock supply to the biorefinery and resulting in higher operational efficiency (Oke *et al.*, 2016; Ashraf and Schmidt, 2018). Maung *et al.* (2013) demonstrate that processing multiple feedstocks for production of cellulosic fuels result in higher costs savings in addition to decreasing the feedstock supply risks. C. Li *et al.* (2015) assessed the feedstock availability and affordability for cellulosic biorefineries and demonstrated that mixed feedstocks are viable and valuable resources to consider. It has also been demonstrated that given a similar feed rate, processing mixed feedstocks is more advantageous than separate conversion of single feed (Ashraf & Schmidt, 2018).

The complexity of logistics can be increased for mixed feedstocks. However, the delivery costs of MLB can decrease in some cases, as demonstrated by (Sultana & Kumar, 2011) for the combined processing of agricultural and woody feedstocks.

The utilization of mixed feedstocks may significantly increase cost savings in comparison with single feedstocks (Rentizelas *et al.*, 2009). Cost reduction will always be the ultimate goal of any improvements to pretreatment processes, so selecting cheap and abundant feedstocks for mixtures may increase the overall process economics. Improved supply and economics can also be obtained when the different feedstocks are located close to each other and to the processing facility; in addition, the ease of collection (processing industrial/agricultural residues over purpose-grown feedstocks) would ease logistics and potentially cause cost savings.

Mixed feedstocks can also improve aspects of storage. Rentizelas *et al.* (2009) observed that to decrease costs of biomass and storage, choosing a cheap biomass and using simple and cheap storage solutions provides the most economic benefits. Nonetheless, multi-feedstock seems to be a more attractive option when using expensive storage solutions.

Environmental impact

The LCB used may be of the same or different origin, which can decrease costs of transportation and overall supply chain. Raftery & Karim (2017) concluded that a feedstock mixture composed of sugarcane, bagasse and corn stover, plentiful in the southeastern United States, could decrease the harvest area needed for a sustainable feedstock supply, therefore lowering the environmental impact of the plant. Moreover, mixed feedstocks can decrease the need for detoxification processes and nutrient supply, beneficial aspects that can decrease the utilization of fossil-derived products during the conversion of cellulosic material.

Improved pretreatment

The choice of biomass used for pretreatment is often based on both the feedstock price and process efficiency, where increased yield and productivity are important measures. For example, the selection of mixture components based on the ability to decrease the production of inhibitor compounds in the hydrolysate, especially for downstream processing involving microbial fermentation (Vera *et al.*, 2015). Obtaining low concentration of inhibitors may lead to removal of expensive chemical detoxification processes (Yang and Wyman, 2008; Vera *et al.*, 2015), decreasing the complexity and costs of biomass pretreatment. The ultimate choice of mixture feedstocks may be obtained from a balance between higher yield and concentration of desired products.

Although the utilization of mixed lignocellulosic biomass (MLB) has the potential to decrease the costs of lignocellulosic feedstock conversion, minimize environmental impact and improve the pretreatment products, there are only few studies relating the utilization for MLB for bioproducts production (compared to single biomass). This can be attributed to the many limitations and challenges associated when using feedstock mixtures, such as the diverse nature of the biomass sources and the complexity of pretreatment. Factors such as biomass bulk density, transportation distance and biorefinery capacity, and overall costs of cellulosic feedstock processing (harvesting, preprocessing, storage and conversion) should all be considered when dealing with the logistics of mixed feedstocks. Because of the relatively limited literature on conversion of MLB, only few studies are available describing strategies to overcome cost implications of mixed feedstocks; however, some measures successfully employed in some applications of MLB are available, and it is also possible to speculate about some other potential strategies, as discussed in the next section.

Challenges and strategies for utilization of mixed feedstocks

Handling, logistics and storage

Adapting a new type of cellulosic biomass feedstock to an existing plant may be difficult to achieve. For example, bioethanol plants are usually located close to the existing feedstock source, and an increased cost of transportation can arise from combining a new feedstock. More complications may arise in the logistics of multiple feedstock sources, as the different biomass types may require differing types of equipment for the various process involved, such as collection, handling and transportation. Therefore, the potentially challenging logistics and the unsuitability of the machinery for all feedstocks may result in process underperforming and may end up undermining the capital cost savings and improved economics of multi feedstock processing (Rentizelas *et al.*, 2009).

Combining the feedstocks in certain mixture forms (as bales, chips, pellets or chopped) and at appropriate proportions can significantly lower the delivery costs (Sultana & Kumar, 2011). Due to various factors affecting supply chain, lower costs were found for mixed feedstocks delivered in the form of bales containing 70% wood chips and 30% agricultural residues.

Lignocellulosic processing plants often present a centralized nature, being a challenge to bioethanol production from single feedstock due to the inconveniences caused by biomass handling and processing in a single location. The utilization of mixed feedstocks would further complicate this issue. To solve this problem, regional biomass processing depot (RBPD) can be used to obtain, pretreat, densify and deliver feedstock to the plant (Eranki & Dale, 2011). RBDP can be used to uniform the characteristics of feedstock supplied by different sources prior to delivery at biorefinery for pretreatment, hydrolysis and fermentation.

A consistent composition can decrease the complexity of the pretreatment (avoids the variation of acid, enzyme and other chemicals used during processing), and is a key factor in for economic cellulosic fuels production (Hill *et al.*, 2006; Gomez *et al.*, 2008). The RBDP system is comparable to centralized system regarding total energy produced, but with decreased amounts of greenhouse gas emissions.

If a mixed feedstocks biorefinery is to be built, its location should be based on an equidistance from the biomass source to decrease overall transportation costs. This would not be an issue if they originate from the same plant, but a centrally located biorefinery would greatly benefit cases where different categories of feedstocks are being supplied by different sources.

Feedstock characteristics

The feedstock characteristics, such as moisture, acetyl groups and ash content, the amounts of structural carbohydrates such as cellulose, hemicellulose and lignin, and physical characteristics like bulk density and particle size and distribution, all affect how a feedstock is handled and converted. The feedstock composition has also been demonstrated to affect process configuration and reactor designs (Templeton *et al.*, 2009).

The characteristics of the products will change according to the characteristics of the feed, resulting in different optimal conditions for pretreatment, hydrolysis and fermentation; therefore, feedstocks presenting similar composition/characteristics would be preferred for combination due to the convenience of similar process parameters and equipment. In real life, however, the feedstock characteristics will likely vary, which becomes an issue when studying mixed feedstock, as different combinations present different optimal conditions for pretreatment and downstream processes (Williams *et al.*, 2016). Therefore, understanding the variations in lignocellulosic feedstock characteristics is an asset, and it can based on the effects of the environment on the feedstock composition. This relationship is useful for determining the ideal soil, climate and time used for a given biomass growing and harvesting, aiming to produce higher quality feedstock in respect to ease of transformation into bio-based fuels, chemicals and energy.

The performance of lignocellulosic biorefinery can also benefit from the understanding the effects of temporal and spatial variation in feedstock quality (composition) and quantity (productivity).

Harvesting time, soil fertility, temperature, nitrogen status and fertilization rate have all been demonstrated to alter the cell wall composition and therefore a feedstock's conversion potential. For example, it has been demonstrated that soils with high sand and low clay accumulates the most biomass.

Harvesting time can affect the cell wall composition (Arundale *et al.*, 2015; Templeton *et al.*, 2009), and these chemical characteristics affect the potential for conversion of some feedstocks (Bals *et al.*, 2010; Godin *et al.*, 2013); therefore, residues obtained from crops with consistent harvesting time would be beneficial as feedstocks.

Soil carbon and soil type can alter lignin biosynthesis and can potentially be a tool to improve the economic and environmental aspects of lignocellulosic processing, while it would be interesting to identify feedstocks that present low lignin and high growth rates and productivity.

The nitrogen fertilization also affects composition, as it was demonstrated to decreases cellulose and hemicellulose in the stem and a decrease in the proportion of cellulose in the leaf (Hodgson *et al.*, 2010). It has also been found that increasing the rate nitrogen fertilization (kg N ha^{-1}) cased increased cellulose and lignin and decreased hemicellulose and ash in the bulk material.

Intercropping (or mixed cropping) is a farming strategy that can be used to improve the feedstock characteristics for bioconversion. It involves growing, simultaneously on the same field, two (or more) types of plants strategically selected to achieve sufficient nutrient composition for microbial fermentation, decreasing the need for additional nutrient supplementation (usually obtained from fossil derived materials). For instance, intercropping of wheat and clover grass may result in extra 1000 kg per hectare compared to if the plants were grown separately (Thomsen & Haugaard-Nielsen, 2008). Besides decreasing the utilization of fertilizer (clover fixes nitrogen) and improving lignocellulosic feedstock characteristics, this strategy also increases food yield.

Technology

Several methods have been developed for plant cell wall deconstruction, such as physical, chemical and biological processes. The technology and conditions used for pretreatment will change based on the feedstock characteristics and downstream processes (hydrolysis and fermentation). Therefore, a technological challenge for cellulosic conversion is to determine the pretreatment method and parameters that will provide the cheapest "optimum" conditions. This challenge may increase for biomass mixtures, as changing from a single biomass based process to a mixed feedstock approach may require revisiting the conversion mechanisms of the technology used. Therefore, assessing how i) feedstock characteristics (sugars/lignin content, susceptibility to hydrolysis) affect the pretreatment products and downstream processes and ii) the appropriate feedstock components and mixture ratio can improve the pretreatment efficiency.

Once the technology is established for a single feedstock, improved yields of bioproducts can be obtained from the utilization of mixed feedstocks, so a common first step used for mixed biomass research is comparing the different effects of single and mixed feedstocks on the pretreatment products. It may be found that better products are obtained either when a biomass with improved characteristics is presented in higher proportions (Imamoglu & Sukan, 2014) or that intermediate mixture ratios result in improved pretreatment products (Vera *et al.*, 2015). Alternatively, mixed feedstocks may present yields similar to the single components (Ashraf *et al.*, 2017). The different results are attributed to (i) feedstock components presenting different susceptibility to hydrolysis (Imamoglu & Sukan, 2014); (ii) synergistic effects caused by different biomass characteristics, such as acetate content and buffering capacity (Vera *et al.,* 2015), or (iii) the balance between components of single and mixed feedstocks, causing little change to the products (Ashraf *et al.*, 2017). These results demonstrate that biomass mixtures are a suitable feedstocks for lignocellulosic biorefineries.

Following the analysis of best feedstock components/ratio, further improvements to the conversion technology can be obtained by adjusting the feedstock prior to delivery to the plant. In that regard, Regional Biomass Processing Depot (RBPDs) can be used to blend or aggregate different biomass types, which are formulation strategies able to decrease the technical challenges that arise from mixed feedstocks (Eranki *et al.,* 2011). Blending (or densification) is done to combine feedstocks with similar characteristics, while aggregation is done for structurally different feedstocks to level out their differences. These processes would allow the RBPDs to produce feedstocks with uniform and consistent characteristics ideal for downstream processing. Another advantage is that these methods partially pretreat the feedstock, increasing the energy density and making other logistics easier.

The most important aspect is that mixing feedstocks will not decrease the viability of the process. Once a technology is determined, studies of mixed feedstocks can be developed by analyzing the effects of the feedstocks available on the products, and choosing the feedstocks that make more sense economically to process. It should be easier to select feedstocks based on the technology available than changing a technology based on a new feedstock being processed. Similarly, altering process parameters to provide higher yields from mixed material should also be studied. To simplify the issues related to the high chemical variability in cellulosic feedstock, the effects of feedstock composition could be analyzed as a function of either the specific amount of compounds in the feedstock and the interaction between certain compounds, such as acetate content, hemicellulose and lignin.

2.1.7 Conclusion

A pretreatment step is necessary in biorefineries to decrease biomass recalcitrance and increase sugar accessibility to enzymes. The advantage of autohydrolysis pretreatment includes cheaper construction material, and safer and cleaner process, as only water is used. Lignocellulosic biomass (LCB) presents different characteristics that result in different products being obtained after pretreatment. Acetate content increases catalytic activities, while high ash content decreases the pretreatment severity. Different types of LCB can be mixed, and undesirable characteristics in one biomass may improve the processing of another feedstock. Further improvements on autohydrolysis pretreatment can be developed to take advantage of the economic technologic aspects of using multiple LCB. Mixed feedstock can present a synergistic effect due to different biomass characteristics, such as avoiding degradation, potentially decreasing costs associated with enzymatic hydrolysis and fermentation.

The challenge in the technological aspect of using mixed feedstock is based on the time-consuming preliminary work needed to find the ideal pretreatment and hydrolysis conditions that would result in a balance between the highest amount of sugars released and lowest generation of fermentation inhibitors. Compromises have to be made to overcome these challenges; therefore, the aspects of increasing cost-savings in pre-biorefinery logistics motivates more empirical studies for mixed feedstocks.

This review indicates that mixed feedstocks may i) increase certain characteristics of the autohydrolysate, ii) present improved autohydrolysate at higher fraction of best feedstock, and iii) keep the pretreatment products similar to single feed. Therefore, mixing feedstocks can be seen as a method to control and improve the pretreatment products in biorefineries, in addition to its potential to decrease feedstock handling costs. Nonetheless, the benefits of stable supply caused by multiple feedstocks has to be analyzed in a case-by-case basis to determine how their mixtures used may affect the downstream bioprocessing (enzymatic hydrolysis and fermentation).

Section 2 – Experimental data and analysis

3 Thesis overview and objectives

3.1 Layout

This thesis investigates the autohydrolysis pretreatment of various feedstocks and different methods for products valorization. The experimental sections presented here are structured in three subsections outlining the development of investigative feedstock analysis for production of various fuels and chemicals. Firstly, biomass characteristics are investigated for the effect of different feedstocks on xylo-oligosaccharides (XOS) recovery (Chapter 4). Chapter 5 demonstrates the potential utilization of MLB hydrolysate to produce biobutanol and other solvents, and Chapter 6 analyzes the total sugars recovered from single and mixed feedstocks after enzymatic hydrolysis and the potential for utilization of MLB for bioethanol production. Each of these subsections are being restructured for peer-reviewed journals.

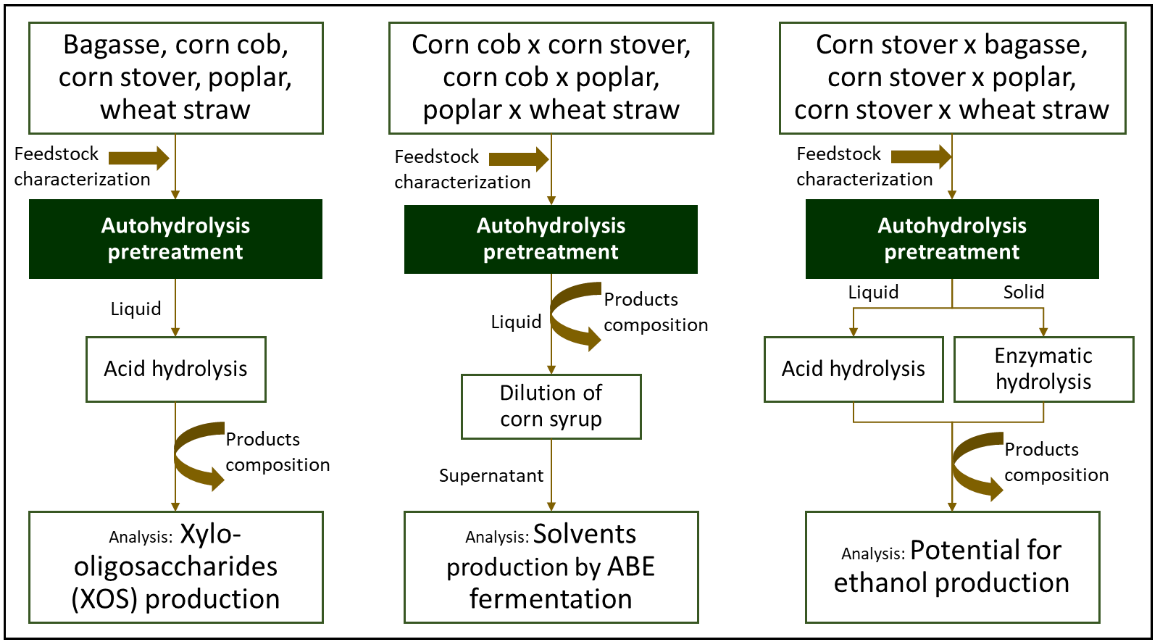

Figure 3-1 – Overview of the research, major tasks and applications

3.2 General objective

The general objective of this thesis is to explore the effects of feedstock characteristics on the products from autohydrolysis pretreatment. The biomass composition is used to study the susceptibility of different lignocellulosic feedstocks to hemicellulose solubilization, allowing the production of sugars that can be converted into high-value products. Some of the commercial products analyzed in this work are xylo-oligosaccharides (nutraceutical / prebiotic), acetone (solvent) and ethanol and butanol (solvents/biofuels). Single feedstock and mixtures were selected to increase the production of the different products studied, as explained in Section 3.3.

3.3 Specific objectives

1. Analysis of lignocellulosic biomass characteristics affecting xylo-oligosaccharides (XOS) production by autohydrolysis

The feedstocks bagasse, corn cobs, corn stover, poplar, and wheat straw were evaluated for the production of xylo-oligosaccharides. The chemical analysis of the autohydrolysate from single feedstocks was used to study the influence of different feedstocks' composition on the pretreatment products. A new index named C5HI (C5-Hydrolysis Index) will be used to explain the production of XOS production of both single and mixed feedstocks. Finally, an ideal C5HI range for XOS production will be evaluated.

2. Effects of mixed cellulosic biomass on the production of solvents by ABE fermentation

Different mixtures of corn cob, corn stover, poplar and wheat straw were selected based on their C5HI. The autohydrolysates from the different feedstock mixtures were used to dilute corn syrup and used as fermentation medium for solvents production. The utilization C5HI of single biomass as a parameter to select feedstock mixtures that can result in higher butanol production is discussed.

3. Potential for ethanol production by autohydrolysis pretreatment mixed cellulosic biomass residues

The autohydrolysate of single bagasse, corn stover, poplar, wheat straw was used for analysis of xylan recovery based on the feedstock C5HI. The enzymatic hydrolysis of the solid fraction is evaluated for monomeric and total sugars recovery. Mixtures of corn stover with each of the other feedstocks were used for analysis of monomer production and the synergistic effects of the mixed feedstocks. Moreover, the composition of the pretreated solids was used to relate how mixed feedstocks affects the xylan solubilization and consequent effects on enzymatic hydrolysis. The results for total sugar recovery and monomeric sugar production from single and mixed feedstocks were used to analyze the effects of mixed feedstocks on potential ethanol production

4 Analysis of lignocellulosic biomass characteristics affecting xylo-oligosaccharides (XOS) production by autohydrolysis

Preface

Large amounts of lignocellulosic residues are produced yearly in countries like Canada, Brazil and US. Bagasse (sugarcane residue), corn cob and stover (by-product from corn industry), wheat straw and poplar wood chips are examples of unutilized residues suitable for valorization. Section 4 explores autohydrolysis, a process where hemicellulose sugars in lignocellulosic materials are converted into xylo-oligosaccharides (XOS), a high-value co-product that can be prepared for utilization as nutraceutical (Otieno & Ahring, 2012). The effects of the different feedstock characteristics on the autohydrolysate were evaluated for XOS production. A simplified index named C5-Hydrolysis Index (C5HI) was used to reduce three feedstock characteristics into one variable that explains the products obtained. It was found that feedstocks with high C5HI, in this work being represented by poplar and corn cob, presented highest XOS production. The higher recovery is attributed to the different biomass characteristics, such as high acetate and low ash content for poplar and low Klason lignin for corn cob. It has been reported that woody biomass presents high C5 sugars recovery due to its low ash content, which decreases neutralization of the medium (Sannigrahi *et al.*, 2010) and improve their conversion.

Although autohydrolysis pretreatment of the feedstocks used in this work have already been reported, this is the first time that an attempt has been made to explain the different recovery values based on a unified value of feedstock characteristics. Analyses in the present study show that XOS yields of poplar (highest C5HI) were higher at 180°C than the values obtained for bagasse (lowest C5HI) at 200°C, demonstrating how the choice of feedstock reflects on the products yield and energy requirements. The study in Section 4 fulfilled the first objective of this thesis. It explored biomass characteristics and developed a simple variable capable of indicating the suitability of a biomass for XOS production. Besides, it provides valuable information and guidance to the following sections, where the C5HI of single biomass was used to choose different feedstock mixtures for pretreatment and their effects autohydrolysis products.

4.1 Introduction

Agricultural residues are widely available in Canada, where it has been estimated that 100.6 million dry mega grams (Mg) of major field biomass are generated per year (X. Li *et al.*, 2012). Some of agricultural biomass residues in Canada are the straw and stover of barley, wheat and corn, while forestry biomass includes roadside harvest residue, mill residues and urban wood waste (Canada, n.d.). These lignocellulosic wastes contain sugars (cellulose and hemicelluloses) and phenolic compounds (lignin) that can be processed in Lignocellulosic Feedstock Based Biorefinery (LCFBR) to produce several marketable products and energy (Moura, Carvalheiro, Esteves, & Gírio, 2008). Xylan is the main compound of hemicellulose, which can be used for production of xylo-oligosaccharides (XOS) as an emerging prebiotic (Surek & Buyukkileci, 2017) from abundant, inexpensive and renewable feedstocks.

XOS are xylan sugar oligomers composed of 2 to 10 xylose units. They present b-1-4 bonds, which are indigestible oligosaccharides for humans. Most of commercial prebiotics are inulin and fructo-oligosaccharides (FOS), while XOS have been recognized as emerging prebiotics (Otieno & Ahring, 2012), opening a new market for cellulosic transformation.

XOS can be applied in in pharmaceutical, chemical and food industries, and they have been produced from several agricultural residues such as corncob (Garrote *et al.*, 2002; Nabarlatz *et al.*, 2007), wheat and barley straw, almond shell, olive stones, rice husk (Nabarlatz *et al.*, 2007) and wheat bran (Manisseri & Gudipati, 2012).

Different approaches can be used to produce XOS from xylan-rich lignocellulosic biomass, including direct enzymatic methods, chemical only or chemical followed by enzymatic methods, and autohydrolytic only or autohydrolysis followed by enzymatic methods.

The production of XOS from processes that use steam or acid tend to produce large amounts of sugar monomers and degradation products (Aachary & Prapulla, 2011), hindering the utilization of dilute acid hydrolysis. Direct enzymatic hydrolysis is only suitable for feedstocks with low recalcitrance, such as fruit peels (Carvalheiro *et al.*, 2004; Samanta *et al.*, 2015), which limits its application to most agricultural residues.

Currently, commercial XOS are mainly produced from the xylan obtained from alkaline pretreatment and subsequent enzymatic hydrolysis. This process deacetylates the xylan; however, reports have shown that XOS with acetyl units present higher prebiotic activity as compared to neutral XOS (Aachary & Prapulla, 2011), demonstrating the benefits of acetylated XOS.

Autohydrolysis pretreatment has been mainly utilized to increase the enzymatic saccharification of the cellulose fraction (Wyman *et al.*, 2004; Yang & Wyman, 2008), although it is also seen as a promising technology to convert agricultural residues into XOS to produce useful food ingredients. Autohydrolysis uses only water, resulting in several advantages compared to acid prehydrolysis, including low by-products generation, lower issues with equipment corrosion and reduction in overall operational costs (Wyman *et al.*, 2004). Autohydrolysis is, therefore, a promising method for biomass pretreatment in biorefineries.

The XOS production by autohydrolysis involves mixing the substrate with water to temperatures between 100 and 250 ºC, which causes autoionization of water and posteriorly removes acetyl groups from the xylan structure, producing acetic acid and increasing the acidity of the medium (Aachary & Prapulla, 2011; Otieno & Ahring, 2012). The hydronium ions from both autoionization and acetic acid catalyze the xylan removal from the lignocellulosic structure and its transformation into XOS with different degrees of polymerization and xylose monomers (Aachary and Prapulla, 2011; Wyman *et al.*, 2004). This mechanism of autohydrolysis can be studied to obtain products with higher amounts of XOS or to provide higher proportions of monomeric sugars.

Hemicelluloses are considered to present two distinct fractions based on their different structure and/or accessibility, where one fraction shows higher susceptibility to hydrolysis (Carvalheiro *et al.*, 2004; Carvalheiro *et al.*, 2008; Aachary and Prapulla, 2011). Nonetheless, both xylan fractions can give oligosaccharides that can be further hydrolyzed to xylose and degraded to furfural (Garrote *et al.*, 2002), demonstrating the need to study the effect of process parameters, such as temperature and time.

The differences in the feedstock characteristics and the pretreatment parameters affect the composition and structure of the XOS obtained (Nabarlatz *et al.*, 2007). Higher pretreatment severities leads to lower molecular weight XOS and increases the formation of xylose and other monosaccharides, also favoring the production of degradation products, such as hydroxymethylfurfural (HMF) and furfural. (Aachary & Prapulla, 2011). Therefore, the feedstock composition in an important variable affecting the XOS production.

The objective of this work is to study the characteristics of four different lignocellulosic feedstocks (bagasse, corn stover, poplar and wheat straw) and their effect on XOS production and the composition of autohydrolysates. A new index is developed to study the effect of feedstock characteristics on XOS production.

4.2 Materials and Methods

Feedstock

Bagasse, corn stover, poplar and wheat straw where characterized and evaluated for xylo-oligosaccharides (XOS) production by autohydrolysis. These feedstocks were kindly donated by Greenfield Specialty Alcohols (Chatham-ON). Corn cob was locally collected and used as an extra biomass to expand the relationships found for the four main biomasses used. All feedstocks were dried in an oven at 45°C for until their water content was < 10wt.%., The biomass feedstock was ground with a Magic Bullet food processor and sieved to obtain a particles size range between 0.5 and 1mm. Samples were then kept in individual zip-lock bags at stored in a 4°C fridge.

Composition analysis of solid fractions

The moisture content was obtained by oven drying the feedstock at 105°C until a constant weight was achieved. Similarly, the total ash content was measure by heating the biomass sample to 575˚C to constant weight. The structural carbohydrates content were determined according to the protocol NREL/TP-510-42618. Briefly, after treatment with 72% H_2SO_4 (w/w), a dilute acid step 4% H2SO4 was used in an autoclave at 121°C for 1h. The acid insoluble residue (AIR), or Klason lignin, was obtained after adjusting for the acid insoluble ash (determined by igniting the AIR at 575℃ for 24h). Monomeric sugars, acetic acid and degradation products were determined by HPLC Waters (Milford, USA) containing an Aminex HPX-87H column (Bio-Rad, Richmond, USA) combined with a cation H^+-guard column (Bio-Rad, Richmond, USA). Elution took place with 5mM H_2SO_4 as mobile phase and at 50℃. Glucose, xylose, arabinose, formic acid, acetic acid, HMF and furfural were detected using a refractive index detector (RID). The experiments were run in triplicate, as shown in Appendix A. Appendix B.1 presents a typical chromatogram obtained for the feedstock composition.

Autohydrolysis pretreatment

The autohydrolysis pretreatment was conducted in reaction tubes built with 6" length thick-walled 316/316L stainless steel pipe nipple with 1.5" internal diameter. The tubes were capped on both ends, and the lid was adapted to contain a 6" length x 3/16" diameter type K thermocouple probe (McMaster-Carr - Aurora, Ohio), which was used to measure the temperature inside the reactors. Prior to the experiments, 2g of biomass was kept soaking overnight with 30mL of deionized water, resulting in a liquid-to-solid ratio (LSR) of 15. The reactors were heated in a convection oven. The temperature used during pretreatment were 180 and 200℃, with holding time of 60mins after the temperature was reached These temperature and time were chosen based on values commonly found in the literature for autohydrolysis pretreatment of lignocellulosic biomass. When the reaction time was reached, the reactors were removed from the oven. Once cooled down, the solid and liquid were separated using vacuum filtration.

Chemical Analysis

The pretreatment slurry was filtered by vacuum filtration system using a glass microfiber filter paper (VWR, USA, catalogue no. 28333-129, particle retention 1.5µm) on a Buchner funnel.

The autohydrolisate was filtered through 0.2μm membranes and directly analyzed by HPLC for quantification of monosaccharides. The amount of oligosaccharides in the autohydrolisate was quantified following NREL (National Renewable Energy Laboratory) procedure NREL/TP-510-42623, based on a dilute acid hydrolysis using 4% H_2SO_4. Appendix B.2 presents a typical chromatogram obtained for the autohydrolysate composition after dilute acid hydrolysis.

Oligosaccharides

The amount of xylo-oligosaccharides was determined from the difference between concentration of monomeric xylan and the xylan concentration after dilute acid hydrolysis of the liquor.

Data analysis

The analysis of feedstock characteristics affecting the autohydrolysis pretreatment products was conducted by pretreating single and mixed feedstocks at two levels of temperature. The calculated values of mixed feedstock composition are the sum of the mass fraction of each structural compound in the two feedstocks used. To analyze the effect of composition on XOS production, the measured values of oligosaccharides produced were compared to calculated values. Pearson correlation (r) was calculated to compare between the measured and calculated values. It is a measure of the relationship between two components vectors A and B, there N is the total number of samples, $\mu_A$and μ_B are the means for the A and B sets and σ_A and σ_B are their standard deviations.

$$\boldsymbol{r = \frac{1}{N-1} \sum_{i-1}^{N} \left(\frac{A_I - \mu_A}{\sigma_A}\right)\left(\frac{B_I - \mu_B}{\sigma_B}\right)} \qquad \textbf{Eq. 1}$$

where

N is the number of samples
$\mu_A$and μ_B are the mean for set A and B, respectively
σ_A and σ_B are the standard deviations for set A and B, respectively.

A positive correlation close to 1 indicates that certain characteristics positively affect the

XOS yields. The hypothesis, “mixing of agricultural and woody lignocellulosic residue does not affect the pretreatment yields”, was tested using p-value. The p-value was measured for the hypothesis that no Pearson correlation existed between the measured and calculated values ($r = 0$). The alternate hypothesis, $r > 0$ (a positive correlation), is evident if it presents $p\text{-value} < 0.05$ at 95% confidence interval. Additionally, the hypothesis was tested using t-test and the coefficient of determination R^2, which is the linear coefficient by minimizing the sum of least squares. All the data in this work was analyzed using RStudio (version 1.1.442).

4.3 Results and discussion

4.3.1 Feedstock composition

Knowing the composition of a given cellulosic material is essential to assess its potential for valorization. Moreover, determining biomass composition can also be important to determine feedstock prices based on quality instead of weight (Otieno & Ahring, 2012). The chemical composition of the feedstocks was used to study relevant biomass characteristics for XOS production. The results for the five feedstocks used are presented in Table 4-1.

Table 4-1 - Chemical composition of feedstocks (wt.% db.)

Compound	Bagasse	Corn cobs	Corn stover	Poplar	Wheat straw
Glucan	41.39 ± 0.14	29.49 ± 0.25	37.40 ± 0.06	47.01 ± 0.20	37.78 ± 0.10
Xylan	23.14 ± 0.06	28.80 ± 0.32	22.12 ± 0.07	19.93 ± 0.13	23.91 ± 0.08
Arabinan	1.40 ± 0.01	4.08 ± 0.09	3.05 ± 0.07	0.94 ± 0.06	2.72 ± 0.02
Total Sugars	65.94 ± 0.19	62.37 ± 0.47	62.57 ± 0.05	67.88 ± 0.27	64.41 ± 0.15
Acetate	3.52 ± 0.31	4.42 ± 0.08	3.84 ± 0.14	6.22 ± 0.21	3.81 ± 0.11
Ac. Sol. Lignin	1.41 ± 0.02	2.54 ± 0.03	1.68 ± 0.01	1.31 ± 0.01	1.56 ± 0.01
Ac. Ins. Lignin	24.37 ± 0.09	19.91 ± 0.17	18.19 ± 0.14	19.02 ± 0.17	20.08 ± 0.10
Total Lignin	25.78 ± 0.11	22.46 ± 0.20	19.87 ± 0.14	20.33 ± 0.16	21.64 ± 0.10
Ash	1.29 ± 0.09	1.50 ± 0.15	5.12 ± 0.39	0.35 ± 0.02	3.36 ± 0.09
Other	3.47 ± 0.32	9.26 ± 0.37	8.61 ± 0.05	5.22 ± 0.20	6.78 ± 0.04

“Other” refers to non-determined fractions, which includes uronic acids, extractives, and other minor compounds considered irrelevant for the purpose of this project.

In general, woody biomass contains higher amounts of cellulose (glucan), while agricultural residues present higher hemicellulose (mostly xylan) and ashes (Garrote *et* *al.*, 1999; Zhao *et al.*, 2012). The high xylan content in the feedstock is of special interest, since XOS are produced from the hydrolysis of the hemicellulose fraction.

Poplar xylan was highly acetylated, as commonly found for hardwood xylans (Surek & Buyukkileci, 2017). Corn stover and wheat straw present relatively similar chemical composition, also seen by other researchers (Lee *et al.*, 2008; Otieno & Ahring, 2012).

Different types of hemicellulose are found in wood (poplar) and agricultural residues. The main hemicellulose in poplar has been identified as glucuronoxylan, while arabinoxylans have been often identified in wheat straw and corn stover (Polizeli *et al.*, 2005; Vera *et al.*, 2015). This may explain the higher amount of glucan and lowest arabinan in poplar, but higher xylan and arabinan in the other feedstocks. The sugars glucan and xylan represented most of carbohydrates in all feedstocks, indicating that over 600kg of sugars could be obtained per tonne of dry biomass.

As xylan is the main compound in the hemicellulosic fraction, its relative abundance determines the XOS production potential of a given feedstock. As shown in Table 4-1, bagasse, corn stover and wheat straw present xylan content around 23%, while poplar presented the lowest and corn cob the highest xylan fraction (20% and 29%, respectively).

In general, it can be considered that feedstocks presenting more than 20% of xylan present great potential for XOS production (Otieno & Ahring, 2012). Low xylan content and high amounts of cellulose and lignin result in low solids solubilization (Parajó *et al.*, 2004), attributed to the low reactivity under autohydrolysis conditions.

Poplar and bagasse presented the highest glucan content (47 and 41%, respectively), whereas corn cobs presented the lowest amount of 30%. Corn stover and wheat straw present around 37% glucan. These results demonstrate an attractive possibility to use the cellulosic fraction leaving the autohydrolysis pretreatment to obtain an integrated utilization of these solid products. Parajó *et al.* (2004) showed that barley husks presented glucan content of 21.4% and was therefore less promising for processes intending this feedstock’s overall utilization.

Poplar presented the highest acetate content (6.22%), followed by corn cob (4.4%). Corn stover and straw presented similar acetate fraction (3.8%), while bagasse showed 3.5% acetate, the lowest value among the feedstocks studied. These results show that the autohydrolysis of poplar and corn cob could proceed under relatively mild conditions, as the higher amount of acetyl groups linked to the hemicellulose structure indicate higher hydronium ion sources and also an enhanced variety of XOS structures (Parajó *et al.*, 2004). From this perspective, bagasse presents the worst feature among the feedstocks used.

The total lignin content for all feedstocks ranged between 20 and 25%. Around 90% of the total lignin consists of Klason lignin (acid insoluble lignin). Bagasse presented the highest Klason lignin content (24.4%), while all the other feedstocks presented similar values between 18 and 20%. Although Klason lignin composes a substantial fraction of many cellulosic feedstocks, this fraction is seen as having minor importance for studies, as no direct food application is envisaged for it (Parajó *et al.*, 2004). In the plant cell wall, lignin acts as a glue that holds together other compounds (including xylan) in the plant structure (Q. Sun *et al.*, 2014); therefore, in this study acid insoluble lignin will also be analyzed for its effect on XOS production.

According to the above ideas, cellulosic biomass presents various characteristics that can benefit or restrict their autohydrolysis for XOS production. The next section presents an analysis of the interaction between different feedstock characteristics and their effect on XOS production by autohydrolysis.

4.3.2 Biomass characteristics affecting XOS production

The analysis of feedstock characteristics affecting XOS production was performed using RStudio. A total of 22 experiments were run, where 15 trials were randomly selected as a training set, leaving the other 7 trials to be used as test trials. Single feedstocks, as well as mixtures of two feedstocks, were used for autohydrolysis pretreatment at 200°C for 60 minutes. The most significant variables are shown in Table 4-2.

Table 4-2 – Significant variables affecting XOS production

Variables	Pr(>F)
Glucan	2.16E-05
Xylan	0.000556
Arabinan	0.000947
Acetate	0.000114
Ac.Ins.Lignin	6.95E-05
Glucan:Xylan	0.000614
Glucan:Acetate	0.005493
Glucan:Ac.Sol.Lignin	0.026431
Glucan:Ac.Ins.Lignin	0.08799
Glucan:TtlAshWt	0.089182
Xylan:Ac.Ins.Lignin	0.006356

Table 4-2 shows that glucan and acid insoluble lignin (Klason lignin) presented the highest significance values for the production of XOS by autohydrolysis, followed by glucan, acetate, xylan and arabinan. Glucan also presented significant effect with other compounds, although not as significant as temperature. The interaction between glucan and xylan present almost the same significant values as xylan and acid insoluble lignin. This relationship will be further analyzed in this work. The modelled values are presented in Figure 4-1.

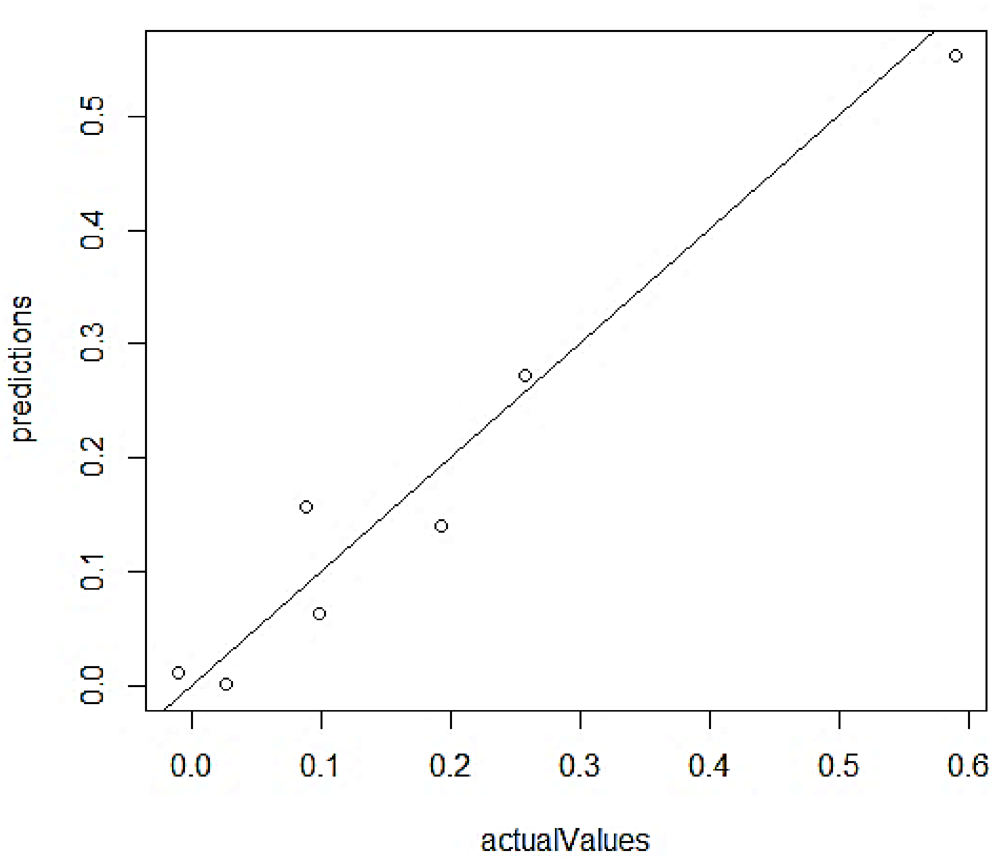

Figure 4-1 – Actual versus predicted values for XOS production based on feedstock composition

Figure 4-1 shows that the experiments presented good correlation between feedstock characteristics and XOS production. The model presents a Multiple R-squared of 0.9726543, while the Adjusted R-squared value is 0.8754249, demonstrating a good predictability of XOS production based on feedstock composition.

- **C5-Hydrolysis Index (C5HI)**

When modeling hemicellulose hydrolysis, the overall xylan-degradation process assumes the existence of two distinct xylan fraction towards hydrolytic degradation, a fast- and a slow-reacting xylan fraction, due to their different structure and/or geometry (Garrote *et al.*, 2002), resulting in different susceptibly of hemicelluloses to hydrolysis.

The concept of "susceptibility", therefore, can be related to feedstock characteristics affecting hemicellulose hydrolysis. In this work, biomass chemical compounds are related to a new indicator, named C5-Hydrolysis Index (C5HI), which relates feedstock composition with hemicellulose hydrolysis.

To analyze this relationship, the ratio of the amount of xylan per lignin in the feedstock can be used as a measure of "susceptibility" of hemicellulose hydrolysis. In this work, this measure is called Xylan Removal Index (XRI). Moreover, the acetyl fraction (HAcF) relates to acetyl groups attached to the xylan backbone, which once released during pretreatment, release hydrogen ions that increase hydrolysis. Therefore, an indication of a feedstock's ease to xylan solubilization can be obtained by increasing the effect of "susceptibility" (used as capacity to hydrolysis) to acetate content in feedstock (seen as ability to autohydrolysis). As around 90% of the total lignin in biomass is composed of acid insoluble lignin (Klason lignin), as shown in Table 4-1, being the most relevant lignin fraction in this work.

The C5HI, therefore, encompasses a relationship between xylan, Klason lignin and acetate, and is used to relate the ability of a xylan-rich cellulosic feedstock to autohydrolysis pretreatment. Eqs. 1-3 show the compounds used for calculation.

$$\boldsymbol{HAcF = \frac{\textbf{Acetate (g)}}{ODW.Feedstock\ (g)}} \qquad \textbf{Eq. 2}$$

$$\boldsymbol{XRI = \frac{Xylan\ (g)}{Ac.Ins.Lignin\ (g)}} \qquad \textbf{Eq. 3}$$

$$\boldsymbol{C5HI = XRI * \textbf{ HacF}} \qquad \textbf{Eq. 4}$$

The C5-Hydrolysis Index (C5HI) takes into consideration physical characteristics of plant cell wall. Based on the Eqs 1-3 above, a simulated C5HI value was calculated for 2g dry weight of feedstock (total feedstock weight used in the autohydrolysis experiments) and the feedstock composition in Table 4-1. The simulated C5HI values for the different feedstocks are presented in Figure 4-2.

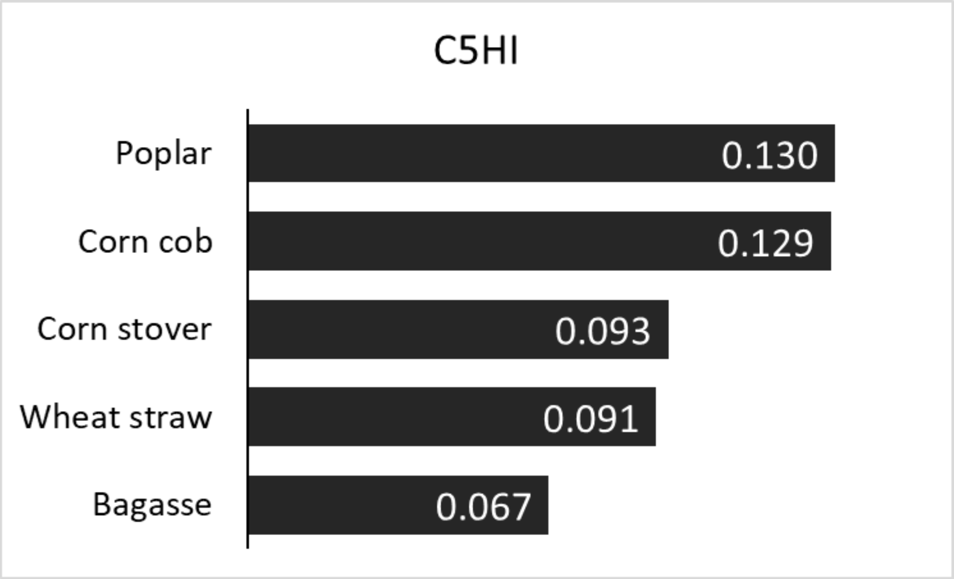

Figure 4-2 – C5HI values of the different feedstocks based on 2g (dry weight) sample.

Figure 4-2 shows that poplar and corn stover presents highest C5HI of 0.13, while bagasse presents over half this value (0.067). Corn stover and wheat straw present similar values around 0.09. The relationship between the variables is seen in Table 4-3 – Factors values used for C5HI calculation.

Table 4-3 – Factors values used for C5HI calculation

	Bagasse	Corn stover	Poplar	Wheat straw	Corncob
HAcF	0.070	0.077	0.124	0.076	0.088
XRI	0.950	1.216	1.048	1.191	1.468

Table 4-3 shows corn cob presents the highest XRI values (1.468), while poplar presents the highest acetate value (6wt.%). Both values led to the highest C5HI for corn cob and poplar. On the other hand, bagasse presents both the lowest acetate (3.5wt.%) and XRI (0.95), resulting in the lowest C5HI among the feedstocks analyzed. Corn stover and wheat straw present similar acetate (3.8wt.%) and XRI (around 1.2), leading to similar C5HI values (about 0.09), where the higher XRI of corn stover reflects in its higher C5HI compared to wheat straw.

The C5HI, being dependent on the amount of xylan and lignin was able to predict the xylan recovery of agricultural residues and poplar, a type of hardwood. On the other hand, softwood would possibly present different behaviors, as their lignin present a different composition compared to hardwood. Softwood lignins present one methoxyl group attached to the phenolic ring (guaiacyl unit), whereas both two methoxyl groups (syringyl) and guaiacyl can also be found in hardwood lignins. The higher presence of methoxyl groups attached to the aromatic ring in hardwoods leads to a less condensed structure and therefore more amenable to autohydrolysis pretreatment. The effect of the C5HI on softwood lignin needs to be further investigated.

4.3.3 XOS yields

The XOS yield is the amount of xylo-oligomers obtained in grams per 100g of the xylan fraction in the feedstock. Usually, the effectiveness of the pretreatment process is determined by the process parameters and is measured by the yields of oligosaccharides obtained, thus the higher yields are obtained, the better is the pretreatment process. Another way to look at it is to determine what feedstocks provide the best yields under determined pretreatment conditions.

Although feedstock characteristics affect XOS production, process parameters, such as temperature, also affects the yields of these sugar oligomers; therefore, the relationship between C5HI and temperature are also studied to explain the autohydrolysate composition and are analyzed for potential production of nutraceutical XOS.

Similar to total xylan recovery, C5HI also showed good correlation with the overall production of XOS. Figure 4-3 presents this relationship.

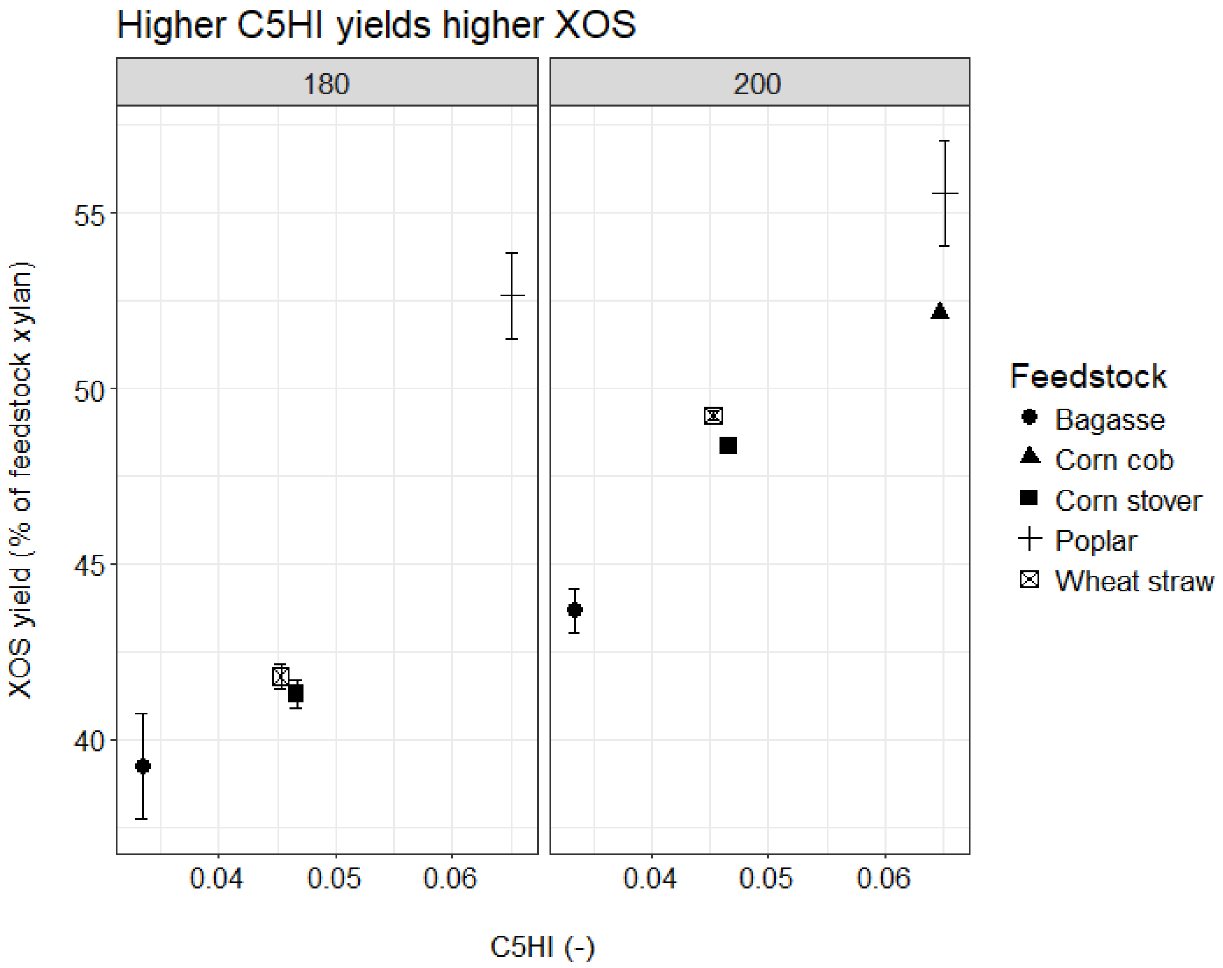

Figure 4-3 – Relationship between C5HI and XOS yields of single feedstocks.

As seen in Figure 4-3, poplar presented the highest XOS production at 200°C (56%), followed by corn cob (52%). Corn stover and wheat straw showed similar values around 48.5%, while bagasse present the lowest XOS yield (43.5%). It is worth noting that the XOS recovery for poplar was higher at 180°C than the values at 200 °C for bagasse, corn stover and wheat straw. This analysis demonstrates how the choice of feedstock greatly affects the process conditions being used and the energy requirements needed for the process. An analysis of Figure 4-3 indicates that using poplar as feedstock for XOS production would require lower temperature and result in higher XOS production than using bagasse, corn stover or wheat straw at a higher temperature.

The XOS yields obtained in this work were similar to yield values (% of feedstock xylan) reported in literature, where the autohydrolysis of corncobs resulted in higher XOS production (59.4%) than wheat straw (41.2%) (Nabarlatz *et al.*, 2007). Other feedstocks present different XOS recovery values, such as almond shells (61.2%), olive stones (53.5%), barley straw (47.1%), rice husk (42.8%) and brewer’s spent grain (61%) (Nabarlatz *et al.*, 2007; Carvalheiro *et al.*, 2004).

The analysis of the pretreatment effects on the feedstock is usually performed in function of process parameters, such as temperature and time (Nitsos, Choli-Papadopoulou, Matis, & Triantafyllidis, 2016). These parameters can be summarized in function of the Severity Factor (log Ro) (Overend *et al.*, 1987), as shown in Eq. 4

$$\boldsymbol{log\ Ro} = \mathbf{log}\ \left(\mathbf{t} * \mathbf{exp}\left(\frac{\boldsymbol{T}-\mathbf{100}}{\mathbf{14.75}}\right)\right) \qquad \textbf{Eq. 4}$$

Where T corresponds to the temperature (˚C) and t is the pretreatment time (min).

Experiments run for 60 minutes at 180 and 200˚C present severity 4.13 and 4.72, respectively. Figure 4-3 shows that the relationship found for C5HI, where higher values result in higher xylan hydrolysis, was valid for both severities used. As mentioned, the Severity Factor is used to study the effects of changing the process parameters on the pretreatment products of a given feedstock. On the other hand, the C5HI developed in this work was used as a new strategy to study the effects of feedstock characteristics on the pretreatment products.

Based on the relationship between the C5HI and XOS yields for single feedstocks shown in Figure 4-3, different biomass mixtures were used for analysis of the effect of single and mixed feedstocks, as shown in Figure 4-4.

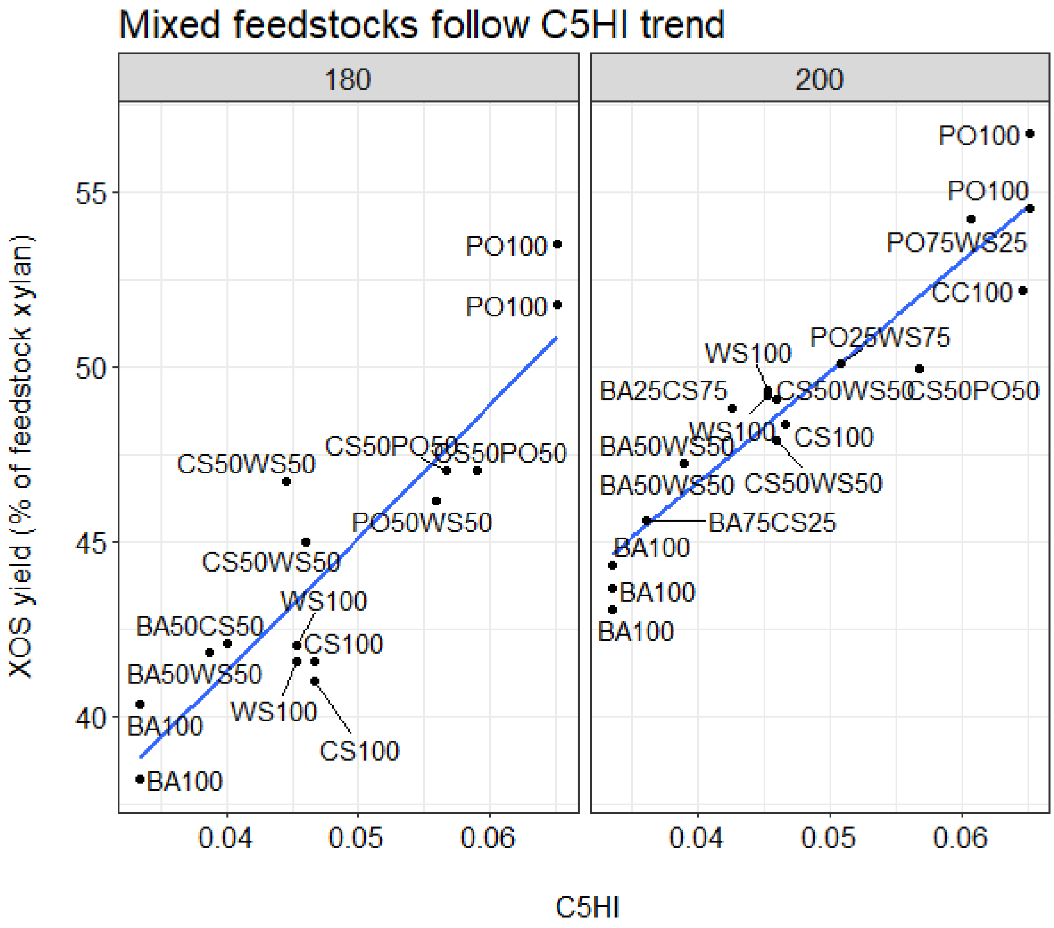

Figure 4-4 – Relationship between C5HI and XOS yields of both single and mixed feedstocks at different temperatures. At 95% confidence level, there is significant difference (p-value = 3.19x10^{-3}) between the two means.

Figure 4-4 shows that the mixed feedstocks follows similar trend compared to their single feedstocks. A Welch two sample t-test was performed for the XOS yield by temperature, resulted in a t-value = 3.7 and. The mean at temperature 180°C was 44.38, while at 200°C it was 48.95. The 95% confidence interval is -7.470063 and -1.668214.

Due to the chemical composition of the mixed feedstocks to be estimated as an interpolation of their single materials, their C5HI values represent the change caused in feedstock composition, therefore resulting in different pretreatment products. Figure 4-5 presents relationship between the real and regression values of XOS recovery.

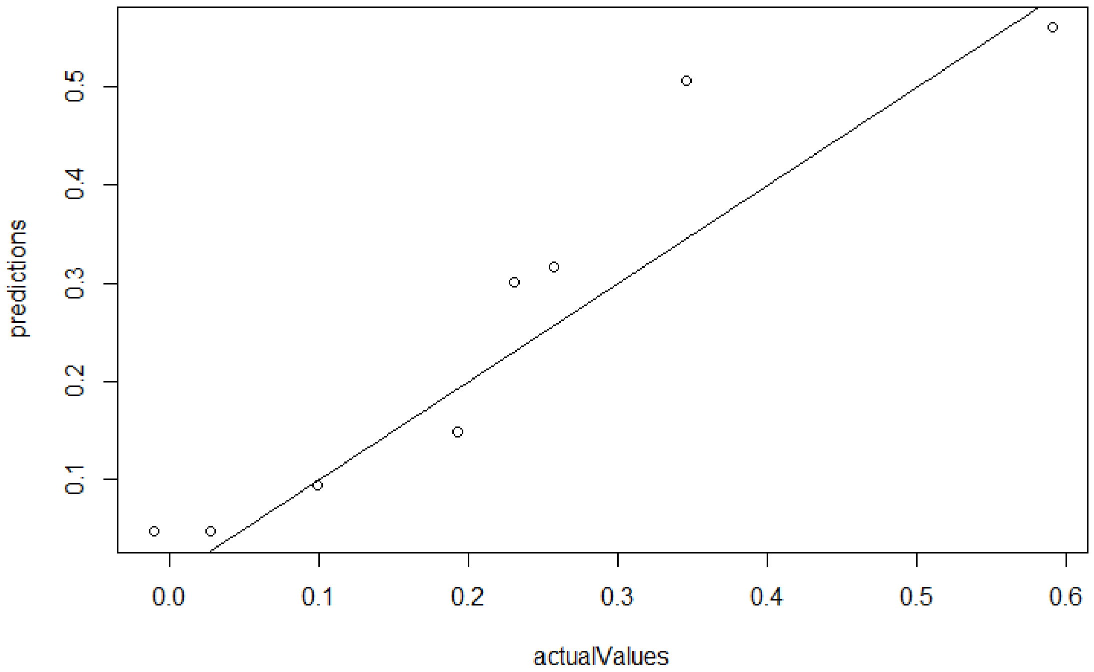

Figure 4-5 - Actual versus predicted values for XOS production based on C5HI.

Figure 4-5 presents the relationship between predicted and actual XOS production values based on the C5HI. The p-values for C5HI was 2.6 x 10^{-9}, demonstrating its significance to explain the XOS results obtained.

The results presented so far demonstrate that feedstock characteristics affect the autohydrolysis pretreatment products, with some compounds presenting higher effect than others. Moreover, it has been demonstrated that the relationship between feedstock characteristics can be used to simplify the analysis of the products obtained. However, besides the total amount of XOS produced, the efficiency of the pretreatment product is also indicated by the ratio of oligosaccharides to monosaccharides, which is important to assess the quality of the pretreatment liquor (Otieno & Ahring, 2012). This relationship is shown in Figure 4-6.

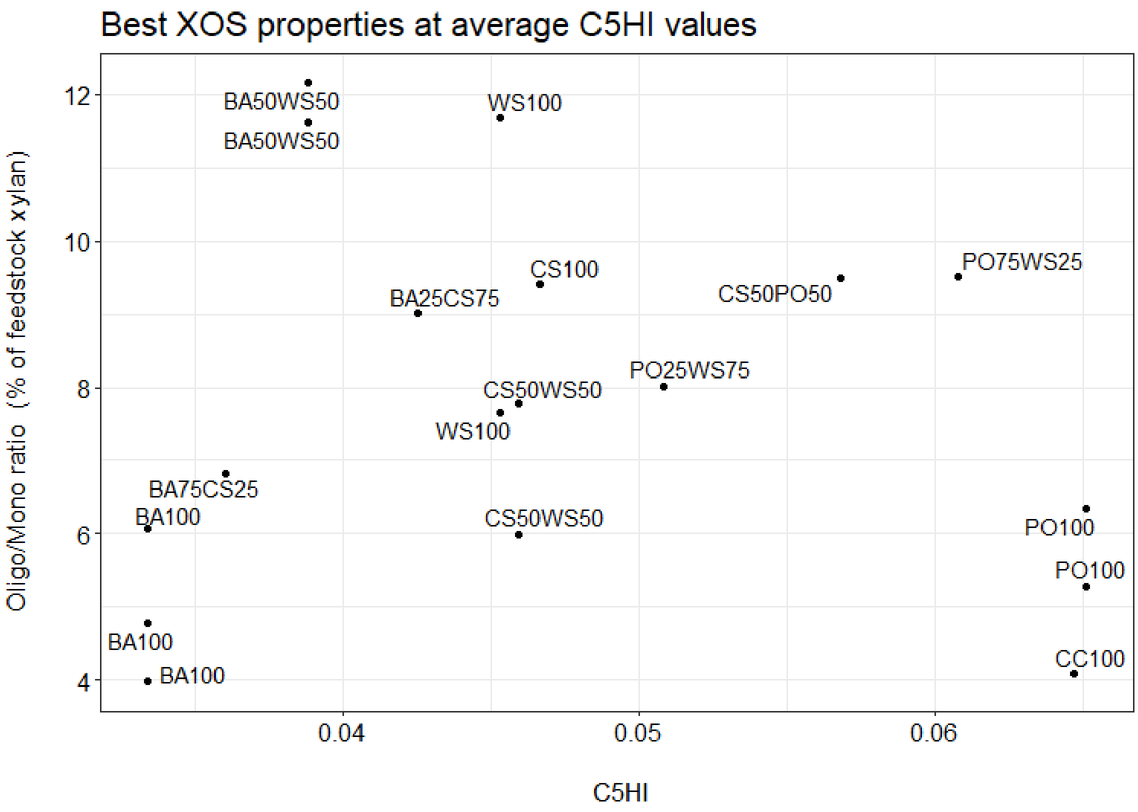

Figure 4-6 – Relationship between C5HI values and the ratio of XOS/monomeric xylose.

An interesting relationship is observed in Figure 4-6, where single feedstocks located at the extremities of the C5HI range studied present the lowest XOS/mono ratio. However, single feedstocks at average C5HI values and their mixtures all present higher XOS/mono ratios. In the case of bagasse, at the lower end of C5HI values studied, the low XOS/mono ratio can be due to its poor xylan hydrolysis. On the other hand, the higher C5HI for corn cob and poplar results in high xylan solubilization and leads to higher monomeric sugars. Therefore, an optimum region of XOS/mono is obtained at medium C5HI values, where a balance between xylan hydrolysis and monomers production is obtained. Corn stover and wheat straw, therefore, presented the highest XOS/mono among the single feedstocks. And all their mixtures with a proportion of 50% and higher also improved the XOS/mono ratio.

It can also be noted that the ratio obtained for the 50% bagasse and wheat straw mixture increased the XOS/mono ratio, achieving approximately twice the value found for single bagasse. This may be caused by the synergistic effect of mixed biomass (Vera *et al.*, 2015). Other feedstocks, such as mixtures of bagasse and corn stover, present a more linear relationship when compared to single feedstocks. Mixing corn stover and wheat straw did not seem improve the XOS/mono ratio, while improvements are observed when any of these two feedstocks are mixed with poplar.

The observations noted above confirm that the characteristics of the feedstocks affect the autohydrolysis products. While some mixtures can increase the production of a desirable compound due to a synergistic effect, other mixtures present a linear products composition when related to their single components, while other mixtures can also decrease the production of a determined product.

It is concluded, therefore, that the autohydrolysis of hemicelluloses at average C5HI values predominantly produces XOS. Low xylan hydrolysis is observed at low C5HI, therefore presenting low XOS production. Nonetheless, high C5HI presents high XOS production; however, these oligomers are also more randomly hydrolyzed, producing sugars monomers from other XOS, which posteriorly generates sugars degradation products.

4.3.4 Degradation products

Higher temperatures leads to higher hydrolysis of hemicelluloses, positively affecting XOS production (Figure 4-3). Nonetheless, the higher severity also results in autohydrolisate with higher monomers and consequently higher sugars degradation, as seen in Figure 4-7.

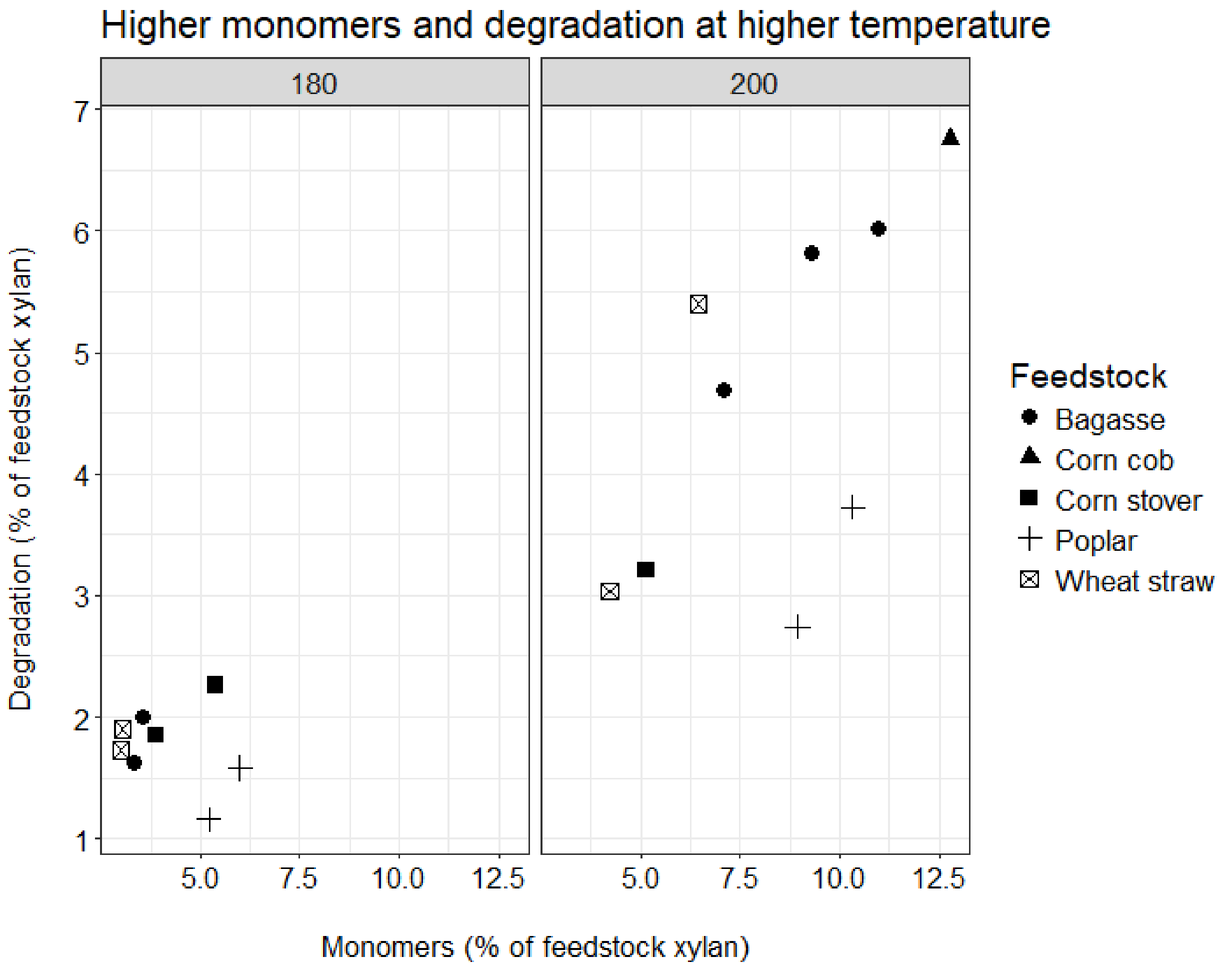

Figure 4-7 - Relationship between monomers and degradation at different temperatures.

Figure 4-7 shows an increase in monomeric xylan with temperature, and consequently higher degradation products. Further increase in temperature (increased severity) leads to a decrease in the degree of polymerization, resulting in increased decomposition of xylo-oligosaccharides into xylose, and consequently increase the production of sugars degradation products. Over temperatures above 180˚C, the XOS production decreases while the production of degradation products (HMF, furfural) increase (Samala *et al.*, 2012). For the production of XOS, it would be desired to operate at lower temperature to avoid monomers production and its degradation.

After pretreatment, a common processing used is enzymatic hydrolysis to transform high molecular weight oligomers into XOS with DP 2-6; therefore, an analysis of how “clean” the autohydrolisate is can be obtained by relating the amount of xylan in feedstock that was recovered as XOS with the amount degraded (based on the furfural concentration in the autohydrolisate). Figure 4-8 shows this relationship.

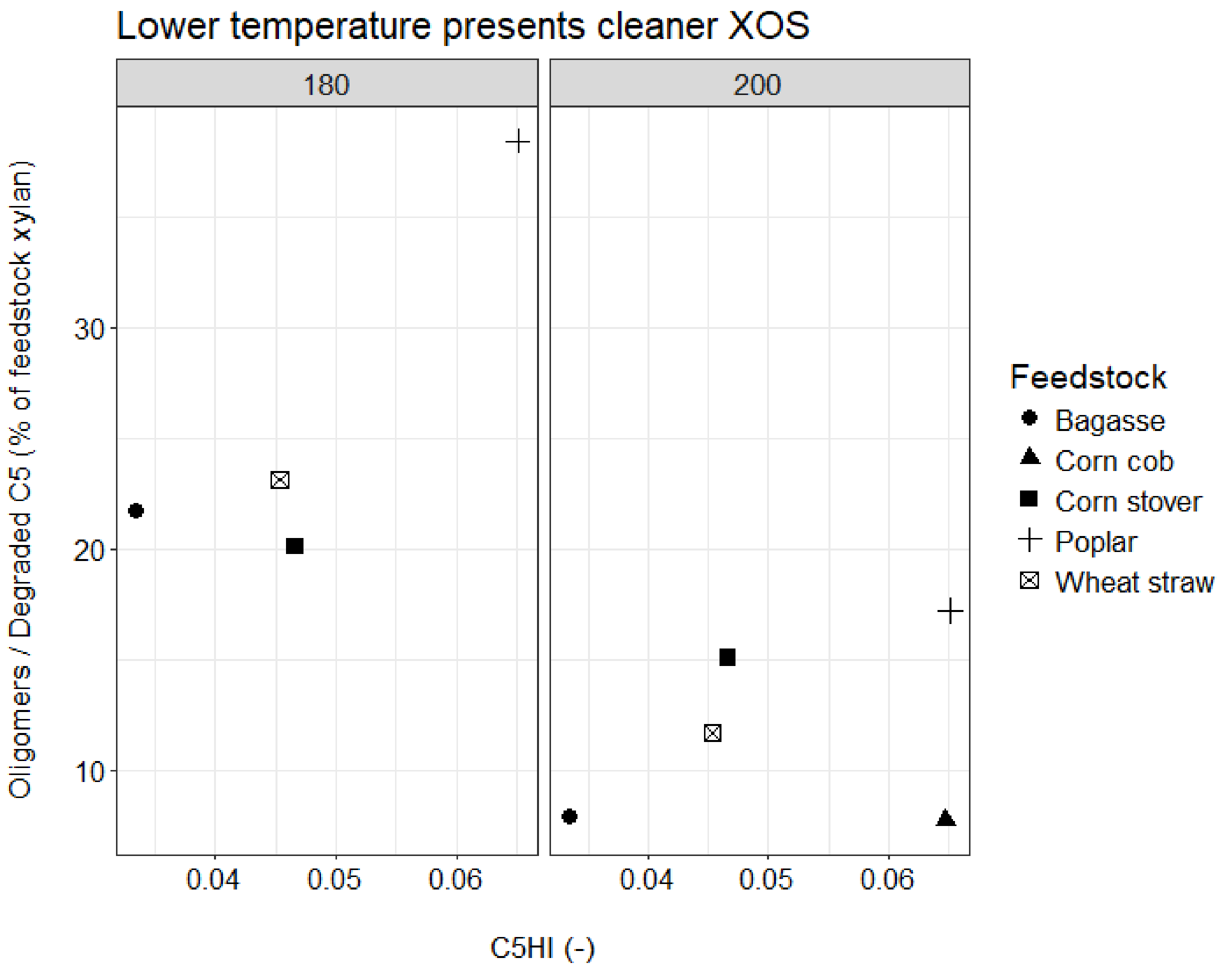

Figure 4-8 - Effect of C5HI on the ratio of XOS to degraded sugars at different temperatures.

Figure 4-8 shows that the average XOS/Furfural is higher at lower temperature, meaning that a cleaner hydrolysate was obtained at 180°C due to higher degradation taking place at 200°C (Figure 4-7). No clear relationship was noticed for C5HI; which is expected, considering that it would be ambitions to obtain a measure of autohydrolisate “cleanness” just based on a relationship between xylan, Klason lignin and acetate in the feedstock.

However, the relationship between higher oligomers at higher C5HI is still observed as a general trend.

Degradation products are known to affect enzymatic hydrolysis; therefore, avoiding degradation is essential for downstream processing of autohydrolysates. The amount of bound acetate also affects enzymatic hydrolysis, as described in the next section.

4.3.5 Acetate

During autohydrolysis, the random hydrolysis of hemicelluloses releases oligomers that contain attached acetyl groups; moreover, some ester bonds that bind acetyl groups to the xylan backbone are also hydrolyzed, increasing the acetic acid concentration in the autohydrolysate. Figure 4-9 shows the dependence of bound acetyl groups to oligomers on temperature.

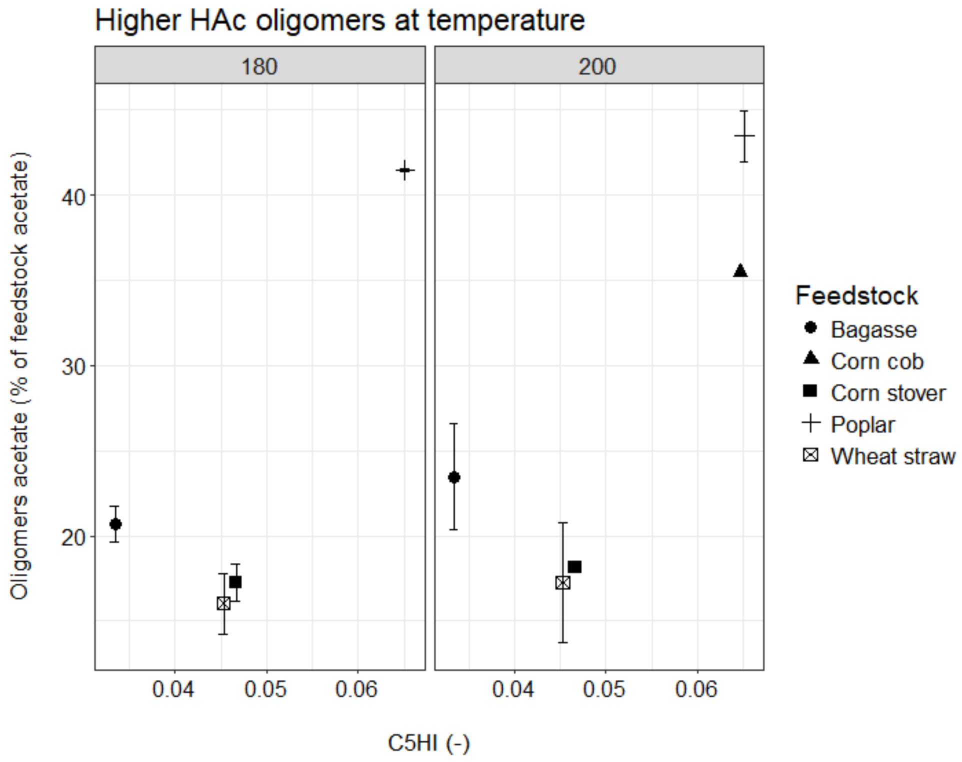

Figure 4-9 - Relationship between C5HI and acetate bound to XOS at different temperatures.

Figure 4-9 presents higher temperature causing increased amount of bound acetyl groups to oligomers. This is due to the higher XOS production, which results in higher release of acetylated oligomers to the reaction media. Poplar presented the maximum amount of acetyl groups linked to oligomers with almost 45% of the initial acetyl groups at 200°C. The higher xylan-bound acetate for bagasse compared to compared with corn stover and wheat straw may indicate that although bagasse presents the lowest acetate content among the feedstock used (7%) but only slight lower than corn stover and wheat straw (roughly 8%), it is possible that it presents higher degree of deacetylation.

Research on biology activities of XOS show that microorganisms metabolism (shown by in vitro fermentation) of acetylated XOS was considerably slower than of neutral XOS, in which more organic acids were produced, resulting in increased prebiotic effect (Pastell *et al.,* 2009). The results presented in Figure 4-9 indicates that a much of the XOS in poplar was substituted with acetyl groups, being a positive result for its prospective utilization. As C5HI takes into account the acetate content in the feedstock, a higher C5HI may also indicate higher degree of acetate groups bound to XOS, as shown for corn cob at 200°C.

4.3.6 Minor sugars

The arabinan in the feedstock can also be an indication of the degree of branching in the feedstock, resulting in increased solubility of the polymers (Wedig *et al.*, 1987).To analyze this relationship, a Branching Index (BrInd) was used relating the ratio of xylan and arabinan in the feedstock, according to Eq. 5, and presented in Figure 4-10.

$$BrIndx = \frac{Xylan\ in\ feed\ (g)}{Arabinan\ in\ feed\ (g)} * Acetate\ in\ feed\ (wt.\%) \qquad \textbf{Eq. 5}$$

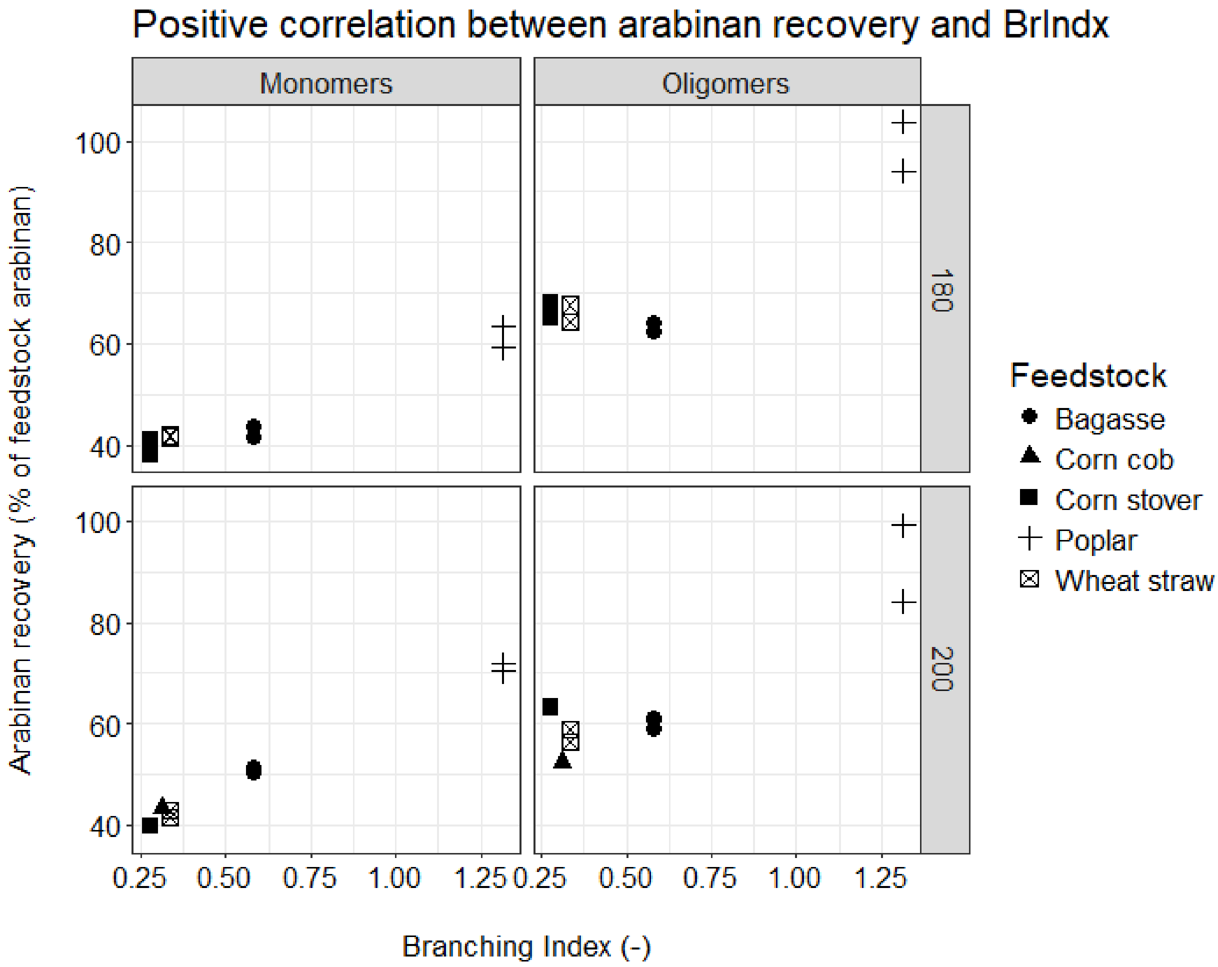

Figure 4-10 Relationship between BrIndx and arabinan recovery in both mono- and oligomeric forms at different temperatures

Figure 4-10 shows high correlation between the Branching Index (BrIndx) and the arabinan recovery. Higher BrIndx means higher xylan/arabinan ratio and increasing solubility when the feedstock presents higher acetate content. It shows that higher amount of arabinan monomers are released at higher temperature, although all arabinan in poplar was released when the combined effect of oligomers is considered. Monomeric xylose and arabinose are susceptible to hydrothermal degradation to form inhibitor by-product compounds such as furfural (Parajó *et al.*, 2004). At 200°C, the average arabinan released from poplar is reduced, possibly due to degradation.

As seen in Figure 4-7, the xylan monomers was lower than 7% at 180°C for all tested feedstocks; however, a minimum of 40% of the feedstock arabinan was recovered at the same temperature, demonstrating that arabinan was more easily cleaved from xylan backbone. Similar results were found by Xiao *et al.* (2015).

The glucan monomers recovered did not increase considerably at higher temperature (data not shown), indicating that once the amorphous cellulose is hydrolyzed, there isn't a difference on the temperatures studied.

4.4 Conclusions

This work demonstrate that autohydrolysis as a promising pretreatment method for production of XOS from lignocellulosic biomass. Biomass structural compounds can be used for evaluating their potential XOS production, as they greatly affect the products after the pretreatment. C5HI was an index used to analyze the susceptibility of different feedstocks to hydrolysis. Out of the five biomasses use in this work, poplar appeared as the best candidate for XOS production. As temperature increases, the acetic acid concentration also increases, causing higher solubilization and higher degradation of hemicellulose sugars. Although higher temperature increases XOS production, there is also increased presence of sugars monomers degradation products. Additionally, the acetic acid concentration also increases, causing higher solubilization and higher degradation of hemicellulose sugars.

5 Effects of various lignocellulosic biomass mixtures on autohydrolysate composition and bioconversion

Preface

This section focuses on conversion of three different biomass mixtures using corn cob, corn stover, poplar and wheat straw. All these feedstocks are lignocellulosic residues with potential for production of fuels and chemicals. US, China and Brazil are the three main corn producers in the world (RFA, 2017), and there has been increased interest in "industrial corn use", which includes the production of high-fructose corn syrup (HFCS – a sweetener used as ingredient for food and beverage manufacturing), ethanol (alcohol that can be used in beverages or converted into biofuels), or other valuable products (Dicostanzo, 2003). Due to its high carbohydrates content (mostly fructose and glucose), HFCS could be considered as a potential carbon source for solvents production by ABE fermentation. The high sugars content; however, inhibits fermenting microorganisms, resulting in the need for dilution of syrup prior to its utilization for fermentation. Water utilization presents issues in biorefineries; therefore, alternative methods can be developed.

In this section, the dilution of corn syrup with autohydrolysate obtained from mixed lignocellulosic feedstocks is studied as a potential supernatant for solvents production by ABE fermentation. As corn syrup is a product a corn refinery, a mixture of corn cob and corn stover was studied. Corn cob was also mixed with poplar wood to study the effects of different feedstock types. Finally, poplar mixed with wheat straw was used to further investigate the effects of feedstock characteristics. This section presents two objectives: i) to study the effects of hydrolysate produced from different mixed feedstocks on bioconversion, and ii) to assess the utilization of corn syrup as a carbon source for solvents production. No prior research has been made on the utilization of autohydrolysate from mixed lignocellulosic biomass to dilute corn syrup for ABE fermentation.

In this study, the same autohydrolysis pretreatment conditions were employed as described in Section 4 to solubilize hemicelluloses. This Section fulfills Objective 2 in section 3. Results obtained from this study can be summarized as three major contributions. First, the ability to use C5HI to obtain insights into the pretreatment products of a given feedstock mixture; second, determine beforehand what will likely be the main fermentation product based on the mixed feedstock used for pretreatment, and third, an overall new perspective is provided on the potential utilization of multiple feedstocks in biorefineries and its effects on the diversification of bioproducts portfolio.

5.1 Introduction

Butanol is a colorless and flammable alcohol produced almost entirely by the petrochemical industry. It has been historically used in various industries as a solvent and chemical. Butanol presents characteristics superior to ethanol, and it is currently recognized as a biofuel that can supplement transportation fuels, such as gasoline and diesel, and kerosene (Bharathiraja *et al.*, 2017).

Industrial production of biobutanol is performed using Clostridium species, which is a microbial species able to biosynthesize multiproduct; furthermore, it presents exceptional appetite for both cellulosic and hemicellulosic polysaccharides, being able to metabolize hexose sugars (glucose) and pentoses (xylose and arabinose). ABE fermentation is usually performed under batch conditions by *Clostridia* species, which presents a metabolism of solvent production that follows a biphasic process of acidogenesis and solventogenesis, as shown in Figure 5-1.

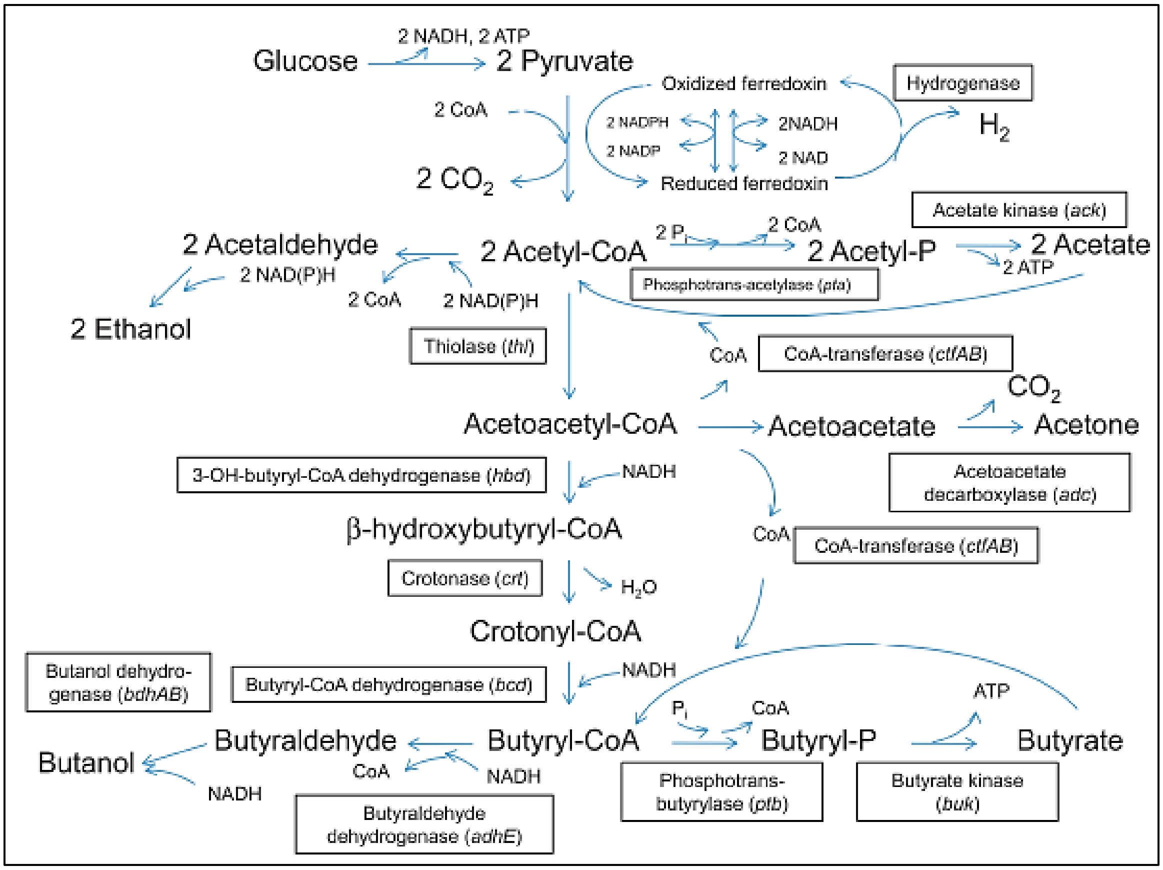

Figure 5-1 – Acidogenic and solventogenic metabolic pathways in clostridia (García *et al.*, 2011)

ABE fermentation must be strictly anaerobic to producing cells, so two of the main challenges in this technology are to manipulate cultures and strains for products specificity improvements and yields and to reduce the toxicity of butanol and O2 (García *et al.*, 2011). It is worth to mention that anaerobic microbes generally produce less energy (< 30 moles of ATP/ mole of glucose) compared to aerobic microbes (30~ 38 moles of ATP/mole glucose). To detoxify toxic compound in the hydrolysate, additional reducing equivalent (e.g. NADH, etc.) or ATP are needed (Su *et al.*, 2018). Several works have been published for the mechanism of detoxification process by microorganism (Adeboye *et al.*, 2014; Almeida *et al.*, 2011; Jönsson *et al.*, 2013). Thus, it is important to produce less toxic and readily to be consumed substrate for biofuel production.

Starch is a fermentable crop product, although it is not seen as economically viable

(Bharathiraja *et al.*, 2017). Agricultural and food residues can also be used as a carbon source that does not compete with food, being presented as a cheap (cost-effective) and easily available feedstock for economic butanol production (Bharathiraja *et al.*, 2017), therefore presenting great potential as feedstocks.

Agricultural residues such as corn stover, what straw and wood have been used as feedstock for the production of ethanol (Demirbaş, 2004) and butanol (Zhang *et al.*, 2014). However, due to their recalcitrant nature, these cellulosic feedstocks have to undergo pretreatment to release sugars for fermentation.

Autohydrolysis is an effective method to solubilize sugars from lignocellulosic material, being recognized as an environmentally friendly and economical technology (Liu *et al.*, 2015). Autohydrolysis pretreatment uses water at elevated temperatures to solubilize hemicelluloses, producing sugars oligomers and monomers. A solid fraction containing mostly cellulose and lignin is also obtained. (Garrote *et al.*, 1999). During autohydrolysis, sugars degradation also take place, generating HMF and furfural, which are known inhibitory compounds; however, it has been demonstrated that the ABE fermentation presents high tolerance to the major sugar degradation products produced during autohydrolysis (García *et al.*, 2011); nonetheless, avoiding the presence of these inhibitors should still be a crucial aspect of pretreatment studies.

The optimization of upstream processing may decrease the concentration of inhibitors in the autohydrolysates for ABE fermentation which can be obtained by mixing different feedstocks, as an undesirable characteristic of a biomass may improve the hydrolysis of another feedstock (Vera *et al.*, 2015), which shows that the utilization of a feedstock with low conversion can improve the pretreatment products if it is mixed with another biomass type.

The objective of this study is therefore to compare three different mixtures of four types of biomass (corn cob and corn stover, corn cob and poplar, and poplar and wheat straw) for autohydrolysis, and their effects on ABE fermentation when using them as diluents to corn syrup for ABE fermentation. All feedstock mixtures were pretreated with autohydrolysis and the composition of feedstocks, autohydrolysate and fermentation products were provided. The autohydrolysates were mixed with HFCS and directly subjected to ABE fermentation by *Clostridium saccharobutylicum* DSM 13864.

5.2 Materials and methods

- Pretreatment

Lignocellulosic feedstock

Corn cob, corn stover, poplar and wheat straw and mixtures were characterized and pretreated for ABE fermentation. These feedstocks were kindly donated by Greenfield Specialty Alcohols (Chatham-ON). All feedstocks were oven-dried at 45˚C until their water content was < 10wt.%., The biomass feedstock was ground with a Magic Bullet food processor and sieved to obtain a particles size range between 0.5 and 1mm. Samples were then kept in individual zip-lock bags at stored in a 4°C fridge.

Composition analysis of solid fractions

The moisture content was obtained by oven drying the feedstock 105℃ until a constant weight was achieved. Similarly, the total ash content was measure by heating the biomass sample to 575˚C to constant weight. The structural carbohydrates content were determined according to the protocol NREL/TP-510-42618. Briefly, after treatment with 72% H_2SO_4 (w/w), a dilute acid step 4% H2SO4 was used in an autoclave at 121°C for 1h. The acid insoluble residue (AIR), or Klason lignin, was obtained after adjusting for the acid insoluble ash (determined by igniting the AIR at 575℃ for 24h). Monomeric sugars, acetic acid and degradation products were determined by HPLC Waters (Milford, USA) containing an Aminex HPX-87H column (Bio-Rad, Richmond, USA) combined with a cation H^+-guard column (Bio-Rad, Richmond, USA). Elution took place with 5mM H_2SO_4 as mobile phase and at 50℃. Glucose, xylose, arabinose, formic acid, acetic acid, HMF and furfural were detected with a refractive index detector (RID). The experiments were run in triplicate, as shown in Appendix A. Appendix B.1 presents a typical chromatogram obtained for the feedstock composition.

Autohydrolysis pretreatment

The autohydrolysis pretreatment was conducted in reaction tubes built with 6" length thick-walled 316/316L stainless steel pipe nipple with 1.5" internal diameter. The tubes were capped on both ends, and the lid was adapted to contain a 6" length x 3/16" diameter type K thermocouple probe (McMaster-Carr - Aurora, Ohio), which was used to measure the temperature inside the reactors. Prior to the experiments, 2g of biomass was kept soaking overnight with 50mL of deionized water, resulting in a liquid-to-solid ratio (LSR) of 25. The reactors were heated in a convection oven. The temperature used during pretreatment was 200℃, with holding time of 60mins after the temperature was reached. These temperature and time were chosen based on values commonly found in the literature for autohydrolysis pretreatment of lignocellulosic biomass. When the reaction time was reached, the reactors were removed from the oven. Once cooled down, the solid and liquid were separated using vacuum filtration.

Chemical Analysis

The hydrolysate was filtered by vacuum filtration system using a glass microfiber filter paper (VWR, USA, catalogue no. 28333-129, particle retention 1.5µm) on a Buchner funnel. The liquors were filtered through 0.2µm membranes and directly analyzed by HPLC for determination of monosaccharides and degradation products following NREL (National Renewable Energy Laboratory) procedure NREL/TP-510-42623.

The HPLC system (Waters, Milford, USA) was equipped with an Aminex HPX-87H column (Bio-Rad, Richmond, USA) in combination with a cation H^+-guard column (Bio-Rad, Richmond, USA) and elution took place at 50℃ with 5mM H_2SO_4 as mobile phase. Glucose, xylose, arabinose, acetic acid, formic acid, HMF and furfural were detected with a refractive index detector (RID).

- Fermentation

Syrup

Corn syrup used in this study was kindly provided by Ingredion Incorporated (London, Ontario, Canada). The main sugars present in this product were glucose (43.46 %w/w), maltose (17.69 %w/w) and fructose (11.33 %w/w). Corn syrup was stored at room temperature in a plastic container and used as received.

Microorganism and media

The *C. saccharobutylicum* DSM 13864 strain used in this study was purchased from DSMZ, Braunschweig, Germany. The following reinforced *Clostridium* media (RCM) was used for all seed cultures: peptone, 10.0 g/L; beef extract, 10.0 g/L; yeast extract, 3.0 g/L; glucose, 5 g/L; NaCl, 5 g/L; soluble starch, 1.0 g/L; sodium acetate, 3 g/L; pH adjusted to 6.8. The seed culture was incubated for 12 h at 37 °C in order to generate an actively growing seed culture. Exponentially growing cells were transferred to production media at 10% (v/v). Production media was prepared mixing corn syrup and supernatant from autohydrolysis pretreatment to obtain a final volume percentage of 5 and 95 for corn syrup and supernatant, respectively. To this media, other chemicals were added: KH_2PO_4, 0.5 g/L; K_2HPO_4, 0.5 g/L; $(NH_4)_2SO_4$, 5 g/L, $MgSO_4•7H_2O$, 0.2 g/L; $CaCl_2•2H_2O$, 0.02 g/L; $FeSO_4$, 0.05 g/L; yeast extract, 1g/L; $CaCO_3$, 2 g/L. pH was adjusted to 6.8 and nitrogen was bubbled (while heating) to drive off oxygen. Then, media was autoclaved for 20 min at 121 °C. After autoclave, media was transferred into an anaerobic chamber (Model 855-ACB, Plas-Labs, Inc., Lansing, MI) and 2 mL of trace element solution (SL7) were added through a 0.2-μm membrane filter. Trace element solution (SL7) was used as followed: $FeCl_2•4H_2O$, 1.5 g/L, dissolved in 10 ml HCl solution (25% solution); $CoCl_2•6H_2O$, 0.19 g/L; $MnCl_2•4H_2O$, 0.1 g/L; $ZnCl_2$, 0.07 g/L; H_3BO_3, 0.062 g/L; $Na_2MoO_4•2H_2O$, 0.036 g/L; $NiCl_2•6H_2O$, 0.024 g; $CuCl_2•2H_2O$, 0.017 g/L.

ABE fermentation

ABE fermentations of corn syrup diluted with supernatant from autohydrolysis pretreatment were carried out in 125 mL shaker flasks containing 50 mL of fermentation medium. All experiments were performed in duplicate under anaerobic conditions. Five milliliters of actively growing cells were inoculated into 45 mL of fermentation medium and kept in an anaerobic chamber at 200 rpm and 37 °C for 120 h. Samples were removed intermittently, centrifuged, filtered using 0.2-μm grade filters, and stored at −20 °C for analysis of sugars and ABE conversion efficiency.

Analytical methods

Concentration of sugars and fermentation products (acids and solvents) was determined via high performance liquid chromatography (HPLC) on an Agilent 1260 infinity (Agilent USA, Santa Clara) using an Agilent Hi-Plex H (7.7×300 mm) column and Cation H+ guard column (Agilent USA, Santa Clara). A refractive index detector (RID) was used for sugars and metabolites detection. H_2SO_4 5 mM was used as the isocratic mobile phase at a constant flow rate of 0.6 mL/min. Before injection, samples were diluted to the appropriate concentration with mobile phase and filtered through a 0.2-μm membrane filter. The analytes were quantified using pure chemicals as standards.

Data analysis

All the data in this work was analyzed using RStudio (version 1.1.442).

5.3 Results and discussion

5.3.1 Composition of single and mixed feedstocks

Table 5-1 presents the chemical composition of the feedstocks used.

Table 5-1 – Composition analysis of single feedstocks (wt.% db.)

Compound	Corn cobs	Corn stover	Poplar	Wheat straw
Glucan	29.49 ± 0.25	37.40 ± 0.06	47.01 ± 0.20	37.78 ± 0.10
Xylan	28.80 ± 0.32	22.12 ± 0.07	19.93 ± 0.13	23.91 ± 0.08
Arabinan	4.08 ± 0.09	3.05 ± 0.07	0.94 ± 0.06	2.72 ± 0.02
Total Sugars	62.37 ± 0.47	62.57 ± 0.05	67.88 ± 0.27	64.41 ± 0.15
Acetate	4.42 ± 0.08	3.84 ± 0.14	6.22 ± 0.21	3.81 ± 0.11
Ac. Sol. Lignin	2.54 ± 0.03	1.68 ± 0.01	1.31 ± 0.01	1.56 ± 0.01
Ac. Ins. Lignin	19.91 ± 0.17	18.19 ± 0.14	19.02 ± 0.17	20.08 ± 0.10
Total Lignin	22.46 ± 0.20	19.87 ± 0.14	20.33 ± 0.16	21.64 ± 0.10
Ash	1.50 ± 0.15	5.12 ± 0.39	0.35 ± 0.02	3.36 ± 0.09
* Other	9.26 ± 0.37	8.61 ± 0.05	5.22 ± 0.20	6.78 ± 0.04

* Other nondetermined fractions include uronic acids, extractives, and other fractions with minor importance for the purposes of this work.

In general, woody biomass contains higher amounts of cellulose (glucan), while agricultural residues present higher hemicellulose (mostly xylan) and ashes, facts that are commonly found in the literature (Garrote *et al.*, 1999; Zhao *et al.*, 2012).

Different types of hemicellulose are found in wood (poplar) and agricultural residues. The main hemicellulose in poplar has been identified as glucuronoxylan, while arabinoxylans have been often identified in wheat straw and corn stover (Polizeli *et al.*, 2005; Vera *et al.*, 2015). This may explain the higher amount of glucan and lowest arabinan in poplar, but higher xylan and arabinan in the other feedstocks. Despite of that, glucan and xylan represented most of carbohydrates in all feedstocks, indicating that over 600kg of sugars can be obtained per tonne of dry biomass.

For comparison purpose, a plot of the amount of acetate and ash for single and mixed feedstocks are shown in Figure 5-2.

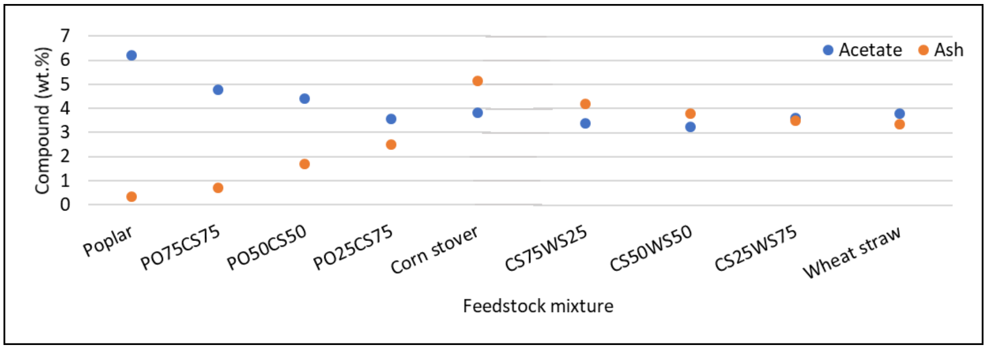

Figure 5-2 – Relationship between amount of acetate and ash of single corn stover (CS), poplar (PO), wheat straw (WS), and mixtures of CS with either PO or WS.

Figure 5-2 shows that the composition of the mixed feedstock is approximately an interpolation of the single feedstocks they are composed of. Vera *et al.* (2015) found a similar relationship for the mixtures of poplar and wheat straw.

5.3.2 Results of biomass composition on xylose recovery

The C5HI values discussed in Section 4.3.2 will be used to explain the results obtained from the composition analysis of the autohydrolysates. In this section, the focus will be on select different feedstocks based on their C5HI, and to investigate the application of the index. For this purpose, a mixture of 25 wt.% corn cob and 75 wt.% poplar

(CC25PO75) were selected due to their high C5HI (0.13). Next, 25 wt.% corncob was mixed with corn stover, as it presents a lower C5HI (0.093). Similarly, wheat straw (C5HI 0.091) was added to 75% poplar to also decrease the final C5HIof for the PO75WS25 mixture. Table 5-2 presents the final C5HI of the different feedstock mixtures used.

Table 5-2 – C5HI of single feedstocks and mixtures

Feedstock mixture	C5HI of single feedstock	Final C5HI
CC25PO75	CC (0.13), PO (0.129)	0.13
CC25CS75	CC (0.13), CS (0.093)	0.102
PO75WS25	PO (0.129), (0.091)	0.12

The feedstocks described in Table 5-2 were pretreated at 200°C for 60min with a LSR 25. The xylan recovery obtained for the different feedstock mixtures is presented in Figure 5-3.

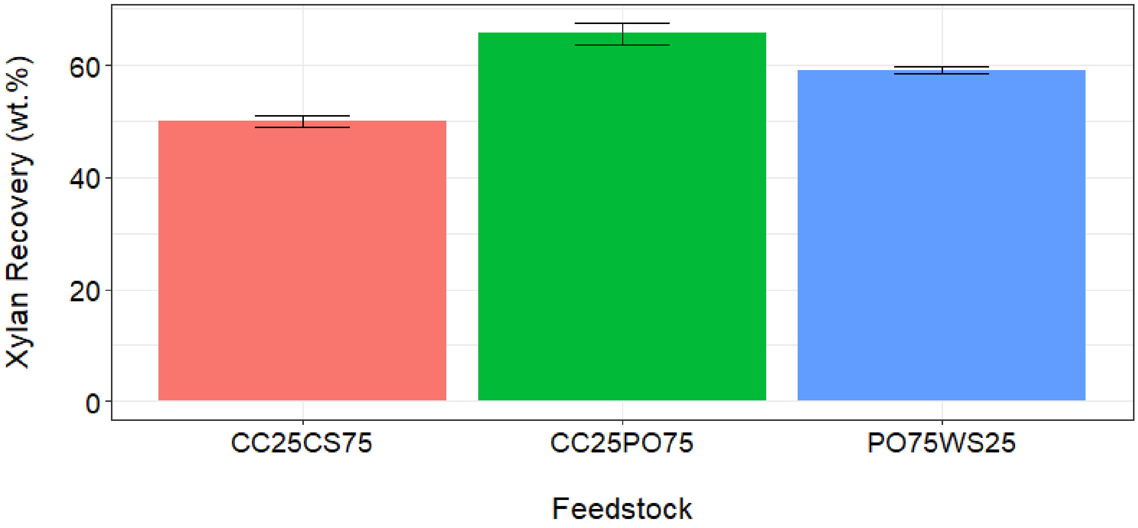

Figure 5-3 – Xylan recovery of the selected feedstock mixtures of corn cobs (CC) corn stover (CS), poplar (PO), wheat straw (WS).

The profile of xylan recovery showed in Figure 5-3 shows the corn cobs and poplar mixtures presenting highest values, while the corn cob and corn stover present the lowest xylan recovery values. The same relationship was noted for the C5HI values.

These relationships will be used to analyze the composition of the autohydrolysate, shown in Figure 5-4.

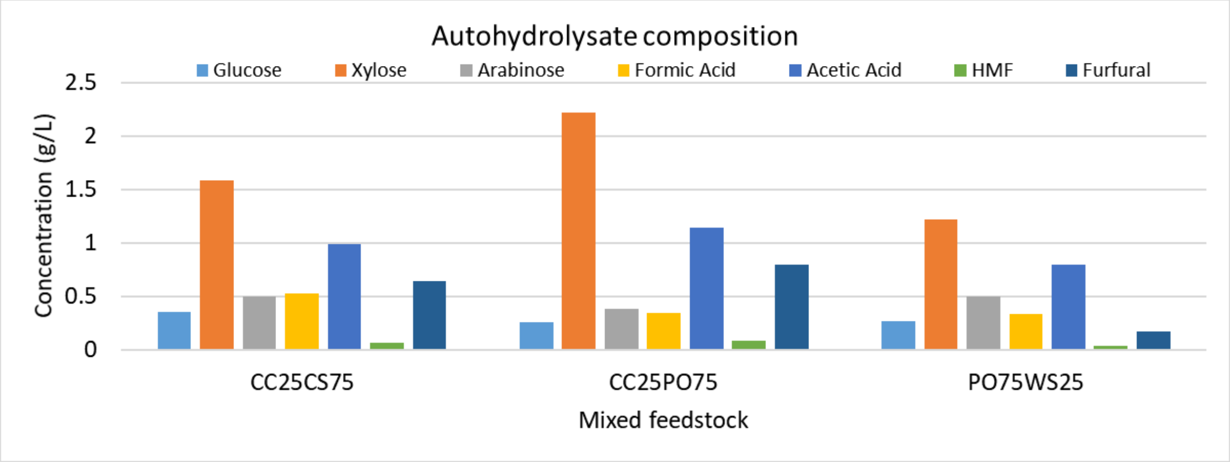

Figure 5-4 – Chemical composition of autohydrolysates used for ABE fermentation for different mixtures of corn cobs (CC) corn stover (CS), poplar (PO), wheat straw (WS).

Figure 5-4 shows that corn cob and poplar present the highest xylose concentration, and as seen in Table 5-2, they also present similarly high C5HI. It becomes evident than when corn cob was mixed with a biomass with similar C5HI (poplar), the xylan removal was higher than when it was mixed with a lower C5HI (corn stover). Similarly, a mixture of poplar and corn cob presents higher recovery than a mix of poplar with wheat straw. The glucose concentration was low for all feedstocks, while arabinose was higher, demonstrating the higher hydrolysis of C5 sugars present in hemicelluloses. The composition analysis of the raw feedstocks shows that poplar and corn cob present the highest acetate fraction, being an indication of higher acetate concentration in the hydrolysate. HMF, a product from C6-sugar degradation, was present in trace amounts, reflecting the low glucose concentration in the media. Furfural, a product of C5-sugar degradation, presented concentration varying according to the xylose amount, reflecting the effect of higher degradation once monomeric sugars are present.

5.3.3 ABE Fermentation

As mentioned in the Materials and Methods section, the autohydrolysate was mixed with corn syrup to a final volume percentage of 95 and 5, respectively. Preliminary experiments using *Clostridium saccharobutylicum* show that this microorganism is able to efficiently produce solvents when the sugars concentration in the fermentation medium is around 50g/L (data not shown); therefore, the fraction of autohydrolysate and corn syrup was chosen to reach this sugars concentration.

Production media was prepared mixing corn syrup and supernatant from autohydrolysis pretreatment to obtain a final volume percentage of 5 and 95 for corn syrup and supernatant, respectively.

The different amounts of varying compounds in the autohydrolysate was used to analyze the products from ABE fermentation, as show in Figure 5-5.

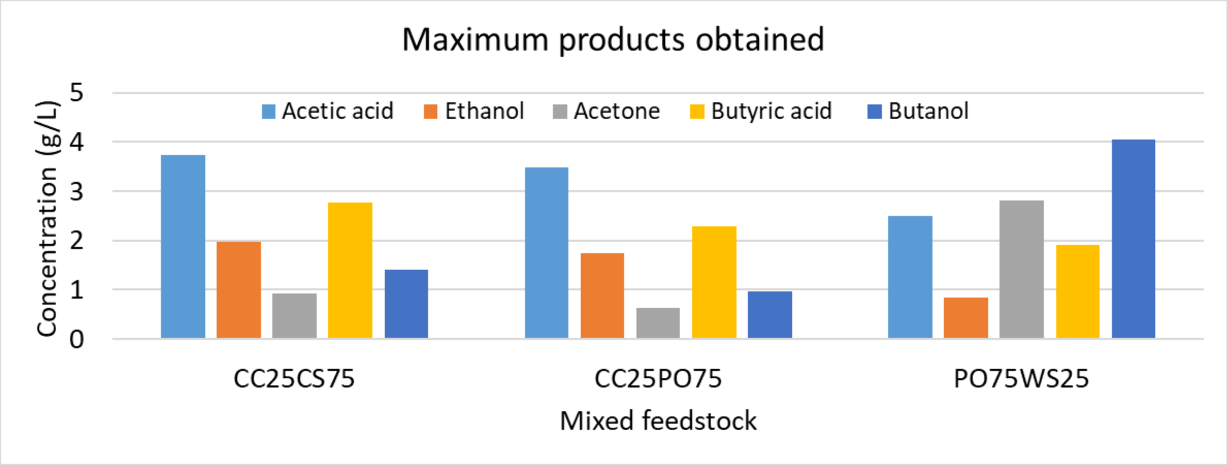

Figure 5-5 – Maximum concentration of acids and solvents produced during ABE fermentation for mixtures of mixtures of corn cobs (CC) corn stover (CS), poplar (PO), wheat straw (WS).

As shown in Figure 5-5, poplar and wheat straw presented the highest butanol production. This may be due to lower degradation products in the hydrolysate. Acetic acid and ethanol and butyric acid followed similar trends, while acetone was also higher for the poplar-wheat straw mixture, again, possibly due to the lower degradation.

In preliminary experiments (data not shown), water was used to dilute syrup to the same ratio used for syrup diluted with autohydrolysate (final volume percentage of 5 corn syrup). In the former case, the butanol, acetone and ethanol concentrations were 7g/L, 4g/L and 1.8g/L, respectively. On the other hand, the highest solvent concentrations from the autohydrolisate diluted samples were acetone 4g/L, butanol 2.8 g/L and ethanol 0.9g/L. These results demonstrate that due to the presence of fermentation inhibitors in the pretreatment liquor, diluting corn syrup with water is better than using autohydrolysate. Nonetheless, improvements to the autohydrolisate can be obtained when feedstocks are selected to decrease sugars degradation.

Autohydrolysis pretreatment produces degradation products that present inhibitory effect on the fermentation process. The toxic effect of these inhibitors to microorganisms reduces the overall productivity (Harmsen *et al.*, 2010). CC25PO presented the highest xylose concentration, and consequently higher furfural, resulting in the lowest butanol and acetone production. Furfural and HMF affect cell growth and respiration (Harmsen *et al.*, 2010). Figure 5-6 shows the profiles for solvents and acids production from autohydrolisate diluted corn syrup.

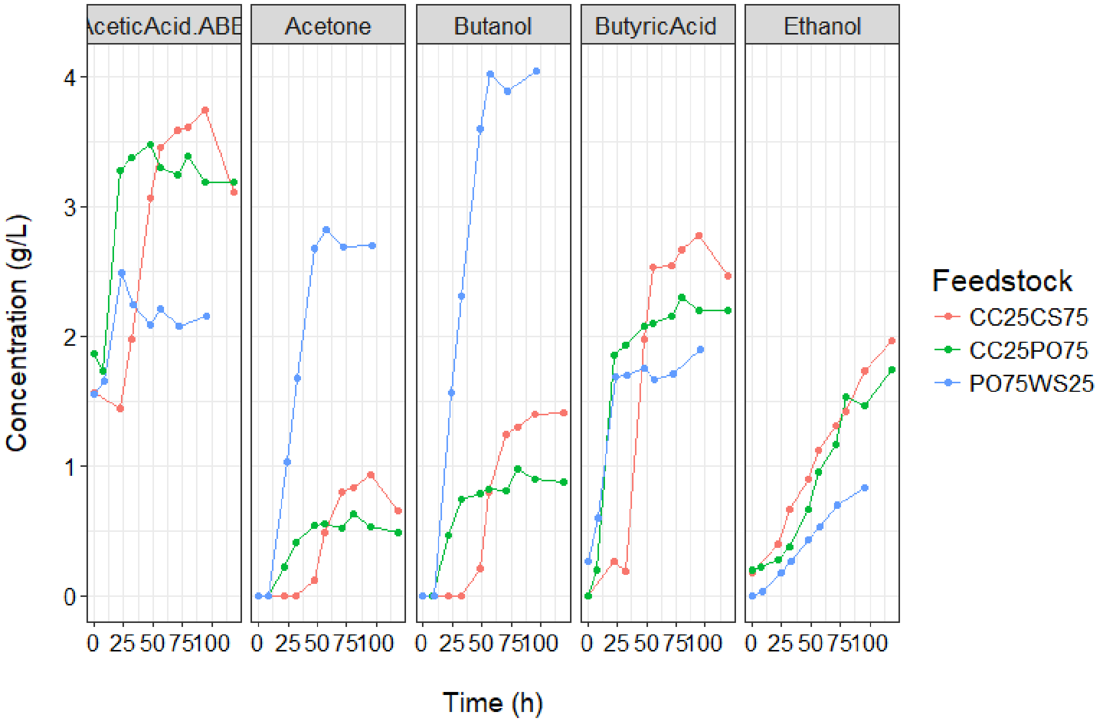

Figure 5-6 – Variation in the concentration of acids and solvents in the fermentation

media for the different mixtures of mixtures of corn cobs (CC) corn stover (CS), poplar (PO), wheat straw (WS).

Corn cob and corn stover presented higher lag phase for all compounds except ethanol, while poplar and wheat straw produced acetone and butanol in roughly 12 hours. Butyric acid for both mixtures of poplar were similar, although CC25PO75 produced higher concentration between the two poplar mixtures, demonstrating how fermenting organisms may be resistant to inhibitors or may become gradually adapted to their presence (Harmsen *et al.*, 2010). The same behavior can be seen for acetic acid. CC25CS75 presented the longest lag-phase for almost all compounds (except ethanol), but this mix also produced the highest amount of butyric acid. The corn cob mixtures produced similar amounts of ethanol, while PO75CS25 presented the lowest ethanol production.

5.3.4 Tuning the feedstock for bioconversion

One of the advantages of mixing biomass is the ability to alter the feedstocks characteristics to improve the pretreatment process (Imamoglu & Sukan, 2014; Vera *et al.*, 2015); therefore, the C5HI can be used as a parameter to tune the feedstock mixures. This relationship in shown in Figure 5-7.

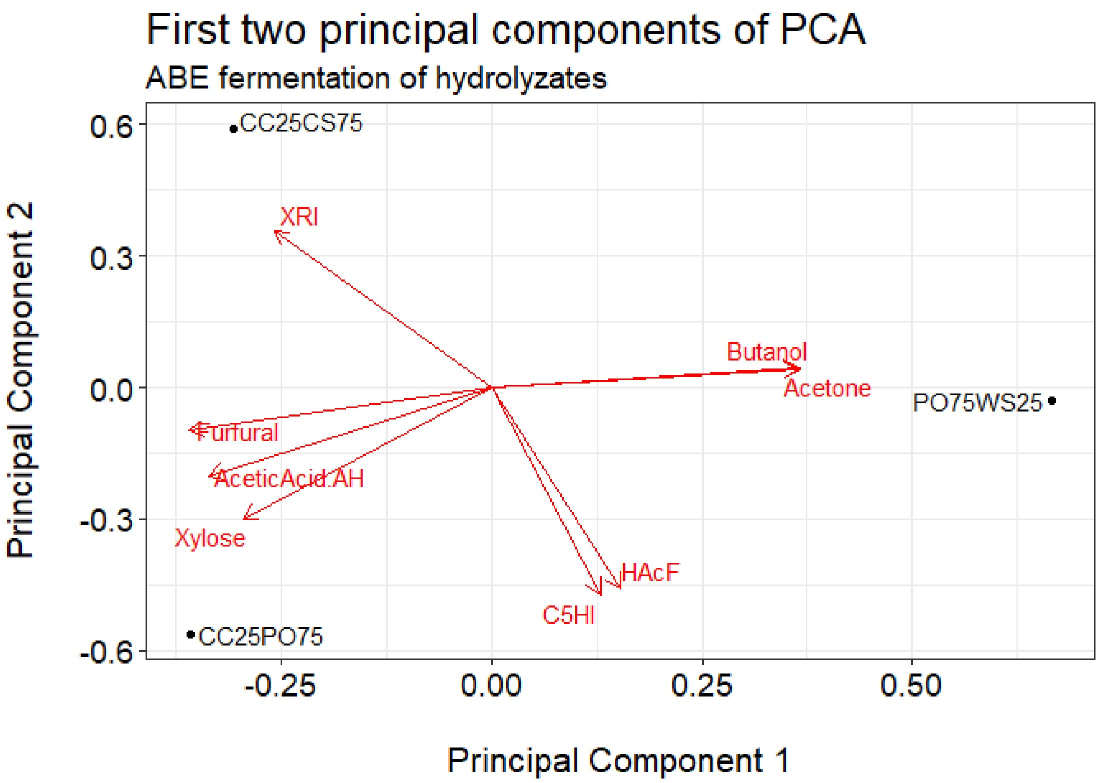

Figure 5-7 – PCA relating feedstock characteristics (C5HI, XRI, HAcF), autohydrolysate composition (Xylose, AceticAcid.AH, Furfural) and fermentation products (Butanol, Acetone) based on mixtures of mixtures of corn cobs (CC) corn stover (CS), poplar (PO), wheat straw (WS).

Figure 5-7 presents the relationship between the autohydrolysate composition and the ABE fermentation products. It becomes apparent that the solvents acetone and butanol were produced from the hydrolysate with the least amount of degradation products. The figure also shows that no tended to high production in the direction of CC25PO75, while all degradation products tended to this direction, demonstrating how high hemicellulose solubilization may not be desired, depending on the targeted product. An ideal approach to fermentation is to prevent the formation of fermentation inhibitors as much as possible through (adaptation of) the pretreatment process conditions of other measures (Harmsen *et al.*, 2010). In this work, the prevention of fermentation inhibitors can be obtained by selecting a feedstock mixtures with low C5HI, which results in lower xylose production and consequently lower furfural, and demonstrate the ability to tune the feedstock to improve bioconversion.

5.4 Conclusion

The choice of mixed feedstock used for autohydrolysis pretreatment can improve the production of solvents by ABE fermentation. Section 5 demonstrates that a mixture of poplar and wheat straw results in higher butanol production than the same mix proportion of poplar with corn cob. This could be attributed to the lower xylose production, and therefore lower formation of degradation products when a feedstock with low C5HI is present. Similarly, adding corn stover to corn cob resulted in higher solvents production compared to when corn cob was mixed (in the same proportion) with poplar. In general, it can be concluded that feedstocks with lower C5HI improve the autohydrolysate characteristics for fermentation, being attributed to the lower formation of degradation products. Using feedstock mixture with low C5HI can be seen as a strategy to increase solvents production by ABE fermentation.

6 Potential for ethanol production by autohydrolysis pretreatment of mixed lignocellulosic biomass feedstock

Preface

The previous two sections dealt with relating feedstocks characteristics (and the C5HI) with the autohydrolisate composition; moreover, it was discussed how C5HI can be used to provide insights into “good” feedstock mixtures for producing XOS and solvents. Biorefineries take advantage of the wide scope of products possible to be obtained and ethanol is another common commercialized product. Ethanol, an alcohol commonly used in the beverage and fuel industries, can be produced from lignocellulosic biomass by using the similar concept in the brewing industry. Similar to converting barley into glucose prior the fermentation, a pretreatment step is needed. However, converting the cellulosic biomass into sugar monomer is much more challenging than releasing the glucan from barley. The pretreatment step in lignocellulose conversion needs to decrease the biomass recalcitrance and increase the accessibility of cellulose fibers to hydrolytic enzymes, resulting in the release of glucose from the feedstock. The operational parameters used for autohydrolysis pretreatment, such as temperature and time, will depend on the feedstock used. Optimization efforts are made focusing on obtaining i) high hemicellulose sugars recovery (ideally in monomeric form) in the liquid fraction and ii) increase solids accessible surface area for glucose production by enzymatic hydrolysis; therefore, process efficiency are based on the total sugars recovery after the combined hydrolysis processes.

In Section 6, the C5HI of single and mixed feedstocks are used for analysis of total sugars recovery by autohydrolysis pretreatment followed by enzymatic hydrolysis. The same pretreatment conditions employed in Sections 4 and 5 were used to reduce the feedstock recalcitrance and increase sugar yields from enzymatic hydrolysis. The composition analyses of feedstocks before and after pretreatment were carried out to investigate the effects of pretreatment of single and mixed feedstocks on the liquid and solid fractions. Finally, the pretreated solids were subject to enzymatic hydrolysis, and the resulting glucose recovery was used to assess the pretreatment of the different effects.

The potential for ethanol production was based on the ratio of total sugars recovered from both hydrolysis steps and total sugars in the feedstock. Corn is a common feedstock in current biorefineries, resulting in great amounts of corn residues being readily available; therefore, corn residues, such as corn stover, is seen as a potential feedstock for lignocellulosic ethanol production. Due to this high potential, corn stover was mixed with bagasse, poplar and wheat straw to test the different products obtained. The C5HI was used to analyze the products, considering that corn stover is mixed with feedstocks presenting lower (bagasse), similar (wheat straw) and higher (poplar) C5HI values.

This part fulfills the Objective 3 described in section 3. Results in this section demonstrate the synergistic effects taking place when feedstocks are mixed, resulting in higher sugars monomers in the autohydrolysate and higher accessibility of cellulose to enzymes. In general, mixed feedstocks presented higher xylan removal, resulting in higher enzymatic hydrolysis takes place. On the other hand, it was found that higher temperature decreased enzymatic hydrolysis, probably due to lignin relocation. Therefore, there must be a balance between xylan removal and glucose hydrolysis.

6.1 Introduction

Lignocellulosic biomass is a potential feedstock for biofuels production. The composition of lignocellulosic biomass ranges from 35-50 wt.% cellulose, 15-30 wt.% hemicellulose, 5-20 wt.% lignin, which indicates that theoretically around 80% of cellulosic biomass can be converted into sugars in biorefineries for fuels and chemicals production. Other compounds in biomass include acetic acid (2-8% wt.%), and ash (0.5-20%) (Garrote *et al.*, 1999). Autohydrolysis pretreatment is used in biorefineries to fractionate biomass, solubilize high amounts of hemicellulose sugars and avoid their degradation to furfural, resulting in high yields of hemicellulose sugars and improving enzymatic digestibility of the pretreated solids to obtain high yields of glucose (Wyman *et al.*, 2004).

During autohydrolysis pretreatment, hydronium ions derived from autoionization of water cause the catalytic depolymerization of hemicellulose to xylo-oligomers and xylose, and the cleavage of acetyl groups. Acetyl groups are removed during autohydrolysis as acetic acid or attached to solubilized hemicelluloses. The minerals in the biomass could neutralize acid and decrease hydrogen ion activity, lowering the pretreatment severity (Lloyd & Wyman, 2004) and requiring harsher pretreatment conditions.

Generally, herbaceous substrates and agricultural residues (e.g., wheat straw and corn stover) have higher ash content than woody materials (such as poplar), and several researchers have correlated high ash content with a greater neutralizing capacity of acid. Similarly, high buffering capacity prevents acid production and makes the pretreatment appear to be less severe. Therefore, neglecting neutralizing and buffering may lead to lower hemicellulose yields for same parameters using different feedstocks (Springer & Harris, 1985; Yang & Wyman, 2008). Therefore, understanding the effects of the feedstock characteristics is as important as the knowledge about the pretreatment process itself and should be analyzed.

Improvements the pretreatment products can be obtained from mixed feedstocks. The combination of different biomass may produce a hydrolysate with lower concentration of inhibitors and higher sugars recovery. However, most literature up to date has mainly focused on single lignocellulosic biomass (SLB) feedstock, and the literature on autohydrolysis of mixed lignocellulosic biomass (MLB) is scarce. Thus, this report presents preliminary studies to assess an ideal pretreatment and hydrolysis conditions for maximizing the sugar release and lowering formation of degradation products. The main objectives of this research are to compare the autohydrolysis of single and mixed lignocellulosic biomass feedstock (SLB and MLB, respectively), and to study their effects on the efficiency of both the pretreatment and enzymatic hydrolysis.

6.2 Materials and methods

Feedstock

Bagasse, corn stover, poplar and wheat straw where characterized and evaluated for xylo-oligosaccharides (XOS) production by autohydrolysis. These feedstocks were kindly donated by Greenfield Specialty Alcohols (Chatham-ON). Corncob was locally collected and used as an extra biomass to expand the relationships found for the four main biomasses used. Feedstocks were oven-dried at 50◦C for until they presented water content < 10wt.%., The biomass feedstock was ground with a Magic Bullet food processor and sieved to obtain a particles size range between 0.5 and 1mm. Samples were then kept in individual zip-lock bags at stored in a 4°C fridge.

Composition analysis of solid fractions

The moisture content was obtained by oven drying the feedstock 105℃ until a constant weight was achieved. Similarly, the total ash content was measure by heating the biomass sample to 575˚C to constant weight. The structural carbohydrates content were determined according to the protocol NREL/TP-510-42618. Briefly, after treatment with 72% H_2SO_4 (w/w), a dilute acid step 4% H2SO4 was used in an autoclave at 121°C for 1h. The acid insoluble residue (AIR), or Klason lignin, was obtained after adjusting for the acid insoluble ash (determined by igniting the AIR at 575℃ for 24h). Monomeric sugars, acetic acid and degradation products were determined by HPLC Waters (Milford, USA) containing an Aminex HPX-87H column (Bio-Rad, Richmond, USA) combined with a cation H^+-guard column (Bio-Rad, Richmond, USA). Elution took place with 5mM H_2SO_4 as mobile phase and at 50℃. Glucose, xylose, arabinose, formic acid, acetic acid, HMF and furfural were detected with a refractive index detector (RID). The experiments were run in triplicate, as shown in Appendix A. Appendix B.1 presents a typical chromatogram obtained for the feedstock composition.

Autohydrolysis pretreatment

The autohydrolysis pretreatment was conducted in reaction tubes built with 6" length thick-walled 316/316L stainless steel pipe nipple with 1.5" internal diameter. The tubes were capped on both ends, and the lid was adapted to contain a 6" length x 3/16" diameter type K thermocouple probe (McMaster-Carr - Aurora, Ohio), which was used to measure the temperature inside the reactors. Prior to the experiments, 2g of biomass was kept soaking overnight with 30mL of deionized water, resulting in a liquid-to-solid ratio (LSR) of 15. The reactors were heated in a convection oven. The temperature used during pretreatment were 160, 180 and 200℃, with holding time of 60mins after the temperature was reached These temperatures and time were chosen based on values commonly found in the literature for autohydrolysis pretreatment of lignocellulosic biomass. When the reaction time was reached, the reactors were removed from the oven. Once cooled down, the solid and liquid were separated using vacuum filtration.

Chemical analysis

The hydrolysate was filtered by vacuum filtration system using a glass microfiber filter paper (VWR, USA, catalogue no. 28333-129, particle retention 1.5µm) on a Buchner funnel.

The solid product was thoroughly washed with deionized water ranging from 100x biomass weight to ensure complete removal of sugars from the solid residues, which were posteriorly dried at room temperature and subjected to the same chemical analysis as the feedstock material.

The autohydrolysate was filtered through 0.2µm membranes and directly analyzed by HPLC for quantification of monosaccharides. The amount of oligosaccharides in the autohydrolysate was quantified following NREL (National Renewable Energy Laboratory) procedure NREL/TP-510-42623, based on a dilute acid hydrolysis using 4% H_2SO_4 for 1h. Appendix B.2 presents a typical chromatogram obtained for the autohydrolysate composition after dilute acid hydrolysis.

The HPLC system (Waters, Milford, USA) was equipped with an Aminex HPX-87H column (Bio-Rad, Richmond, USA) in combination with a cation H^+-guard column (Bio-Rad, Richmond, USA) and elution took place at 50℃ with 5mM H_2SO_4 as mobile phase. Glucose, xylose, arabinose, acetic acid, formic acid, HMF and furfural were detected with a refractive index detector.

Enzymatic hydrolysis

Batch enzymatic hydrolysis of the pretreated solids was conducted in 125 mL Erlenmeyer flasks. Each flask contained 5 grams of treated sample (dried weight) mixed with 50 mL of distilled water. The pH was maintained at 5.0 using 1 mol L-1 NaOH until the end of hydrolysis. Cellic CTec2 (kindly donated by Novozyme) was dosed to result in a filter paper activity of 15 FPU g-1 dry matter. The enzyme properties were reported by Cannella et al, (2012). Cellic CTec2 is composed of a blend of cellulases, high levels of β-glucosidase and hemicellulose. These enzymes have been used for cellulose hydrolysis of pretreated solids from several feedstocks types and pretreatment methods, presenting high effectiveness to convert cellulose into glucose for fermentation. Hydrolysis was performed in a Multitron incubator (InforsMulitron, Infors Switzerland) at 50 °C, 2.5 Hz (150 rpm) for 72 h. Samples were removed periodically for sugar analysis.

Data analysis

The analysis of feedstock characteristics affecting the autohydrolysis pretreatment products was conducted by pretreating single and mixed feedstocks at different temperatures. The calculated values of mixed feedstock composition are the sum of the mass fraction of each structural compound in the two feedstocks used. To analyze the effect of composition on xylan recovery, the measured values for amount of feedstock xylan recovered after the pretreatment were compared to calculated values. Pearson correlation (r) was calculated to compare between the measured and calculated values. It is a measure of the relationship between two components vectors A and B, there N is the total number of samples, $\mu_A$and μ_B are the means for the A and B sets and σ_A and σ_B are their standard deviations.

$$\boldsymbol{r = \frac{1}{N-1}\sum_{i-1}^{N}\left(\frac{A_I-\mu_A}{\sigma_A}\right)\left(\frac{B_I-\mu_B}{\sigma_B}\right)} \qquad \textbf{Eq. 5}$$

where

N is the number of samples
$\mu_A$and μ_B are the mean for set A and B, respectively
σ_A and σ_B are the standard deviations for set A and B, respectively.

A positive correlation close to 1 indicates that certain characteristics positively affect the xylan recovery yields. All the data in this work was analyzed using RStudio (version 1.1.442).

6.3 Results and discussion

6.3.1 Biomass characterization

The composition analysis of single bagasse, corn stover, poplar and wheat straw were performed. Their results are presented in Table 6-1.

Table 6-1 - Chemical composition of feedstocks (wt.%, db.)

Compound	Bagasse			Corn stover			Poplar			Wheat straw		
Glucan	41.39	±	0.14	37.40	±	0.06	47.01	±	0.20	37.78	±	0.10
Xylan	23.14	±	0.06	22.12	±	0.07	19.93	±	0.13	23.91	±	0.08
Arabinan	1.40	±	0.01	3.05	±	0.07	0.94	±	0.06	2.72	±	0.02
Total Sugars	65.94	±	0.19	62.57	±	0.05	67.88	±	0.27	64.41	±	0.15
Acetate	3.52	±	0.31	3.84	±	0.14	6.22	±	0.21	3.81	±	0.11
Ac. Sol. Lignin	1.41	±	0.02	1.68	±	0.01	1.31	±	0.01	1.56	±	0.01
Ac. Ins. Lignin	24.37	±	0.09	18.19	±	0.14	19.02	±	0.17	20.08	±	0.10
Total Lignin	25.78	±	0.11	19.87	±	0.14	20.33	±	0.16	21.64	±	0.10
Ash	1.29	±	0.09	5.12	±	0.39	0.35	±	0.02	3.36	±	0.09
* Other	3.47	±	0.32	8.61	±	0.05	5.22	±	0.20	6.78	±	0.04

*Other are non-determined fractions that include uronic acids, extractives, and other fractions with minor importance for the purposes of this work.

In general, woody biomass contains higher amounts of cellulose (glucan), while agricultural residues present higher hemicellulose (mostly xylan) and ashes (Garrote *et al.*, 1999; Zhao *et al.*, 2012).

Corn stover presents the lowest acetate content (2.85 wt.%), while bagasse and wheat straw show similar values (3.19 and 3.44 wt.%, respectively), followed by poplar with the highest acetate of 5.65%. Conversely, poplar presented lowest ash content, followed by bagasse, corn stover and wheat straw (1.82, 3.47 and 4.75 wt.%, respectively). Bagasse and wheat straw present acetate content slightly higher than corn stover, while their ash content is lower in bagasse and higher in wheat straw. Poplar presents considerably less ash and much higher acetate content than corn stover; therefore, in order to study the effect of mixed feedstocks with different characteristics, corn stover was chosen to be mixed in different proportions (0, 25, 50, 75, 100 wt.%) with each of the other biomass.

Different types of hemicellulose are found in wood (poplar) and agricultural residues. The main hemicellulose in poplar has been identified as glucuronoxylan, while arabinoxylans have been often identified in wheat straw and corn stover (Polizeli *et al.*, 2005; Vera *et al.*, 2015). This may explain the higher amount of glucan and lowest arabinan in poplar, but higher xylan and arabinan to all other biomass studied. Despite of that, glucan and xylan represented most of carbohydrates in all feedstocks, indicating that over 600kg of sugars per tonne of dry biomass.

Figure 6-1 presents the C5HI values used for pretreatment.

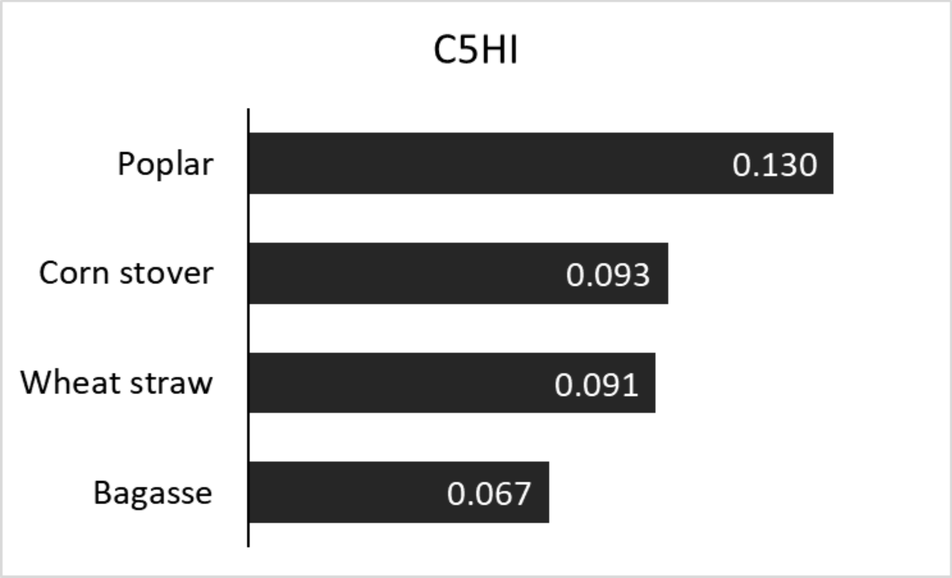

Figure 6-1 – C5HI values for the different feedstocks used.

Figure 6-1 shows that poplar presents the highest C5HI of 0.13, while bagasse presents over half this value (0.067). Corn stover and wheat straw present similar values around 0.09.

6.3.2 Autohydrolysis pretreatment

The ultimate goal of the pretreatment is recover as much of sugars in the feedstock as possible. In the case of autohydrolysis pretreatment (and other pretreatments at low pH), the objective is to obtain high yields of the xylan in the biomass used. C5HI was used to relate biomass characteristics with xylan recovery, as shown in Figure 6-2.

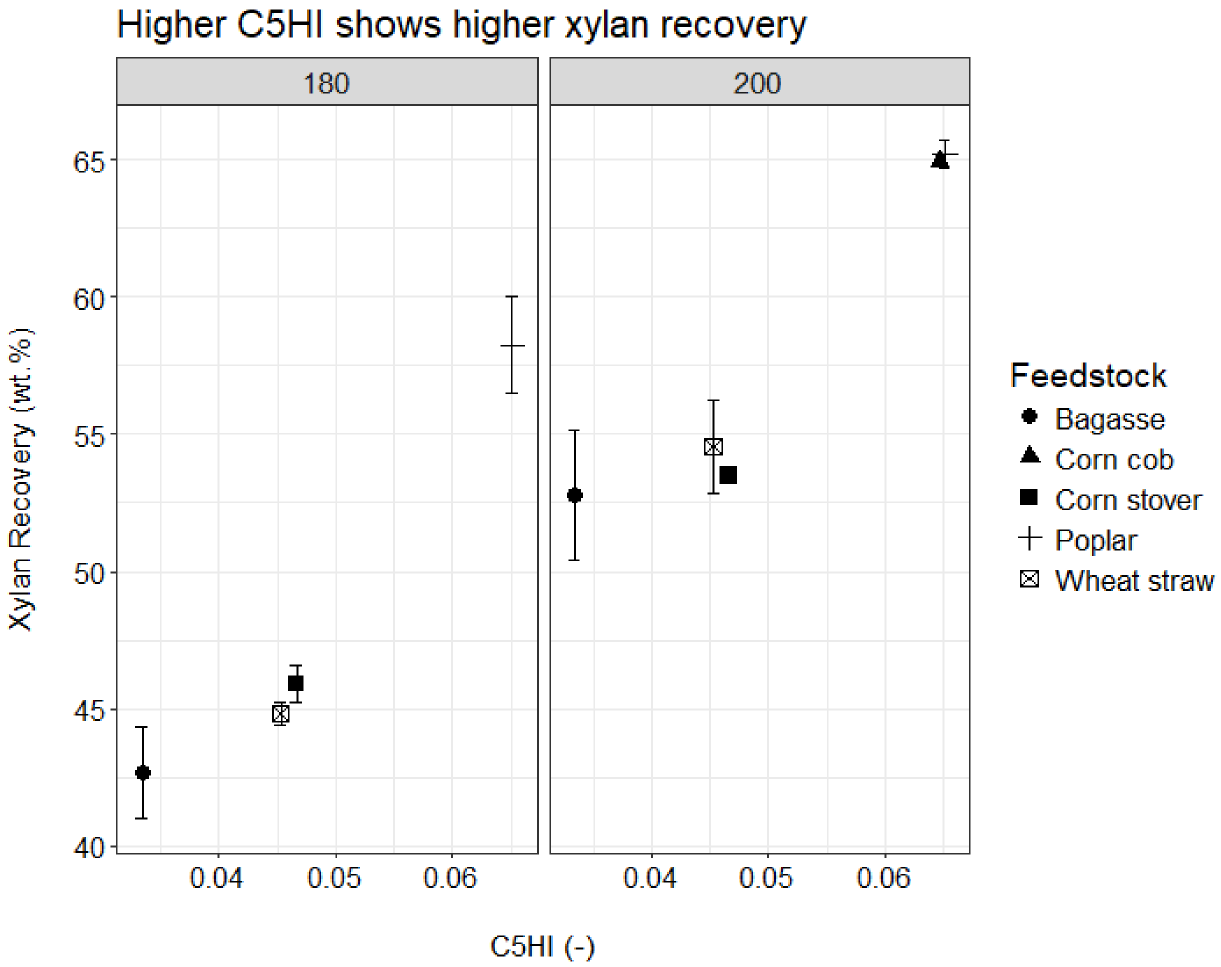

Figure 6-2 - Relationship between C5HI and total xylan recovery of single feedstocks at different temperatures.

Figure 6-2 demonstrates that both the biomass source as substrate and the pretreatment parameters are important factors for hemicellulose sugars recovery. The feedstock characteristics, as represented by the C5HI, shows good relationship with xylan recovery after pretreatment of various biomass types. For all feedstocks, the xylan recovery was higher at 200°C due to higher xylan hydrolysis. These results are similar to observations from Aoyama (1996), Garrote *et al.* (1999), and Carvalheiro *et al.* (2004), all showing dependence of xylan recovery on temperature.

Bagasse presented lowest recovery at both temperatures, with an increase from 43% to

53% at 200°C, while poplar showed increased recovery from 58% to 66%. Corn cob was used as an extra biomass at 200°C to further test the relationship between feedstock and hydrolysate. The xylan recovery for corn cob was 65%, which is similar to poplar, and demonstrating that both feedstocks presenting similar high C5HI also presented higher xylan recovery. Nitsos *et al.* (2016) obtained a maximum xylan recovery of around 60% for poplar and grapevine.

Maloney *et al.* (1985) used dilute acid hydrolysis and proposed that the first 60-70% of the xylan hydrolyzes according to apparent first-order kinetics, while the remaining xylan is hydrolyzed at a decidedly slower rate. This behavior of xylan hydrolysis has been demonstrate by several other studies (Garrote *et al.*, 2002; Otieno and Ahring, 2012). For the feedstocks tested in this project, it can be speculated that C5HI can also related to the fast-reacting xylan, as both are representation of how easily xylan is hydrolyzed.

The linear relationship found for single biomass can also relate to the aspect of mixed feedstocks, as shown in Figure 6-3.

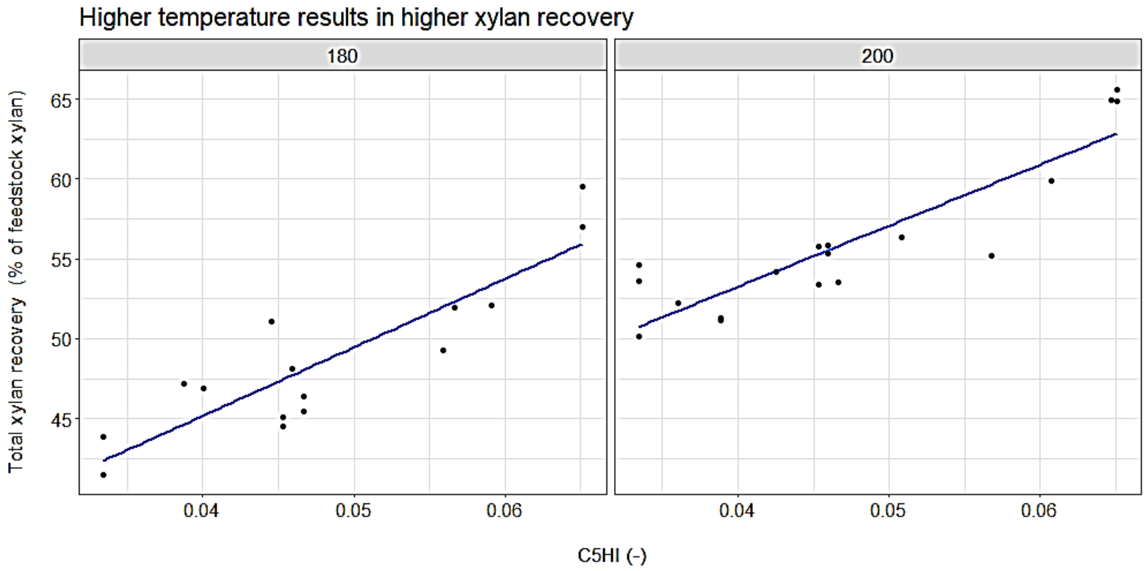

Figure 6-3 – Effect of C5HI on total xylan recovery of single and mixed feedstocks at different temperatures. At 95% confidence level, there is significant difference (p-

value = 1.6x10^{-4}) between the two means

Figure 6-3 shows that the effect of mixed feedstocks follows a similar trend to their single feedstocks, as demonstrated by the linear relationship found for total xylan recovery based on the feedstock C5HI. To validate the results, a regressions analysis of the feedstocks pretreated at 200°C for 60 minutes was performed, as seen in Figure 6-4.

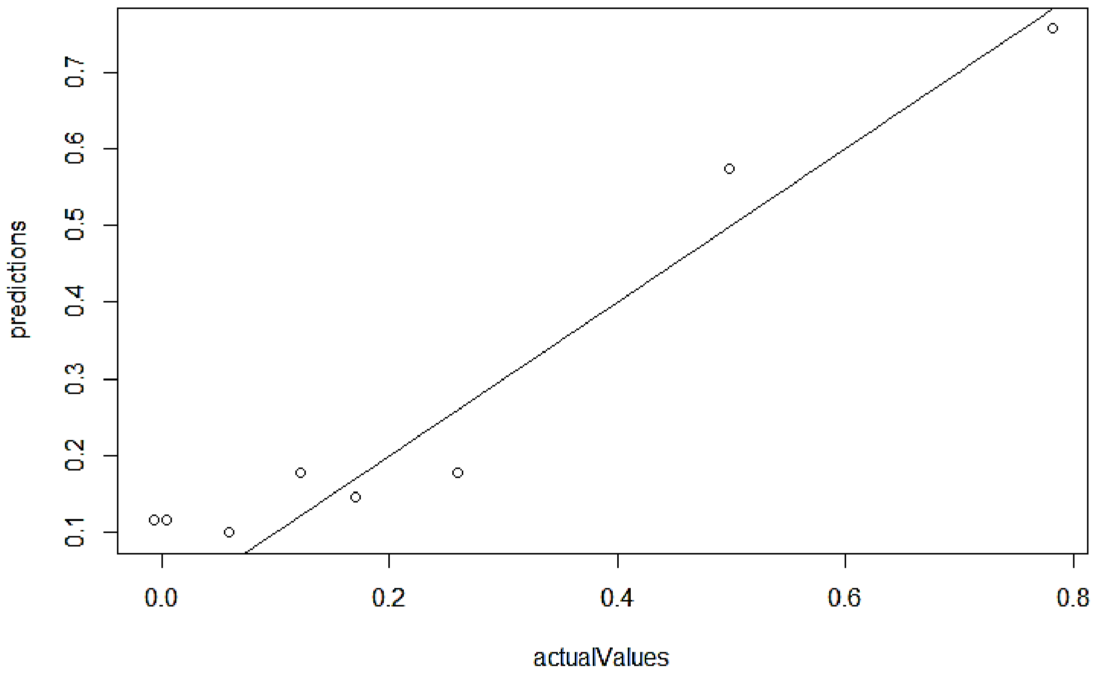

Figure 6-4 - Actual versus predicted values for xylan recovery based on the C5HI values of single and mixed feedstocks

Figure 6-4 shows the relationship between modelled and actual values found for xylan recovery. The model resulted in a p-value < 0.05, indicating the significance of the C5HI variable to explain the xylan recovery.

The results presented so far demonstrate that feedstock characteristics affect the autohydrolysis pretreatment products, with some compounds presenting higher effect than others. Moreover, it has been demonstrated that the relationship between feedstock characteristics can be used to simplify the analysis of the products obtained.

The yeast used for bioethanol production uses sugars monomers for fermentation. Therefore, a ratio of sugars monomers and oligomers can be used to assess the quality of the pretreatment liquor (Figure 6-5).

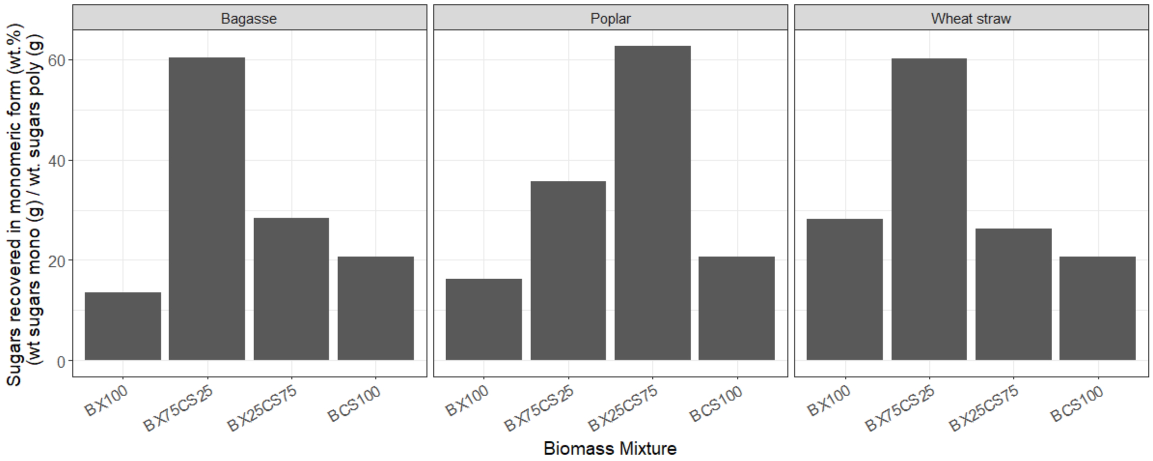

Figure 6-5 – Effect of single feedstocks and mixtures on monomeric sugars recovery. Corn stover (BCS) was mixed in varying weight fractions with other feedstocks (BX), namely bagasse, poplar and wheat straw.

Figure 6-5 demonstrates increased amount of xylan monomers for mixed feedstocks, where values of up to 60% monomers are obtained for mixtures, compared to around 20% monomers found for single feedstock. The synergistic effect of mixed feedstocks was also found by Vera *et al.* (2015) for mixed poplar and wheat straw. The authors attributed this synergy to the interaction between the high acetic acid in poplar and higher presence of ash in wheat straw. Similarly, the corn stover used in this work presents the highest ash content among the feedstocks used, so the buffering capacity provided by the higher ash in corn stover may interact synergistically to counteract the effect of acetyl groups derived from the added biomass.

Figure 6-5 shows bagasse and poplar presenting the lowest monomers content (around 15%). These feedstocks present the lowest and highest C5HI values, respectively, resulting in bagasse presenting low xylan hydrolysis and poplar showing high hydrolysis (higher degradation). The relationship between monomeric sugars and degradation is shown in Figure 6-6.

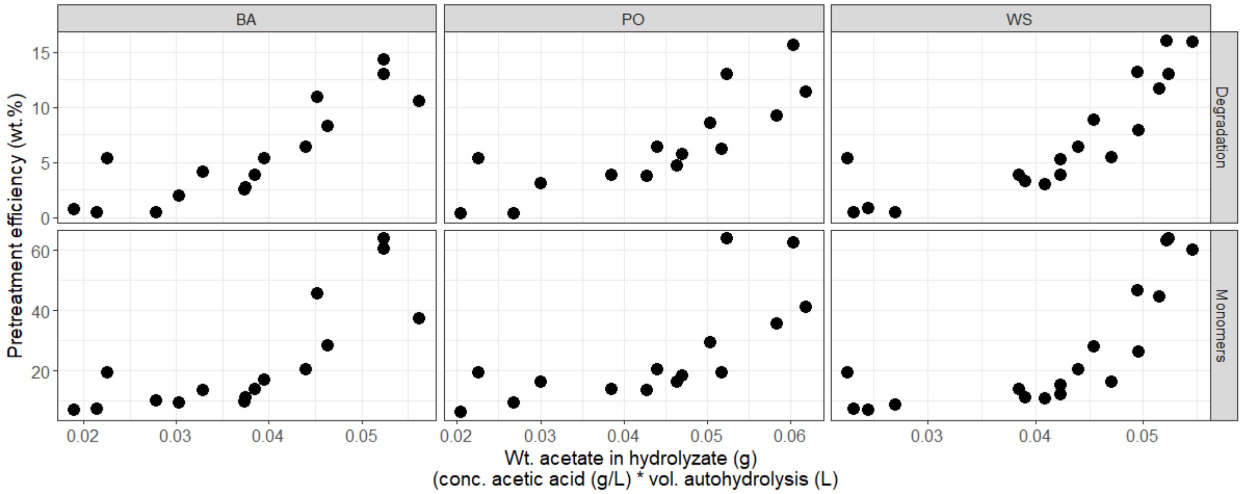

Figure 6-6 – Effect of acetic acid in the autohydrolysate on the production of monomeric and degraded sugars for mixtures of poplar with bagasse (BA), poplar (PO) and wheat straw (WS)

Figure 6-6 demonstrates that higher acetate in hydrolysate results in a more acidic environment, causing the xylose to further degrade to furfural and decreasing the overall process efficiency (Wyman *et al.*, 2004).

According to Gregg and Saddler (1995), when more than 70% of the original xylan is a feedstock can be obtained as xylose after pretreatment, a separate enzymatic hydrolysis step may not be required. The benefit would be to directly send the autohydrolysate to the pentose fermentation step. However, an inhibitor removal step would be required is high concentration of inhibitors are present, such as acetic acid and sugars degradation products.

The utilization of batch presents a challenge to obtain high sugar monomers while presenting low degradation and low acetic acid. As it has been demonstrated, high acetate results in higher solubilization (good for enzymatic hydrolysis of solids), but it also results in higher monomers and higher degradation. This indicates that although batch processing of cellulosic biomass may improve cellulose hydrolysis, challenges will often be found for downstream processing of the hemicellulosic stream.

Several autohydrolysis pretreatment tests were performed for single and mixed feedstocks with temperatures between 160 and 220°C for 60 minutes. Figure 6-7 shows some important trends found for the products.

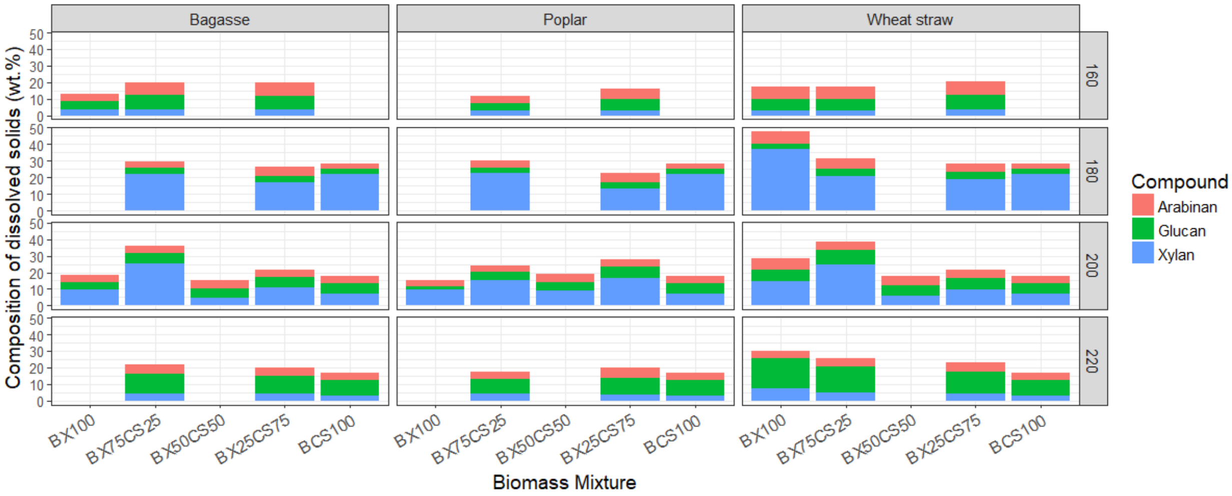

Figure 6-7 – Effect of temperature on the autohydrolysate sugars composition. Corn stover (BCS) was mixed in varying weight fractions with other feedstocks (BX), namely bagasse, poplar and wheat straw.

The results in Figure 6-7 show a decreased amount of xylose with temperature, where at the harshest conditions, enhanced hydrolysis of xylo-oligosaccharides toward monomeric xylose and subsequent transformation/degradation of xylose to furfural and formic acid. It also shows a decreasing amount of arabinan for all feedstocks as temperature increases, probably caused by increased degradation as the pretreatment severity increases. Glucan is relatively high at 160°C, and its fraction increases when temperature increased from 180 to 220°C. This can be explained by the release of glucan molecules existing in the hemicellulose chain and parts of amorphous cellulose.

At higher temperatures, hydrolysis of more crystalline cellulose takes place, therefore increasing the glucan content recovered in the hydrolysate. Similarly, the xylan content of the dissolved solids also increases and then decreases with temperature, achieving maximum values at 180°C and presenting similar fractions at 160 and 220°C. This behavior can be explained by two distinct mechanisms, where at 160°C there is low solubilization of xylan molecules, while at 220°C the decreased xylan is caused by higher degradation (Yoon, 1998; Wyman *et al.*, 2004). Figure 6-6 and Figure 6-7 demonstrate that the choice of pretreatment parameters depends on the balance between sugars solubilization and degradation.

These relationships are also reflected on the composition of the insoluble solids, as seen in Figure 6-8. For simplicity, only data from solids pretreated at 180°C are presented.

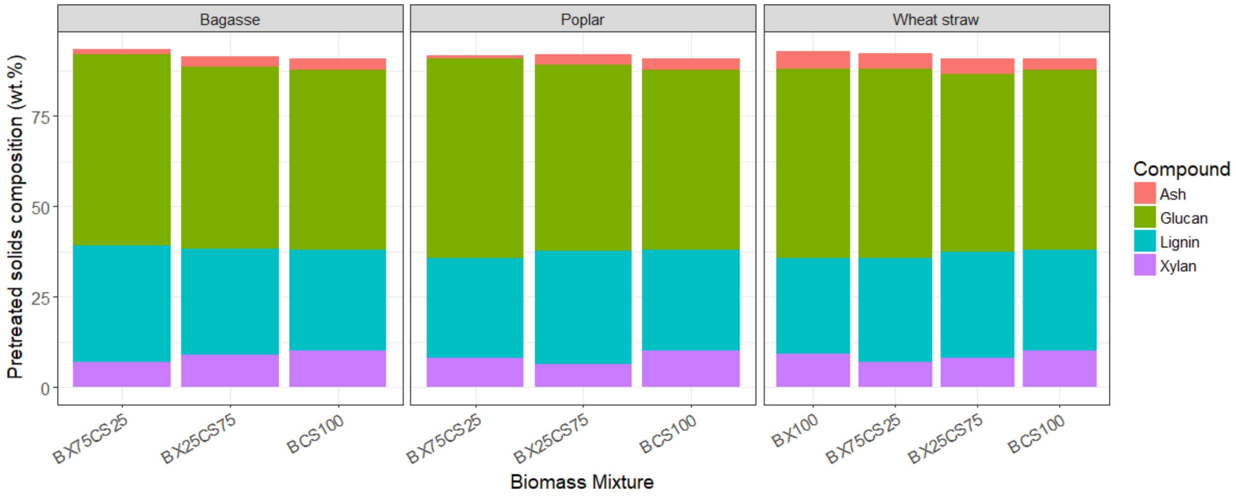

Figure 6-8 – Effect of single feedstocks and corn stover mixtures on the composition of the pretreated solids. Corn stover (BCS) was mixed in varying weight fractions with other feedstocks (BX), namely bagasse, poplar and wheat straw.

Figure 6-8 shows the composition of pretreated solids, where glucan and lignin were the main compounds. It can also be noticed that the composition of the mixtures presented lower xylan content compared to corn stover alone. In general, this demonstrates the advantages of mixing corn stover other lignocellulosic biomass; furthermore, the comparable chemical composition of solids after pretreatment demonstrate that autohydrolysis is able to produce a homogeneous slurry from a diverse mixture of biomass containing combinations of different feedstocks. Similar relationship was found by Vera *et al.* (2015).

Most of the hemicellulose can be extracted from the autohydrolysis of cellulosic residues. The autohydrolysate contains fermentable sugars and breakdown products of hemicellulose and lignin. The recovery yield of fermentable sugars depends on process parameters, such as temperature and time, where higher severity decreases the yield due to more breakdown products of hemicellulose and lignin which are inhibitory to yeast fermentation (D. Gregg & Saddler, 1995). However, high severity is usually required to increase enzyme accessibility of the cellulose. Therefore, an optimal pretreatment condition exists that results in minimum overall ethanol production costs, which can be obtained by high extraction yield and dissolved solid concentration in the extract, minimizing costs of extraction and subsequent conversion.

Similarly, based on the different composition of autohydrolysate obtained for different feedstocks, an optimum feedstock mixture could be used to increase the sugars yields at specific process parameters.

6.3.3 Enzymatic hydrolysis

Different mixtures of corn stover with and bagasse, poplar and wheat straw were used for enzymatic hydrolysis. Although the enzymatic hydrolysis was analyzed for samples run at 200°C, the glucan recovery presented similar patterns of solids composition, where mixed feedstocks presented higher glucan recovery. The results for all feedstocks are shown in Figure 6-9.

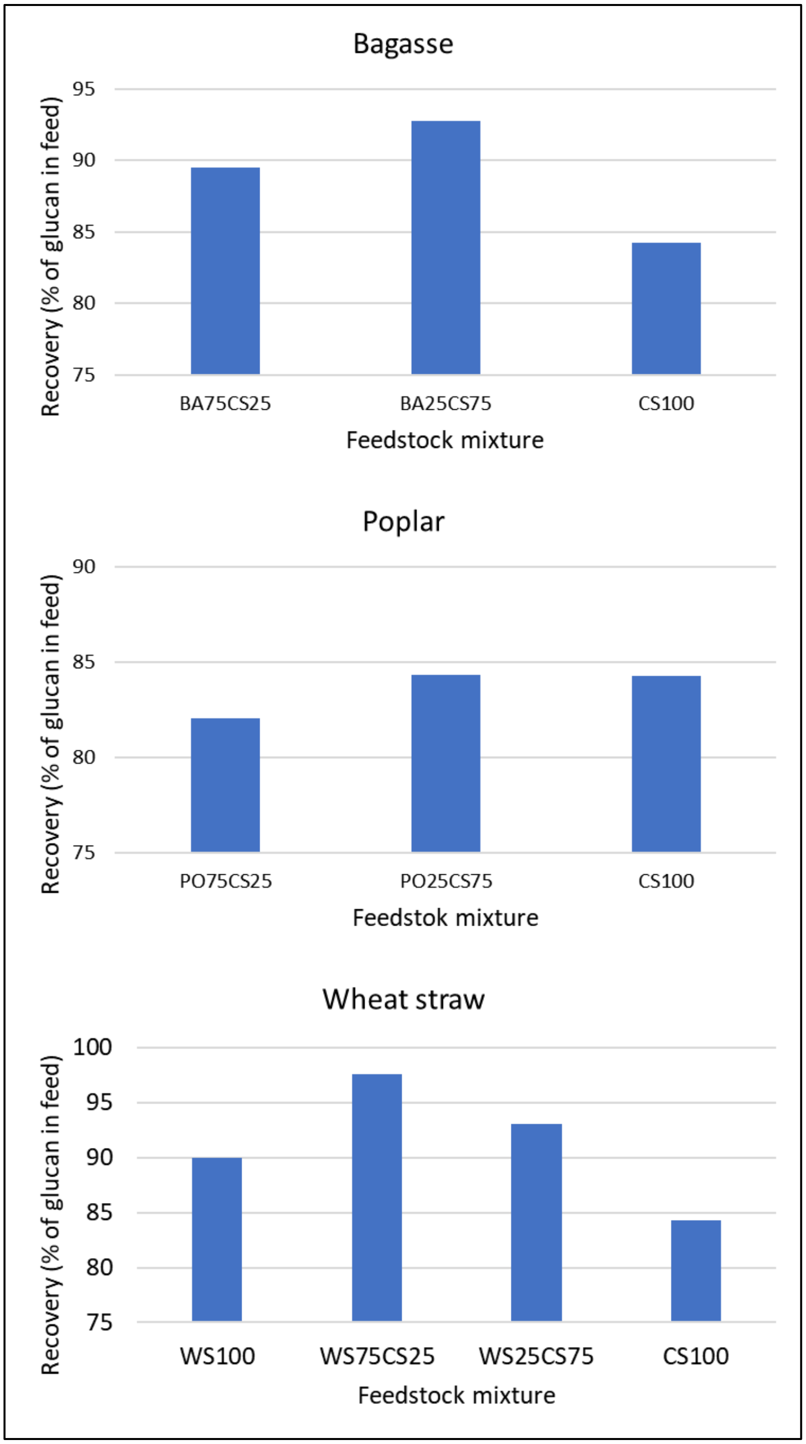

Figure 6-9 – Total glucan recovery of single feedstocks and corn stover mixtures. Corn stover (CS) was mixed in varying weight fractions with bagasse (BA), poplar (PO) and wheat straw (WS).

Figure 6-9 shows that corn stover mixed with bagasse and wheat straw presented higher recovery compared to the single feedstocks. The mixture with poplar, however, showed little variation when 25wt% poplar was used, but a lower recovery was obtained when 75% poplar was used in the feedstock. This behavior may be caused by the higher lignin redistribution in poplar at 200°C (Kristensen *et al.*, 2009). This relationship is seen in the effect of temperature on enzymatic hydrolysis, as seen in Figure 6-10.

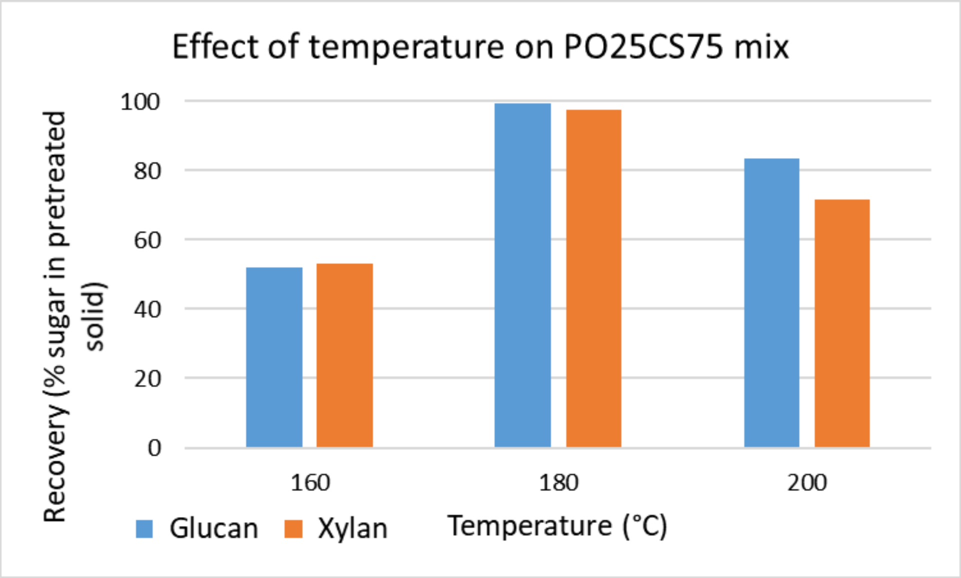

Figure 6-10 – Effect of temperature on the sugars recovery from pretreated solids from a feedstock mixture containing corn stover (CS) with 25wt% poplar (PO).

Figure 6-10 shows that 180°C presents the highest sugars recovery. The decreased glucan recovery at 160°C may be caused by the lower xylan solubilization, while the lower recovery at 200°C may be due to lignin redistribution commonly seen at high pretreatment severities (Zheng *et al.*, 2014).

(Pielhop *et al.*, 2015) demonstrates how lignin repolymerization in autohydrolysis pretreated spruce increases cellulose deactivation.

The results show that the optimum temperature for monomeric hemicellulosic sugars differs from that for enzymatic hydrolysis for cellulosic sugars. This relationship was also found by Silva-Fernandes *et al.* (2015), and demonstrate the need for research intended to find the balance required for total sugars recovery.

6.4 Conclusion

Higher sugar recoveries and lower degradation products were obtained during pretreatment of mixed lignocellulosic biomass when compared to a single feedstock. Pretreatment at 180°C gives the highest sugar recovery due to optimum balance between xylan solubilization and cellulose hydrolysis. It also demonstrates that mixing corn stover with corn cob (other corn processing residue), wheat straw (other agricultural residue) or poplar chips (woody residue) can improve the pretreatment products from corn stover by changing the feedstock characteristics.

Section 3 – Summary and conclusions

7 Summary

7.1 Analysis of lignocellulosic biomass characteristics affecting xylo-oligosaccharides (XOS) production by autohydrolysis

In Chapter 4, lignocellulosic feedstocks including bagasse, corn cob, corn stover, poplar and wheat straw were studied for XOS production. The feedstock characteristics were analyzed, and relationship between the amount of xylan, Klason lignin and acetate were used to develop the C5HI factor, which was defined as a measure of a feedstock's susceptibility to hydrolysis. The C5HI was used to explain the XOS content in the autohydrolysate. It was found that feedstocks with higher C5HI (poplar and corn cob) resulted in higher XOS production. The relationship of C5HI with XOS did not vary with temperature, as higher XOS for high C5HI were found at both 180 and 200°C. Higher temperature presented higher production of XOS; however, lower temperature showed "cleaner" hydrolysate, determined by the ratio of oligomers and degraded xylan. Another aspect of this section is the demonstration that processing high C5HI feedstocks (demonstrated by poplar) at lower temperature was more efficient than using low C5HI (the case of bagasse) at higher temperature. This result shows that a more energy efficient process is possible to be obtained when the feedstock (single or mixed) is carefully selected.

7.2 Solvents production by ABE fermentation of corn syrup diluted with autohydrolysate from mixed lignocellulosic biomass

In Chapter 5, the supernatant from autohydrolysis pretreatment of mixed lignocellulosic biomass was used as a diluting agent for anaerobic fermentation of corn syrup. There had been no previous research in the literature using this strategy for ABE fermentation of corn syrup. This method can be thought of as a way to i) valorize corn syrup by producing solvents or ii) improve autohydrolysate characteristics for production of butanol. Results showed that mixing feedstocks with high C5HI (poplar and corn cob) resulted in the lowest butanol production, possibly caused by high degradation. On the other hand, high butanol production was obtained when a lower C5HI was used (poplar and wheat straw), indicating that it may be possible to determine what type of feedstocks to mix based on the feedstock characteristics (and their related C5HI) and desired products.

7.3 Potential for ethanol production by autohydrolysis pretreatment of mixed lignocellulosic biomass feedstock

The utilization of mixed feedstocks can increase the total amount of sugar recovered from the feedstock used, increasing the possible yield of ethanol produced per kg of feedstock. The analysis of different fractions of corn stover mixed with bagasse and wheat straw show improvement in the total sugar recovery after enzymatic hydrolysis compared to single feedstock. Moreover, the higher amount of monomeric sugar from mixed samples is another positive effect of processing multiple feedstocks.

8 Conclusions and recommendations

8.1 Conclusions

All five lignocellulosic feedstocks used in this project (bagasse, corn cob, corn stover, poplar and wheat straw) are suitable for autohydrolysis pretreatment and have the potential to be converted into nutraceutical xylo-oligosaccharides, solvents (acetone-butanol-ethanol) and second-generation ethanol. Depending on the feedstock characteristics and process conditions, different pretreatment products are obtained, while mixing different feedstocks may help to improve feedstock characteristics and assist in obtaining pretreatment products with more desirable characteristics. The C5HI appears to be a good indicator of xylan hydrolysis, and may be able to inform decisions regarding feedstock choice in order to produce ideal pretreatment products for further conversion.

8.2 Future work and recommendations

The results shown in this work indicate that various lignocellulosic biomass types (herbaceous, wood) are suitable for conversion into diverse valuable compounds. The process temperature is a main factor affecting xylan solubilization, where at higher temperatures, higher xylan recovery takes place, and also higher sugar degradation. Future work should focus on the advantages of using lower pretreatment temperatures to obtain “clean” autohydrolysates (containing less toxic compounds), and hemicellulose enzymes to increase xylan and total sugars recovery. To study the overall pretreatment efficiency, different fermentation strategies - separate or combined saccharification and fermentation processes – could be tested. Another potential topic is the separation of the pretreatment from the bioprocess in biorefineries and to analyze the potential for commercialization of cellulosic sugars. Finally, studies on the effect of the fertilizer on the cellulosic feedstock composition could help to produce crops with the desirable characteristics for valorization of the residues (e.g., decreases lignin production).

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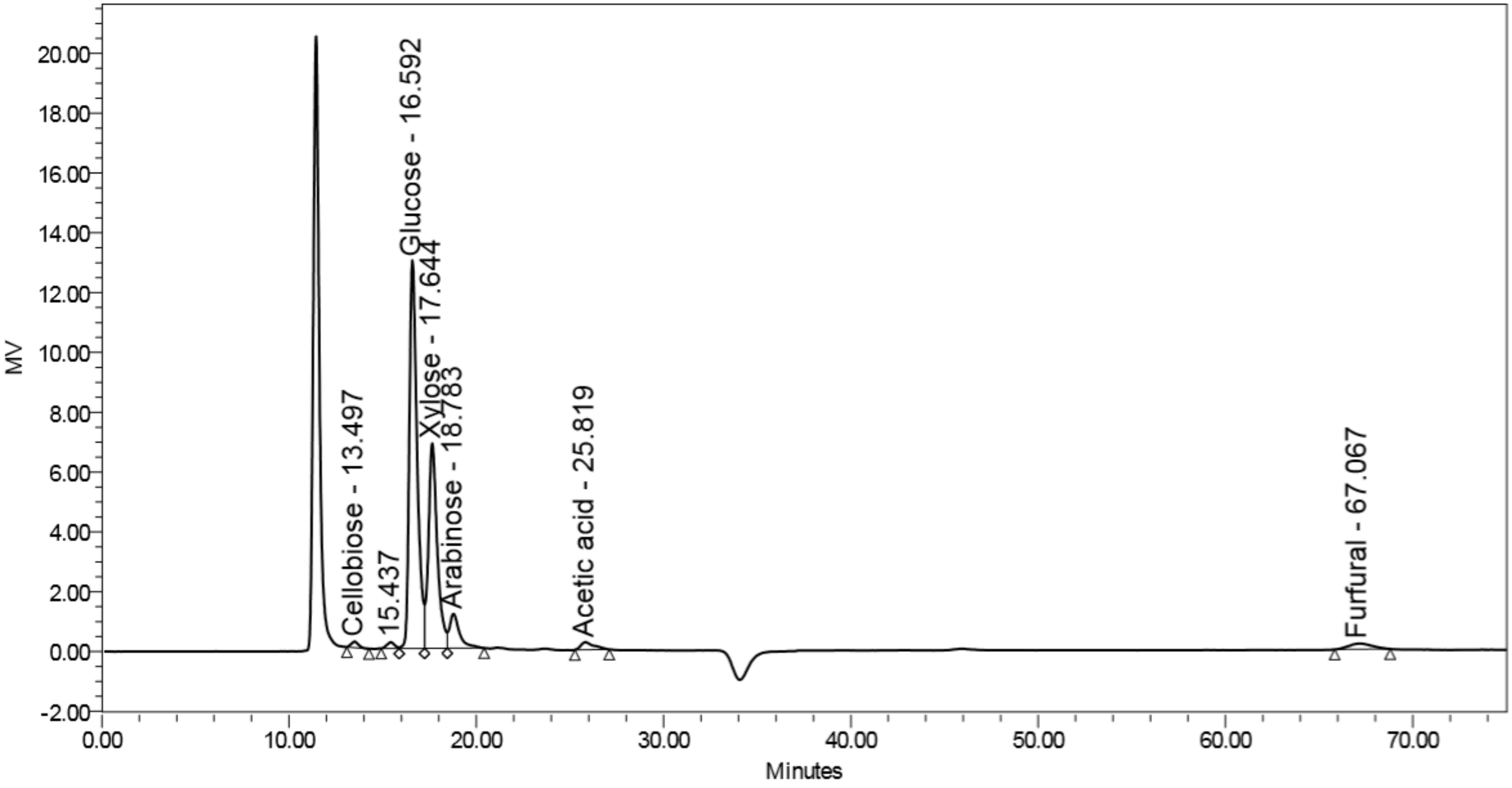
Appendix A – Composition analysis data

Feedstock composition analysis (wt.% db.)

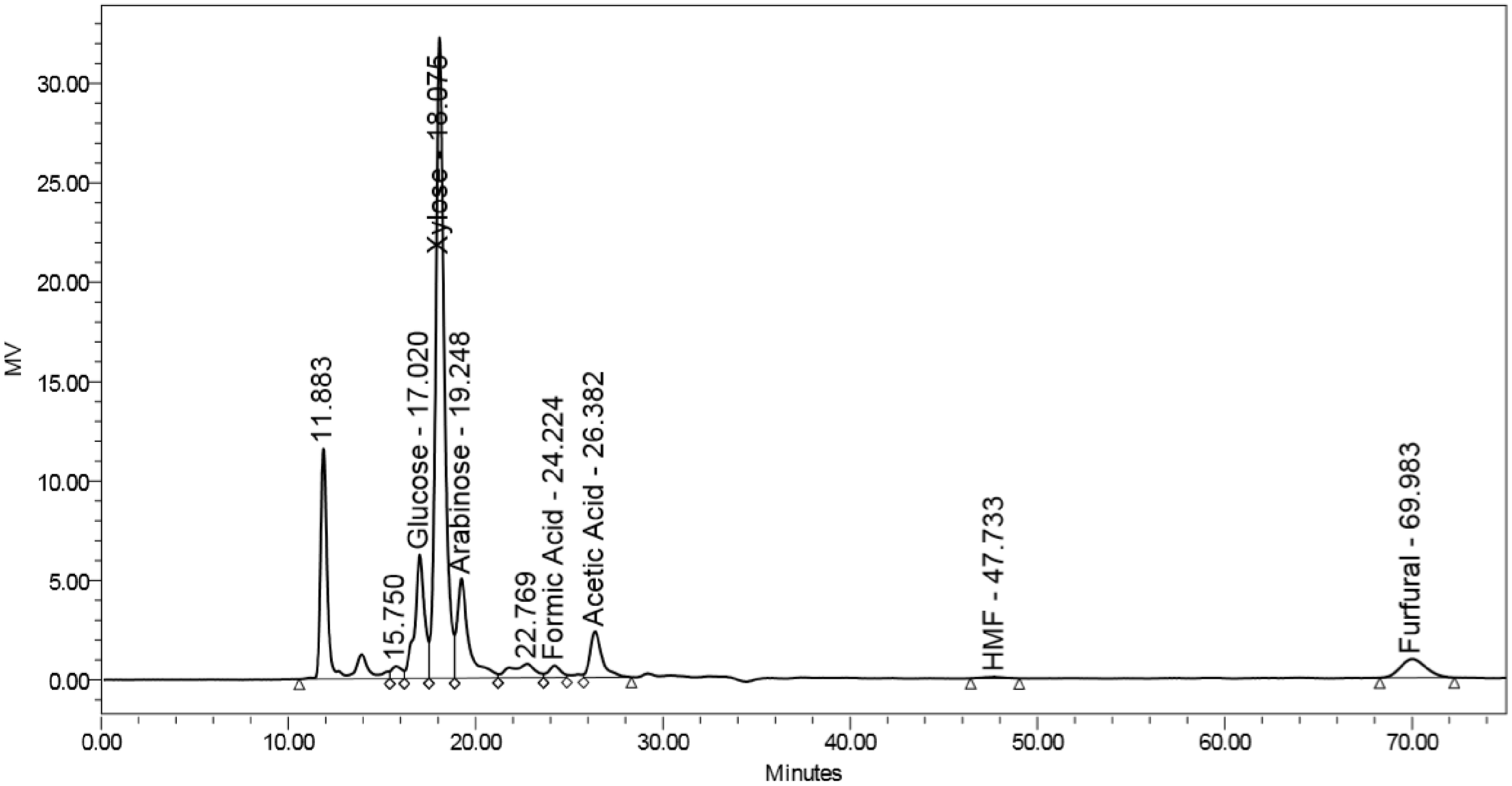
Feedstock	Rep.	Glucan	Xylan	Arabinan	Total Sugars	Acetate	Ac. Sol. Lignin	Ac. Ins. Lignin	Total Lignin	Ash	Other
Bagasse	1	41.23	23.07	1.41	65.71	3.89	1.43	24.47	25.91	1.40	3.08
	2	41.56	23.21	1.39	66.17	3.14	1.39	24.26	25.65	1.18	3.86
	3	41.39	23.14	1.40	65.94	3.52	1.41	24.37	25.78	1.29	3.47
Corn cobs	1	29.79	28.91	4.09	62.79	4.41	2.50	19.70	22.19	1.67	8.94
	2	29.17	28.36	4.19	61.72	4.52	2.56	20.12	22.68	1.30	9.78
	3	29.52	29.12	3.97	62.61	4.33	2.57	19.92	22.49	1.52	9.05
Corn stover	1	37.47	22.20	2.96	62.63	4.01	1.69	18.36	20.04	4.65	8.67
	2	37.33	22.04	3.14	62.50	3.66	1.67	18.02	19.69	5.60	8.55
	3	37.40	22.12	3.05	62.57	3.84	1.68	18.19	19.87	5.12	8.61
Poplar	1	47.26	20.09	0.87	68.22	5.96	1.30	19.23	20.52	0.33	4.98
	2	46.76	19.77	1.01	67.55	6.48	1.32	18.81	20.13	0.37	5.47
	3	47.01	19.93	0.94	67.88	6.22	1.31	19.02	20.33	0.35	5.22
Wheat straw	1	37.67	23.82	2.74	64.23	3.95	1.57	19.95	21.52	3.48	6.83
	2	37.90	24.01	2.69	64.60	3.67	1.56	20.21	21.76	3.25	6.73
	3	37.78	23.91	2.72	64.41	3.81	1.56	20.08	21.64	3.36	6.78

Appendix B – Selected chromatograms

B.1 - Typical chromatogram profile for feedstock composition



B.2 - Typical chromatogram profile for autohydrolysate after dilute acid hydrolysis



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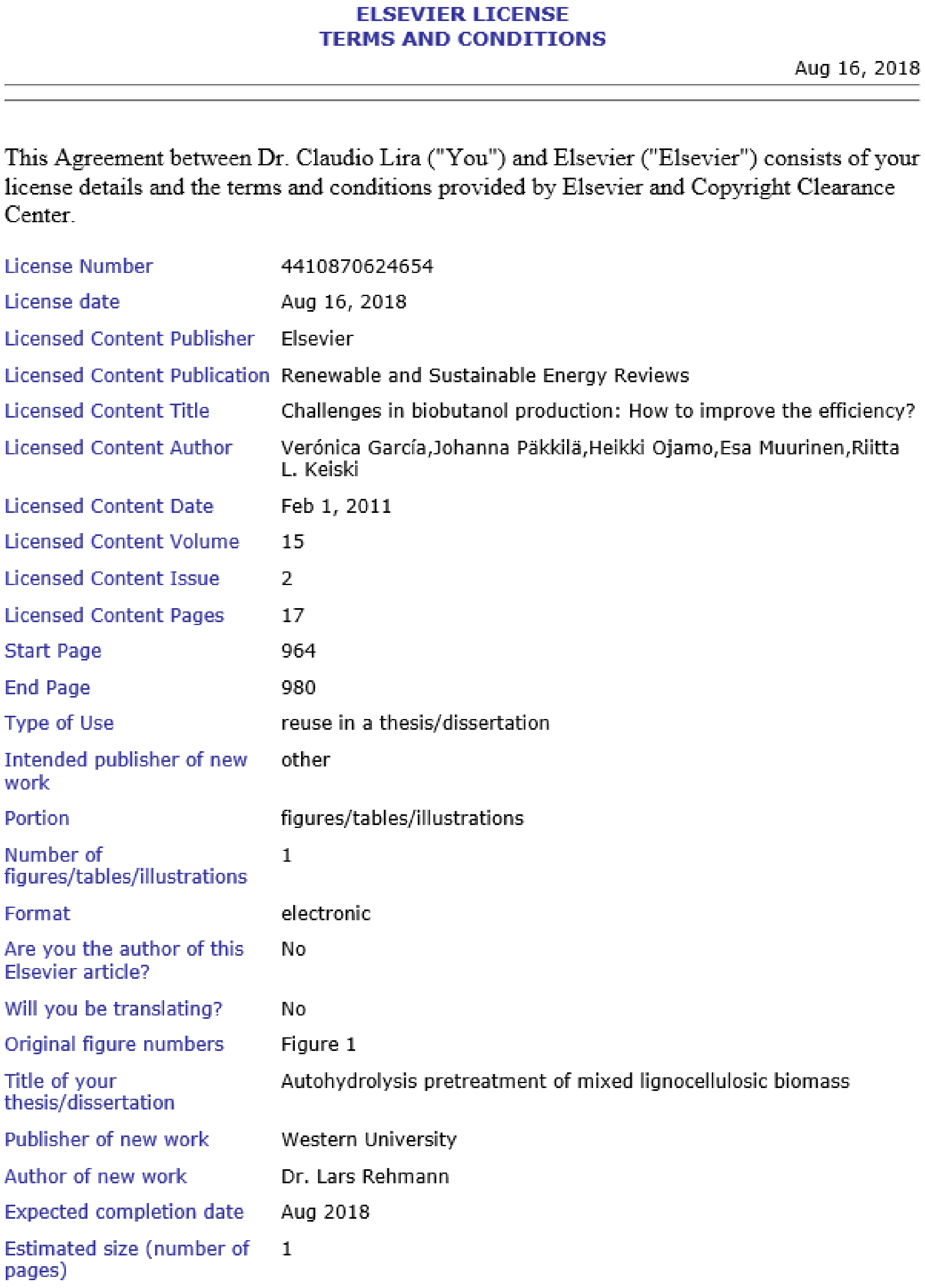

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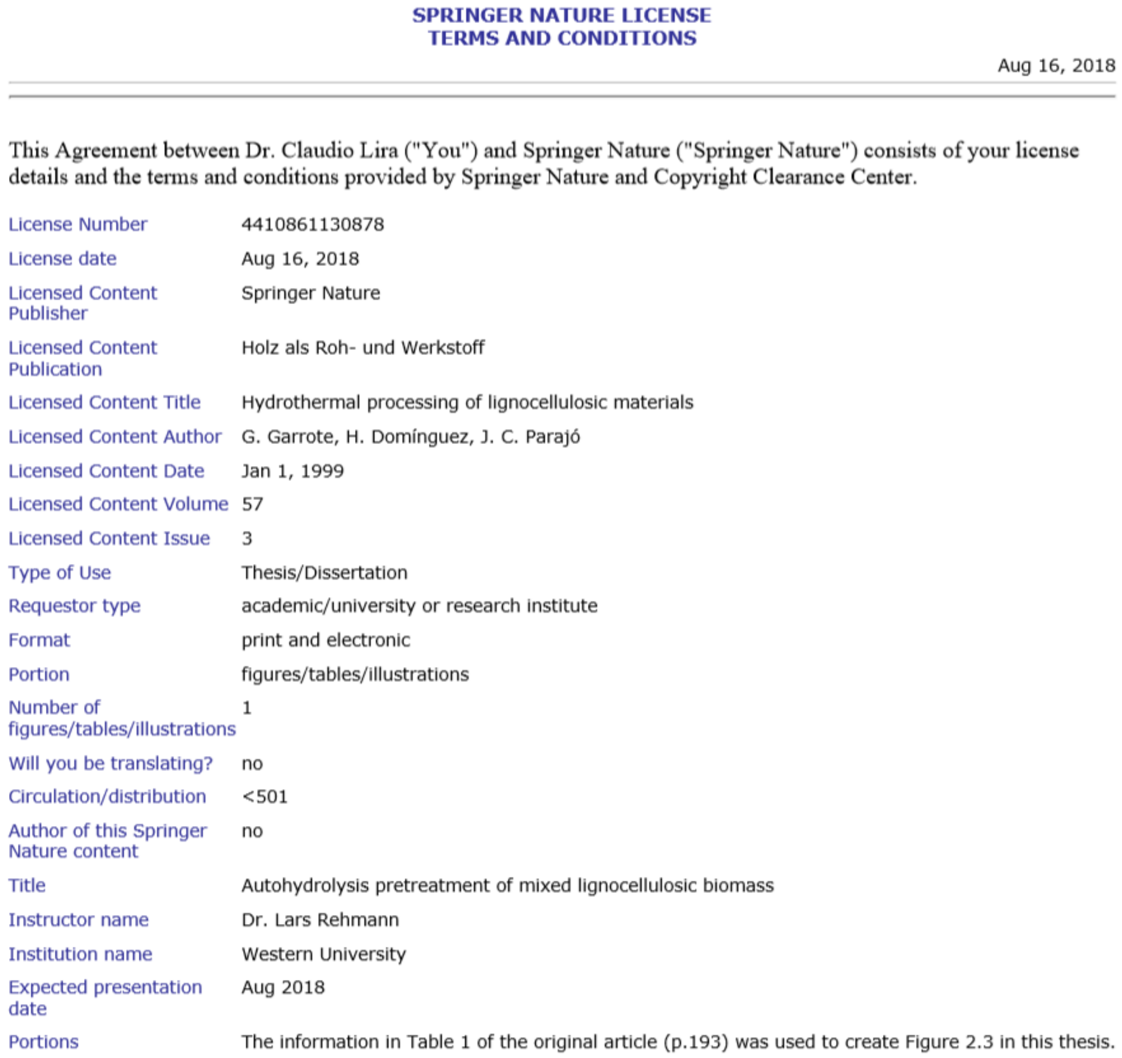
C.2 – Garrote *et al.* (1999)



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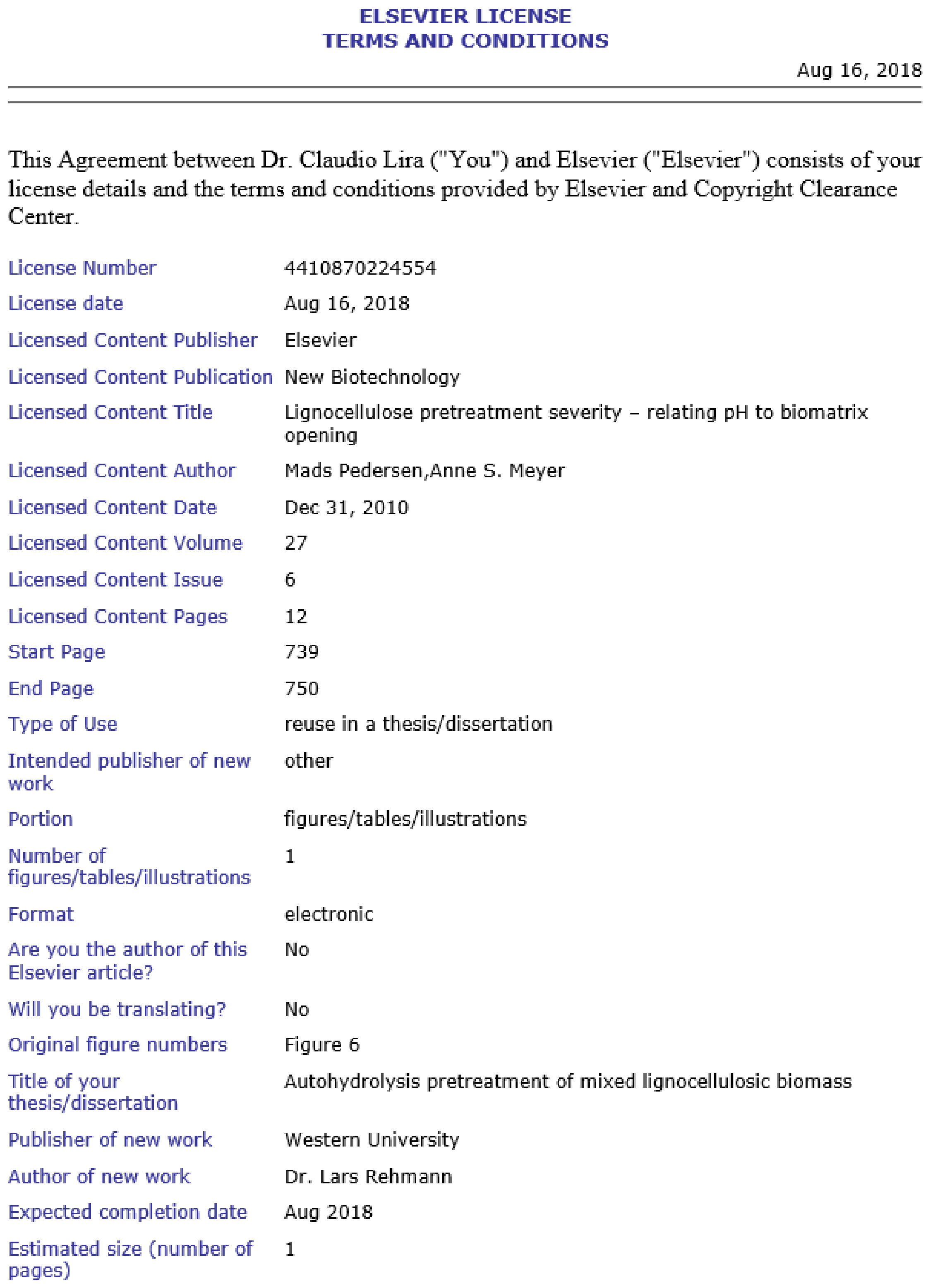
C.3 – Pedersen and Meyer (2010)



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