Development of a propylene glycol-based organosolv process for biomass fractionation

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Chemical Engineering

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Declaration

I declare that this document is an original work of my own authorship and that it fulfills all the requirements of the Code of Conduct and Good Practices of the Universidade de Lisboa.

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Resumo

A etapa de pré-tratamento representa uma fração substancial dos custos operatórios do processamento da biomassa, representando um enorme impacto no desempenho económico e ambiental das biorefinarias. Assim, o desenvolvimento das estratégias de pré-tratamento é visto como uma peça-chave no impulsionamento da competitividade económica da conversão da biomassa.

Nomeadamente, o propilenoglicol é considerado um solvente renovável, seguro, não-inflamável e não-tóxico, preferível a outros solventes mais tradicionais, como o etanol e a acetona.

Neste trabalho, um processo organosolv com propilenoglicol foi avaliado para a desconstrução de resíduos de eucalipto e palha de trigo. Foi estudado o efeito das condições de pré-tratamento na solubilização dos diferentes componentes da biomassa e na sua digestibilidade enzimática, bem como o efeito de catalisadores e da transição para operação em contínuo.

No tratamento com uma solução aquosa com 75% de propileno glicol e 0,5% de ácido sulfúrico, a 160ºC durante 15 minutos, obteve-se solubilizações de xilano e lenhina acima de 80%, em simultâneo com recuperações de celulose de 90%, revelando uma digestibilidade enzimática de 89%. A operação em contínuo resultou numa maior solubilização de xilano e lenhina, acompanhada por uma menor formação de produtos de degradação. Além disso, permitiu ainda a recuperação de 60 a 80% da lenhina inicial, na forma sólida, simplificando os processos de separação e recuperação de solvente a jusante.

Estes resultados revelam o potencial do processo proposto para competir com outros processos organosolv, dado que apresenta um desempenho semelhante em condições operatórias mais suaves, com o respetivo ganho em custos de capital e operatórios.

Palavras-chave: bioeconomia circular; biomassa lenhocelulósica; biorefinaria; pré-tratamento organosolv; propilenoglicol; solvente não-volátil.

Abstract

Biomass pretreatment represents a substantial fraction of biomass processing costs and has great impact on economic and environmental performance of the biorefinery. Hence, improvements in pre-treatment strategies are widely recognized as a key way to improve the economic competitiveness of biomass conversion.

Particularly, propylene glycol (PG) is a green, high-boiling, safe, non-toxic and non-flammable solvent, less hazardous than most commonly used solvents in organosolv processes, such as ethanol and acetone.

In this work, a PG-based organosolv pretreatment was evaluated for the deconstruction of eucalyptus residues and wheat straw, as model feedstocks. Pretreatment conditions and their effect on the solubilization of lignocellulosic components and enzymatic digestibility of pretreated solids were discussed, as well as the effect of catalyst use and transition to flow-through operation.

Xylan and lignin solubilization values above 80% were obtained for pretreatment at 160ºC for only 15 minutes with 75%PG and 0.5% of sulfuric acid. For those conditions, cellulose recovery reached 90% in the pretreated solids, resulting in 89% enzymatic digestibility. Flow-through operation mode enabled the solid recovery of 60-80% of total lignin in feedstocks, simplifying downstream process design. Furthermore, flow-through regime allowed an increased lignin and xylan solubilization, as well as reduced formation of degradation products, when compared to batch operation.

These results suggest the ability of the proposed process to compete with other organosolv methods, as it originates similar yields at much milder operation conditions, with the respective gains in capital and operational costs.

Keywords: circular bioeconomy; lignocellulosic biomass; biorefinery; organosolv pretreatment; propylene glycol; high-boiling solvent.

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Notation

AFEX	Ammonia fiber explosion
AOS	Arabino-oligosaccharides
ASAM	Alkaline-sulfite anthraquinone methanol pulping
ASE	Accelerated Solvent Extractor
CS	Combined Severity factor
DAD	Diode array detector
DP	Degree of polymerization
ER	Eucalyptus residues
FPU	Filter paper units
FT	Flow-through
GOS	Gluco-oligosaccharides
HMF	Hydroximethylfurfural/ 5-(hydroxymethyl)furfural
HPLC	High-pressure liquid chromatography
IL	Ionic liquids
LHW	Liquid hot water
LM	Lignocellulosic materials
Log R_O	Severity factor
LSR	Liquid-to-solid ratio
MIBK	Methyl isobutyl ketone
NREL	National Renewable Energy Laboratory (USA)
PG	Propylene glycol/ Propane-1,2-diol
R^2	Coefficient of correlation
RID	Refractive index detector
TFA	Trifluoroacetic acid
UV	Ultraviolet radiation
VOC	Volatile organic compounds
WS	Wheat straw
wt%	Mass fraction of a component, in percentage
XOS	Xylo-oligosaccharides

Framework

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Thesis structure

This dissertation comprises seven chapters. The first Chapter includes a general introduction to the biorefinery concept as a way to upgrade biomass into high-value products. The composition of lignocellulosic biomass is enlightened and the state of the art of lignocellulose fractionation is presented, with focus on the organosolv technology. The chapter ends by establishing the objectives of the developed work.

Chapter 2 describes the common materials and experimental procedures applied throughout the following chapters, including sample preparation and analytical methods applied.

Focusing on the study of biomass pretreatment, the results section is divided into Chapters 3, 4, 5, and 6. Chapter 3 presents the evaluation of propylene glycol organosolv pretreatment for the deconstruction of two model lignocellulosic materials, while Chapter 4 discusses the effect of the use of catalysts in combination with the organosolv process.

Chapter 5 aims to make the transition from batch to flow-through mode of operation, by comparing results of both configurations for autohydrolysis of a model lignocellulosic material and as a way to contribute to process intensification. Chapter 6 picks up the conclusions from Chapter 5 and pursues

the application of the flow-through advantages realized for auto-hydrolysis to the organosolv process developed in Chapters 3 and 4.

Finally, Chapter 7 summarizes the main findings and discusses future work.

Publications by chapter

The present work has already resulted in the following publications.

Chapter 3

Sampaio B., Moniz P., Roseiro L.B., Pinto F., Carvalheiro F., Duarte L.C. 2019. 'Propylene glycol-based biomass deconstruction'. In Carvalho MdG, Scarlat N, GrassI A, Helm P (ed), *European Biomass Conference and Exhibition Proceedings* doi:10.5071/27thEUBCE2019-3CV.5.21.

B. Sampaio, F. Pinto, C. Oliveira, C. F, D. L.C., Avaliação do potencial de valorização de biomassa agroflorestal residual por um processo organosolv baseado em propilenoglicol, submitted to the Congresso CIES2020Lisboa, Portugal, 2020.

Chapter 4

B. Sampaio, F. Pinto, F. Carvalheiro, L.C. Duarte, Catalyzed propylene-glycol -based organosolv process for efficient lignin and hemicellulose recovery, submitted to the 28th European Biomass Conference & Exhibition. Marseille, France, 2020.

Chapter 5

Sampaio B., Vicente D., Duarte LC., Pinto F., Oliveira C., Carvalheiro F., Van-Dúnem V., Marques J., Costa P., André R. 2019. Autohydrolysis of Lignocellulosic Biomass Under Diverse Operation Regimes, Bioenergy International Conference 2019, Portalegre, Portugal.

Chapter 6

B. Sampaio, F. Pinto, C. Oliveira, F. Carvalheiro, L.C. Duarte, Intensification of a propylene glycol-based organosolv process under continuous flow-through operation, submitted to the 28th European Biomass Conference & Exhibition. Marseille, France, 2020.

1. General introduction

For centuries, since the industrial revolution in the 19th century, the world has been dominated by an economy in which fossil fuels take center stage. However, these days, fossil fuels suffer from major drawbacks, such as their finite supply, their role on greenhouse gas emissions and climate change and increasing price and unexpected fluctuations (Haghighi Mood et al. 2013). Furthermore, the global population keeps growing to unprecedented numbers and with it the demand for energy and chemical supplies (Zhang et al. 2016). Moreover, global concern about sustainability and environmental preservation has become a necessity in response to the escalation of extreme climate events. In fact, the Paris' Agreement, signed by 196 state parties, devised the objective of limiting the increase in average global temperature to 1.5ºC when compared to pre-industrial levels (UNFCCC 2015), while the EU has defined a long-term strategy to become climate-neutral by 2050. So far, the EU is to meet the target of 20% reduction of greenhouse gas emissions of its 2020 climate & energy framework (European Commission 2008), with already a reduction of 22% between 1990 and 2017, in spite of a 58% growth of the economy in the same period, proving that economic growth is not coupled with GHG emissions and that climate action can be associated with the development of new industries, jobs and technologies. By doing so, the EU leads the world in the fight against climate change, a world that has been seeing an increase in the mobilization of extensive scientific effort to find alternatives to the common fossil fuel-based economy. Namely, biomass is taking a center role as it is a renewable, cheap, widely available, versatile, carbon neutral resource with the potential to provide the wide range of products traditionally obtained from fossil fuels via the implementation of the biorefinery concept (Figure 1). Biorefineries are to play a major role in key strategic aspects for climate neutrality, of the nine proposed by the EU, namely, maximization of deployment of renewable energies, maximization of energy efficiency, transition to a circular economy, investment in clean and safe mobility and taking the most benefit from the bioeconomy (European Commission 2019).

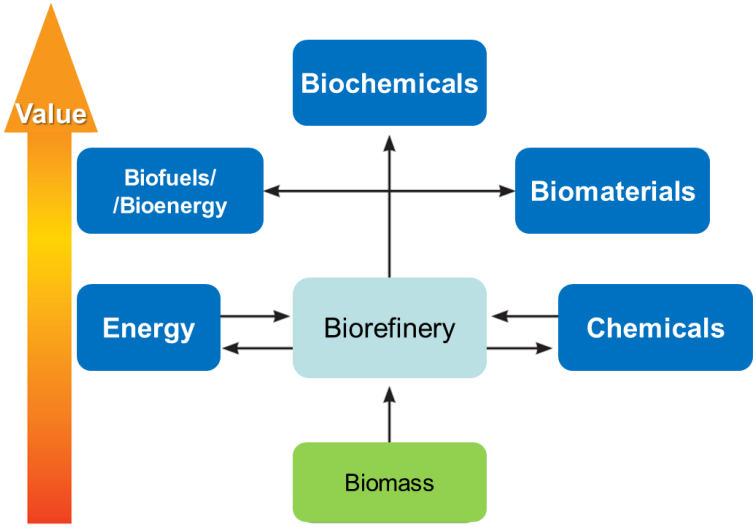

Figure 1. The biorefinery concept according to (Carvalheiro, Duarte, and Gírio 2008)

The International Energy Agency (IEA), in IEA Bioenergy Task 42 “Biorefining in a Circular Economy” which main purposes are to facilitate the commercialization and market deployment of biorefinery systems with a benefic impact on environment, society and economy and to advise policy and industrial decision makers accordingly (IEA Bioenergy July 2019), defines biorefinery as “the sustainable processing of biomass into a spectrum of marketable products (food, feed, materials, chemicals) and energy (fuels, power, heat)”, analogous to the petroleum refineries it aims to substitute. Furthermore, IEA acknowledges the “important role of biorefineries in the transition towards a sustainable circular economy”, which entails to the mitigation of waste and pollution, the maintenance of products and materials in use and the regeneration of natural systems, contrasting to a linear economy paradigm. In fact, biorefineries have the potential for the reuse and valorization of waste biomass, while achieving a more balanced carbon cycle and being able to produce renewable energy as well as a wide range of well cascaded bioproducts, which even include feedstocks for plastics, allowing for a complete replacement of the petroleum refineries (Bos, Annevelink, and Ree 2017). As illustrated in Figure 1, the main purpose of biorefineries in the circular economy is to add value to widely available biomass feedstocks. Additionally, the represented recycling of energy and chemical used to convert biomass into high-value products in the biorefinery is the key aspect which decides its sustainable character.

Biorefineries can be grouped accordingly to their main processing strategy in three groups: biochemical, thermochemical and microorganism (Hwang et al. 2014). The thermochemical platform uses direct gasification or pyrolysis of biomass to produce syngas, bio-oil and char to be used as fuels directly or converted and upgraded to other products. The microorganism platform involves the production of biofuels from algae and other live microorganisms, such as the production of biodiesel from algae and methane gas by anaerobic microorganisms. However, the most widely spread platform is the biochemical platform, also known as sugar platform, which focus on the recovery of sugars from biomass that can then be subjected to enzymatic and fermentation steps to yield a variety of products and fuels, typically separated via distillation, such as for bioethanol. The biochemical platform was the focal point of this work’s scope and was further analyzed in the following sections.

In terms of the feedstocks applied in the biorefineries, which include plant-based materials, waste biomass and compost, and resulting products, it is believed that each biorefinery process should be able to accommodate the widest range possible of both groups, as a key way to improve its robustness to changes in feedstock supply and cost and its economic viability by complete valorization of the whole biomass ('Bio-based industries Joint Undertaking (BBI JU)' 2016). As such, the optimum design of a biorefinery should be able to accommodate the concepts of multi-feedstock, multi-process and multi-product (Kamm and Kamm 2007).

1.1. Lignocellulosic Feedstocks

Lignocellulosic biomass is the most widely available type of raw material that can be upgraded to various products in a biorefinery. As raw material for the biorefinery, it presents the advantages of renewable, low cost and available worldwide. It is composed mainly of three biopolymers (cellulose, hemicellulose and lignin) linked together in a complex structure (Figure 2) that resists (bio)chemical conversion – biomass recalcitrance.

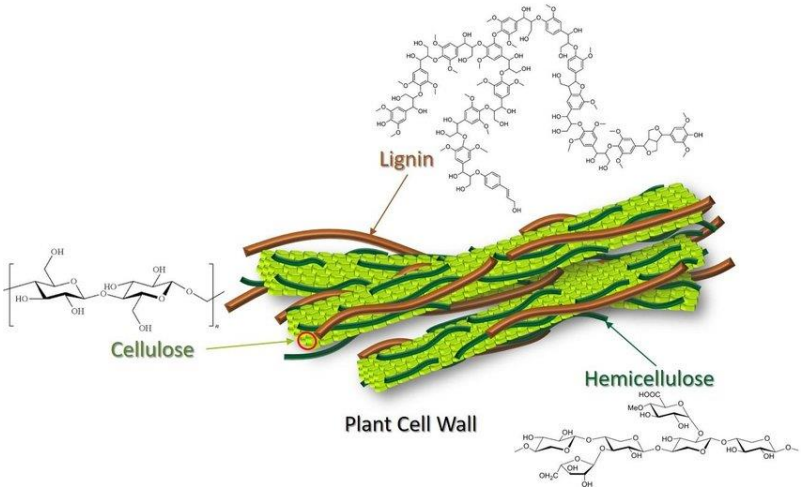

Figure 2. Lignocellulosic structure (Jensen et al. 2017)

Cellulose, the most abundant biopolymer in the world and the major structural fiber in plant biomass is an unbranched homopolymer of glucose monomers (hexoses) linked by β-1,4-glycosidic bonds. It constitutes 40-50% of dry biomass (Finch 1986). Cellulose chains are aggregated into microfibrils of 3-5 nm (diameter) by hydrogen and van der Waals bonds, alternating between crystalline (predominant) and amorphous regions. The microfibrils are then encapsulated into larger structures, macrofibrils of 50-250 nm (diameter), in which cellulose is interconnected to hemicellulose via hydrogen bonds, residing within a complex non-crystalline matrix of hemicellulose and lignin (Zhang et al. 2016).

Hemicelluloses are branched heteropolymers formed mainly by pentoses (xylose, arabinose), but also by hexoses (mannose, glucose, galactose) and acids (uronic acids, acetyl groups). These polysaccharides form up to 25-35% of biomass dry weight (Finch 1986). Differently from cellulose, hemicellulose is an amorphous polymer that presents a very variable composition, in terms of both backbone and substituents, from plant to plant and is able to form multiple hydrogen bonds with itself and with cellulose. Hemicelluloses are also shorter than cellulose chains and are much easily hydrolysable. Hemicelluloses include xyloglucans, xylans, mannans, glucomannans, arabinoxylan and β-(1→3,1→4)-glucans. The major component of hemicelluloses in LM is xylose, although in softwoods mannose can be the most abundant sugar (Zhang et al. 2016).

Lignin has an aromatic three-dimensional amorphous crosslinked polymer structure of alkylphenols, with molecular weight ranging from 2000 to 15000g/mol (Demirbas 2004). Three basic units are found in lignin structure (Figure 3): p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S). These units are derived from three monomeric precursors: p-coumaryl, coniferyl and sinapyl alcohols, with zero, one and two methoxy groups in the aromatic ring, respectively. Those structural units are linked by a variety of ether, (e.g., α-O-4, β-O-4, 4-O-5) and carbon-carbon bonds (e.g., β-β, β-5, 5-5). Lignin is covalently linked to hemicellulose, creating the lignocellulosic network rigidity. From all the lignocellulose constituents, lignin is the one that varies the most in composition and complexity between different biomass feedstocks and its structure is not yet perfectly studied, mostly due to the inability to isolate lignin in its native form as each pretreatment type results in a different lignin structure.

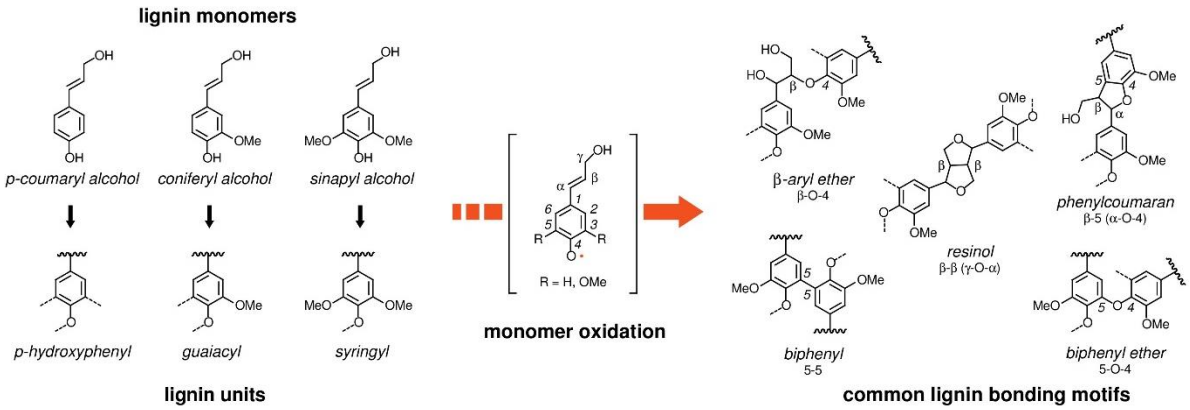

Figure 3. Lignin structure: monomeric precursors and typical bonds (Brown and Chang 2014)

Lignin accounts for 20 to 30 percent of total lignocellulosic materials, providing cell walls with mechanical support, waterproofing and resistance to pathogen attacks (Nge et al. 2018). It is insoluble in water and strongly resistant to chemical reactions. The higher the content in S units, the greater the solubility of lignin in water and the lower its re-condensation during pretreatment, as a result of increased polarity conferred by the methoxy groups. As such, softwoods are more resistant to fractionation than hardwoods, given the combined effect of higher content in G units of softwood lignins and generally higher lignin percentage in softwoods (Nitsos, Rova, and Christakopoulos 2017). Lignin is covalently linked with xylans in hardwoods and with galactoglucomannans in softwood (Demirbas 2004). It has applications in the production of glues, foams, emulsifiers, adsorbents, polymer composites, plywood, raw material for carbon nanofibers, among many other applications (Schutyser et al. 2018). Demirbas (Demirbas 2004) evaluated lignin potential as adsorption material to remove heavy metals from waste waters. Lignin depolymerization and targeted upgrading can even give a wider variety of chemicals and fuel components in the future (Schutyser et al. 2018; Renders et al. 2017).

1.2. Pretreatment Review

The use of lignocellulosic biomass to produce valuable products (exemplified in Figure 4), namely fuels, chemicals and bio-ethanol, through the biochemical platform, is heavily constrained by its recalcitrant nature induced by the intricate structure in which it appears. Particularly, the enzymatic saccharification of biomass structural polysaccharides to originate monosaccharides, a key step for the production of a variety of bioproducts from lignocellulose via microbial fermentation or chemical reactions, is impractical to be applied directly to the raw feedstock, resulting in very low yields of sugars (Zhang et al. 2016). In fact, cellulose breakdown to glucose is inhibited by its crystallinity and as the lignin and hemicellulose rigid structure prevent the cellulolytic enzymes from accessing the cellulosic substrate (Haghighi Mood et al. 2013). Accessible surface area, pore size and volume, particle size, specific surface area, chemical compositions in lignin, hemicelluloses, acetyl group and cellulose, cellulose's degree of polymerization, lignin structure (S and G units content and aryl ether linkages content) also play an important role in biomass recalcitrance (Zhao et al. 2017; Herbaut et al. 2018).

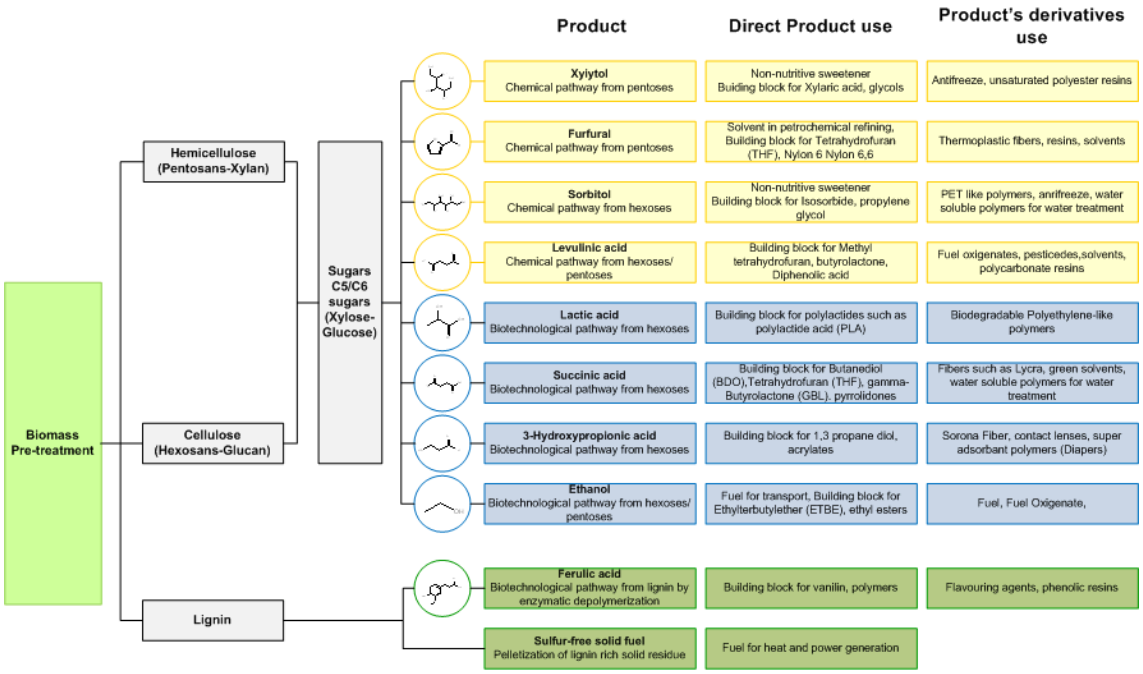

Figure 4. Examples for applications/valorization of products obtained from lignocellulosic materials (adapted from (Villegas and Gnansounou 2008))

Therefore, a pretreatment step is required to disrupt the cell wall structure of lignocellulosic biomass and separate its components, making them more susceptible to further processing and valorization, drastically enhancing the economic viability of enzymatic saccharification and, consequently, of the biorefinery. An effective pretreatment should disrupt the hydrogen bonds of crystalline cellulose, breakdown the matrix of lignin and hemicelluloses and increase the surface area and porosity of cellulose for enzymatic hydrolysis (Haghighi Mood et al. 2013). Delignification increases enzymatic digestibility of substrate by removing a physical barrier to cellulolytic enzymes, but also because lignin/phenolic compounds can non-productively adsorb those enzymes, acting as inhibitors to enzymatic hydrolysis (Zhao et al. 2017).

For the pretreatment process to be effective and economically viable, it must ensure: (i) high sugar yields for the fermentation step; (ii) low concentration of fermentation steps inhibitors (aliphatic acids, furans and phenolic compounds typically generated during pretreatment – acids and furans from sugar degradation/dehydration and phenolic compounds from lignin breakdown) (dos Santos et al. 2011); (iii) recovery of hemicellulose and lignin for later use in the production of valuable by-products; (iv) low demand of post-pretreatment operations such as washing and neutralization; and (v) minimal energy, chemicals and water inputs (Kucharska et al. 2018). Pre-treatment represents a substantial fraction of biomass processing costs (20-40%) (Beisl et al. 2017) and has great impact on economic and environmental performance (Archambault-Leger, Shao, and Lynd 2012; Liu and Wyman 2004). Hence, improvements in pre-treatment strategies are widely recognized as a key way to improve the economic competitiveness of biomass conversion (Archambault-Leger, Shao, and Lynd 2012).

Pretreatments can be divided into mainly four categories: mechanical, chemical, physicochemical and biological pretreatment. Table I provides a general comparison between existing pretreatment processes, in terms of the most relevant parameters related to their performance.

Table I. Comparison of available processes for pretreatment of lignocellulosic materials (Haghighi Mood et al. 2013; Carvalheiro et al. 2013; Girio et al. 2010)

Pretreatment Parameters	Mechanical Pretreatments	Chemical Pretreatments					Physicochemical Pre-treatments				Biological Pretreatments
		Acid	Alkaline	Organosolv	Ozonolysis	Ionic liquids	Steam explosion	LHW	AFEX	CO2 explosion	
Temperature	-	+	-/0	-/+	-	-/0	+	+	0	0	-
Pressure	N/A	+	-	-/+	-	-	+	+	+	+	-
Increase specific area	+	+	+	+	+	+	+	+	+	+	+
Hemicellulose removal	-	+	0	+	-	0	+	+	0	+	-
Lignin removal	-	0	+	+	+	0	-	-	+	-	+
Lignin quality	N/A	-	-	+	ND	+	0/+	+	+	ND	ND
Cellulose removal	-	-	-	-	-	-/+	-	-	-	-	-
Enzymatic digestibility	0	+	+	+	+	+	0	+	+	+	0
Inhibitors formation	-	+	-	-	-	-	+	0	-	-	-
Cellulose de-crystallization	+	-	-	ND	ND	+	-	ND	+	-	0
Energy requirements	+	+	-	-/+	0/+	0	+	+	+	+	-
Equipment corrosion	-	0/+	+	0	+	-	-	-	+	-	-
Residue formation	-	+	-	-	-	-	-	-	-	-	0
Catalyst recovery	N/A	Hard	Easy	M	M	M	N/A	N/A	M	M	N/A
Capital investment	0/+	-	-/0	0	+	-	+	-	+	+	0
Operation costs	+	0	0/+	0/+	+	+	-	-	+	+	+
Pilot plant deployment	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No	Yes	No

+: high; -: low; 0: moderate; N/A: not applicable; ND: non-demonstrated/not-found; M: mandatory

Mechanical pretreatments like grinding, milling, freezing, radiation, or extrusion have the purpose of reducing particle size and increasing the surface are of lignocellulosic biomass, while also decreasing the crystallinity and degree of polymerization of cellulose. Typically, these methods are applied previously or simultaneously to other pretreatments, as their intensive energy requirements make them economical unsuitable to be used on their own (Haghighi Mood et al. 2013; Zhang et al. 2016).

In the radiation pretreatments category, emphasis has been given to microwave retreatment, as

an alternative method to conventional heating, which results in less energy input and therefore lower operation costs. Additionally, microwave pretreatments benefit from shorter residence times. Microwave irradiation has been combined with many other pretreatments as an alternative heating method (Haghighi Mood et al. 2013).

Chemical pretreatment comprises acid, alkaline, organosolv, ozonolysis and ionic liquids pretreatments, all of which are performed at temperatures above room temperature.

In acid pretreatment, mainly the polysaccharides fractions of biomass are affected, as they are hydrolyzed to oligo and monosaccharides in acid media. The mechanism of acid hydrolysis of the glycosidic bonds, illustrated in Figure 5 for cellulose (analogous for hemicelluloses), involves three major steps: 1) quick reversible protonation of the oxygen of the glycosidic bond, followed by 2) the rupture of the same bond with the irreversible slow formation of a stable oxocarbenium ion represented as a resonance structure between the carbocation and the oxonium ion and finally 3) the quick irreversible regeneration of the hydronium cation by reaction of the carbocation with a water molecule (Belkacemi et al. 1991). Hydrolysis of cellulose and hemicelluloses occurs in the same manner, however the highly ordered crystalline structure of cellulose sustained by strong intermolecular hydrogen bonds makes its hydrolysis much more difficult than for hemicelluloses (Girio et al. 2010).

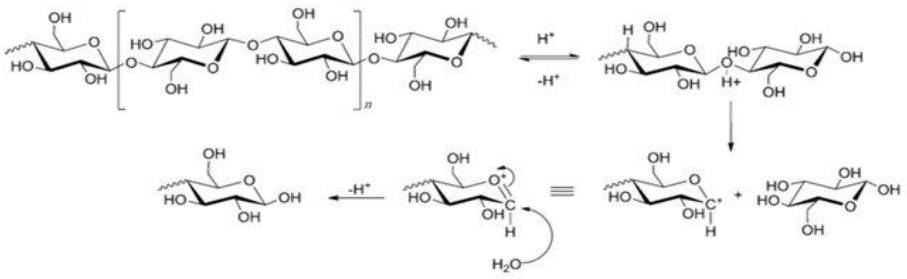

Figure 5. Mechanism of acid hydrolysis of cellulose to glucose via carbocation formation as described in (Muhaimin et al. 2018)

For acid pretreatment of LM, sulfuric acid is the most utilized, though hydrochloric, nitric, fosforic and trifluoroacetic (TFA) acids are also applied (Girio et al. 2010). Either low acid concentrations and high temperatures or high acid concentrations and low temperatures can be applied. Concentrated acid treatment typically utilizes 72% H2SO4, 41% HCl, 85% H3PO4 and 100% TFA at atmospheric pressure and temperatures around 20-50ºC for relatively short times of 20 to 60 minutes. Concentrated acids can disrupt the bonds of lignin-carbohydrate complexes and solubilize both hemicellulose and cellulose polysaccharides via the breakage of their hydrogen bridges, while weakly hydrolyzing them to small oligosaccharides. Concentrated acid pretreatment benefits from the additional advantage of no formation of degradation products (Girio et al. 2010; Zhang et al. 2007), as HMF and furfural. More information on sugar degradation reactions can be found in Supplementary material C. For dilute acid pretreatment, only hemicelluloses are hydrolyzed at typical conditions of 0.5-6% acid concentration and temperatures between 121 and 160ºC. Cellulose hydrolysis is not pursued for dilute acid pretreatment, as it would require very extreme temperatures of 230-240ºC, that would dramatically increase sugar degradation and operation costs. Instead, the remaining solid enriched in cellulose is submitted to enzymatic hydrol-

ysis to recover the hexose sugars. Dilute acid pretreatments are also poorly effective for lignin solubilization, as lignin precipitates in acidic media. In spite of the high operation costs associated with higher temperatures, the dilute acid treatment is preferred due to the toxicity and corrosion problems of using concentrated acids, as well as their difficult and costly recovery. Both concentrated and dilute acid processes require acid removal or neutralization steps before downstream fermentation (as acidic environment inhibits the fermentation step), which results in the negative production of large amounts of waste (Haghighi Mood et al. 2013; Girio et al. 2010).

Alkaline pretreatment is known to improve the removal of lignin and acetyl groups, though the removal of hemicelluloses is less effective than for acid pretreatment. Lignin is more extensively removed in alkaline pretreatment as it is very soluble in alkaline solutions – due to its high hydroxyl content, while being insoluble in acid solutions. Alkaline pretreatment also causes swelling of fibrous cellulose which leads to the disruption of crosslinking between structural components and increased biomass porosity. Comparatively to acid hydrolysis, alkaline pretreatment has the advantages of using lower temperatures (30-130ºC) and producing less or none degradation products. However, long residence times (hours to days) and slurry neutralization are required. The most used alkalis are sodium (soda) or potassium hydroxide, calcium hydroxide (lime) and ammonia (Haghighi Mood et al. 2013), with soda being the most effective. Pretreatment with lime is cheaper, poses less safety hazards compared to soda and potassium hydroxide and is more easily recovered via reaction with carbon dioxide, despite being less effective. Alkaline pretreatment is reported to be more effective for non-woody feedstocks, such as agricultural residues. Addition of an oxidant agent (as oxygen gas or hydrogen peroxide) to alkaline pretreatment can widely improve pretreatment performance by enhancing lignin (Girio et al. 2010). Alkaline pretreatments are very costly due to the costly downstream processes necessary for the recovery/neutralization of alkalis. Some alkali is negatively converted to irrecoverable salts or incorporated as salts into biomass (Carvalheiro, Duarte, and Gírio 2008). Additionally, large amounts of water are applied (Maurya, Singla, and Negi 2015).

An alkaline pretreatment already well established is applied in the pulp and paper industry since 1890 – the Kraft process – to produce a cellulose rich pulp for paper production while yielding a black liquor enriched in lignin which is used as solid fuel after recovery. The Kraft process applies a blend of sodium hydroxide and sodium sulfide at temperatures between 150 and 170ºC. The hydroxide (OH-) and sulfide (SH-) ions are responsible for breaking down the C-O-C bonds of lignin structure, that allow for its removal from the pulp, while preserving the more resistant C-C bonds. The originated lignin, known as Kraft lignin, accounts for 85% of global lignin production and incorporates multiple thiol groups in its structure (-SH) which hinder its valorization (Chen 2015).

Ionic liquids (IL) can also be used as solvents for lignin and hemicelluloses from lignocellulosic biomass. They are organic salts composed typically of small inorganic anions and large organic cations that melt under 100ºC, making them liquids at pretreatment conditions. The selection of different cation and anion combinations allows for great variability of available IL with different properties and behaviors in terms of their effect on the treated biomass. Currently, imidazole-based IL are the focus of a great deal of attention. Advantages of ionic liquids include high thermal and chemical stability, low vapor pressure that makes them environmentally friendly and safer to use, and the requirement of mild operation

conditions, i.e. temperature between 100 and 150ºC. IL are able to form hydrogen bonds with cellulose at high temperatures because of the presence of anions like chloride, formate, acetate or alkyl phosphonate and show great potential for pretreating lignocellulosic materials, resulting in more than 90% cellulose digestibility (Maurya, Singla, and Negi 2015). Industrial application of ionic liquids is limited by their incompatibility with cellulase enzymes, their high prices, the high energy requirements associated with their recycling and the increased viscosity of the reactional mixture during pretreatment (Haghighi Mood et al. 2013).

Physicochemical pretreatments include steam explosion, liquid hot water, ammonia fiber explosion, wet oxidation and CO2 explosion.

Liquid hot water (LHW) resorts to water at high temperatures (160-220ºC) and pressure (above 50bar, used to maintain the liquid state) for about 15min of residence time without the use of any catalysts (Carvalheiro et al. 2016). The underlying mechanism of LHW is auto-hydrolysis, similar to acid hydrolysis (see acid pretreatment), but with no addition of acids, as the hydronium cation from water auto-ionization and the organic acids liberated from the lignocellulosic structure itself (acetic acid from hemicellulose side-branches, and formic and levulinic acids resulting from degradation of hydrolyzed sugars) already act as acid catalysts. The resulting autohydrolysis effect is able to solubilize more than 80% of total hemicellulose, without affecting the more resistant crystalline cellulose structure. The products of LHW pretreatment are a solid fraction containing cellulose and lignin and a liquid hydrolysate containing the solubilized hemicelluloses. Given the lack of an acid catalyst, hemicelluloses are mostly present in the hydrolysate as oligomers, rather than monosaccharides. These oligomers can potentially be used as prebiotic agents (Girio et al. 2010). The severity of LHW pretreatment conditions can be measured by a severity factor defined as the reaction ordinate, R_O (Equation 1) developed by Overend and Chornet in 1987 (Overend and Chornet 1987) and typically represented in logarithmic form, where t corresponds to the treatment time and T(t) to the reaction temperature, which is a function of time. The reference temperature, T_{ref}, is the temperature up to which the hydrolysis is considered to be negligible (usually 100ºC) and the constant ω relates to a conventional energy of activation, typically equated at 14.75.

$$R_O = \int_0^t exp\left(\frac{T(t)-T_{ref}}{\omega}\right) dt \quad \text{(Equation 1)}$$

Harsher pretreatment conditions (higher temperatures for longer residence times) give higher severity factors. The selection of pretreatment conditions (time and temperature) is based on a trade-off between the extent autohydrolysis and hemicellulose removal, which increases with severity factor, and the level of sugar degradation and inhibitors formation and operation costs which also increase with the severity factor (Moniz et al. 2013). Additionally, the adjustment of the severity factor determines the product distribution of LHW, particularly the degree of polymerization of the solubilized polysaccharides, which decreases as the pretreatment conditions become harsher (Branco et al. 2015; Carvalheiro et al. 2016).

Liquid hot water pretreatments benefit from lower costs associated with no-use of chemicals and reduced corrosion problems. The use of only water also represents an important gain in operation

and environmental safety. Additionally, the lack of acid catalyst leads to low formation of inhibitory components. However, this process is yet not developed at commercial scale because of higher water demand and high energy requirement. Moreover, LHW has a limited effect in biomass deconstruction originating low enzymatic hydrolysis yields (Maurya, Singla, and Negi 2015).

Steam explosion consists of subjecting biomass to pressurized steam (20 to 50 bar, 160 to 270ºC) for several seconds to a few minutes, after which pressure is released resulting in the rapid vaporization of biomass moisture that disrupts the lignocellulosic structure. Steam explosion then combines three effects that contribute to breakdown of biomass structure: thermal (heating provided by the hot steam), mechanical (shearing due to sudden decompression and moisture vaporization) and chemical (auto-hydrolysis induced by the presence of water). During steam explosion, hemicellulose hydrolysis occurs, as well as lignin transformation and increase of cellulose crystallinity as a result of high temperatures. The small size of water molecules enables them to enter the small pores of lignocellulosic structure, which upon vaporization effect disrupt the microfibrils and increases the biomass porosity, while the hydrolysis of hemicellulose exposes the cellulose to enzymatic hydrolysis. The existence of an acid environment during steam explosion has the inconvenient of sugar degradation (to HMF and furfural), though to lesser extent than in acid pretreatments for which the acid conditions are harsher. For some types of lignocellulosic feedstocks with low content of acetyl groups (as softwoods), the addition of an acid catalyst might be required, with the drawbacks already mentioned. Such catalysts include sulfuric acid and sulfur dioxide. From the wide range of studied and available options, steam explosion is one of the few cost-effective pretreatment methods for pilot scale and commercial applications, due to its lower capital investment and higher energy efficiency, as used steam can be already produced by a parallel process and further used for power generation to supply the energy requirements of the pretreatment with potential overproduction that can be sold (Haghighi Mood et al. 2013).

Ammonia fiber explosion (AFEX) applies the concept of steam explosion, but the vaporized substance upon decompression is ammonia instead of water. The vaporized ammonia is condensed and reused. The rapid expansion of the ammonia gas breaks the bonds between carbohydrates and lignin while physically disrupting the lignocellulose fibers. The main process parameters that determine the optimization of the process are ammonia loading, water loading, reaction temperature and residence time. Typical operation conditions are 60-120ºC and 17-21bar for less than 30 min. Unlike steam explosion, AFEX produces a solid material (as ammonia vaporizes entirely), which can represent advantageous cost savings, as drying and recycling steps are no longer required. It also has the advantage of not producing any inhibitors. However, during AFEX only the structure of the fibers is altered and not the chemical composition of the fibers, since no lignin nor hemicelluloses are removed. As such, ammonia fiber explosion has only been successfully applied to grass feedstocks such as wheat straw, rice straw, corn stover and switchgrass. Conversely, softwoods and hardwoods, which present higher lignin contents, are more recalcitrant to AFEX pretreatment (Haghighi Mood et al. 2013).

Carbon dioxide explosion pretreatment is another variant of steam explosion, where supercritical CO_2 is applied (31ºC, 74bar). The advantages of this process when compared to steam and AFEX are the lower temperatures used and the lower toxicity and flammability of carbon dioxide. Moreover, carbon dioxide is cheap and has the advantage of an acid catalyzed pretreatment due to the acidity of

CO_2, while benefiting from its less corrosive effect. Recovery of carbon dioxide is as easy as ammonia recovery in AFEX, both occurring via depressurization (Haghighi Mood et al. 2013). CO_2 explosion is more cost effective than AFEX and produces less degradation products than steam explosion (Maurya, Singla, and Negi 2015).

Biological pretreatments consist of conversion of lignocellulosic materials by microorganisms, namely fungi, into more accessible compounds. Despite the advantages of low energy consumption, no chemical requirements and environment friendly character, this type of pretreatments faces several economical limitations that prevent its industrial application, such as long residence times, the need for constant monitoring of microorganism growth, large space requirements, and low yields (Haghighi Mood et al. 2013).

1.3. The organosolv processes

Organosolv pretreatments consist of the use of aqueous solutions of organic solvents and pretreatment temperatures typically in the 120-220ºC range. The use of organic solvents, typically volatile, implies operation at relatively high pressures, varying depending on the solvent vapor pressure. Applied solvents include aliphatic alcohols (ethanol, methanol, butanol), polyols (ethylene glycol, glycerol), ketones (acetone), organic acids (acetic, formic), dioxane, phenol, among others, with or without the addition of a catalyst. An important advantage of some of these solvents is that they can be produced from the pretreated lignocellulosic materials they are applied to, contributing to a more sustainable process overall (Zhang et al. 2016). Newer studied solvents also include alkylene carbonates, γ-valerolactone, methyl isobutyl ketone (MIBK), and others. The catalysts that can be used include inorganic and organic acids, as hydrochloric and sulfuric acids, and bases, as sodium hydroxide, ammonia and lime. The simultaneous presence of water and organic solvents in the reaction medium allows for the simultaneous removal of lignin and hemicelluloses during organosolv pretreatment. In organosolv pretreatment, two fractions are obtained: a solid fraction enriched in cellulose and containing some non-solubilized hemicelluloses and lignin, and a liquid fraction with the solubilized sugars (hemicelluloses in oligomeric and monomeric form) and lignin (acid-soluble lignin), as well as some degradation products, organic acids, extractives and soluble ash. Organic solvents enhance the deconstruction of lignocellulose as they increase solvent penetration and biomass dissolution, when compared to aqueous medium. They also enhance hydrogen transfer, reaction kinetics (by decreasing its activation energy) and product selectivity. Advantageously, the lignin produced during organosolv processes is usually relatively pure, with low sulfur and less condensed than for other pretreatment technologies and can, therefore, be used for other added-value products (Zhang et al. 2016). However, the volatility and flammability of organic solvents constitute a safety and environmental hazard and increase the operation and capital costs due to the high pressures applied. Additionally, another drawback is the need for recycling the solvent, necessary to lower operation costs and prevent the inhibitory effect on enzymatic hydrolysis and fermentation processes. Nonetheless, organosolv pretreatments have been intensively perceived as very promising pretreatment technologies, ever since their original development in the pulp and paper industry (Zhang et al. 2016).

1.3.1. Solvent options

The majority of organic solvents used for lignocellulose pretreatments are either polar protic or polar aprotic solvents. This results in a cellulose-rich fraction, an organosolv lignin fraction, and a water-soluble fraction containing sugars (mainly hemicellulose-based sugars), acid soluble lignin, carbohydrate degradation products, organic acids, and other components. Most of the organic solvents used are bulk commodity chemicals, and so their cost is relatively low when compared to other pretreatment solvents, such as ionic liquids. Despite this, the cost of organosolv pretreatments and corrosion issues continue to be factors affecting their large-scale adoption and effective recovery and recycling strategies are required (Zhang et al. 2016). This section presents a brief description of the most common solvents applied for organosolv pretreatment, focusing on their main advantages and disadvantages. Table II reviews several organosolv research works, resuming their operation conditions and main obtained results.

1.3.1.1. Alcohols

Methanol

Methanol is the simplest alcohol that can be applied to organosolv pretreatment. Methanol pretreatment can be carried out with or without catalyst at temperatures ranging from 170 to 200ºC. Methanol pretreatment was successfully applied in the pulping industry in to produce high quality cellulose fiber. The Organocell and the ASAM processes are examples of methanol organosolv processes developed in the 1990's that saw industrial application (Muurinen 2000). The Alkaline Sulfite Anthraquinone Methanol (ASAM) process adds anthraquinone (AQ) and methanol reagents to conventional sulfite process to improve the delignification rate, pulping selectivity, and process performance. Methanol in the most volatile of alcohols, which makes it the easiest to recycle. However, due to its inherent toxicity and flammability, research into methanol pretreatment for biomass saccharification has been minimal in recent years.(Zhang et al. 2016).

Ethanol

Ethanol is the most frequently used alcohol solvent for organosolv pretreatment of lignocellulosic biomass given that it is a low price, renewable solvent, which presents good solubility of lignin, low toxicity, miscibility with water, and it is easy to recovery. Ethanol organosolv selectively removes lignin and hemicelluloses, while the cellulose fraction remains untouched (Zhou et al. 2018). Ethanol organosolv has been known since 1940 and is the basis of the Alcell and Lignol processes, currently applied in pilot/demonstration plants (Zhang et al. 2016). Ethanol can be applied alone or with the addition of catalysts. Acid catalysts are preferred for ethanol pretreatment of lignocellulose, as they enhance glucan digestibility when compared to ethanol-alone and base-catalyzed ethanol organosolv, while allowing for lower operation temperatures and reaction times. It is suggested that improvement in glucan digestibility with the addition of acid catalyst is not a function of delignification efficiency, but rather a result of a reduced degree of cellulose polymerization, reduced average fibber length, and increased substrate porosity of pretreated biomass, which lead to increased accessibility to hydrolytic enzymes (Zhang et al. 2016). Application of organic acids (e.g. formic, acetic) and inorganic salts ($FeCl_3$, $Fe_2(SO_4)_3$, $FeSO_4$, $AlCl_3$, $Al_2(SO_4)_3$, $MgSO_4$), as opposed to inorganic acids (e.g. sulfuric, or hydrochloric acids), reduces corrosion issues and degradation products, even though higher concentrations are typically required.

Organic acids present the added advantage of not representing an additional contaminant as they already are products released during pretreatment of LM. On the other hand, they can form esters with ethanol (and other alcohols). During ethanol organosolv, ethanol (due to being an alcohol) can react with solubilized sugars, particularly xylose and glucose, to form ethyl-xylosides and ethyl-glucosides (Figure 6). The formation of these compounds may protect xylose and glucose from degradation into furfural and HMF, and can be a new source of high-value chemicals (Zhang et al. 2016). The main disadvantage of ethanol organosolv is the very high energy demand of high temperature and pressure operation and of its recovery by distillation (Zhao et al. 2017).

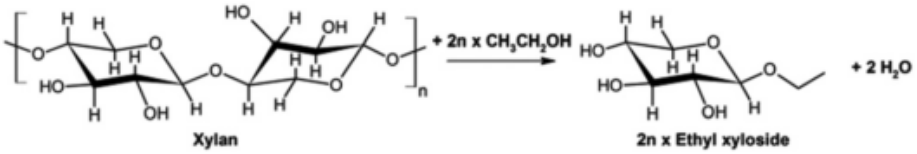

Figure 6. Formation of ethyl-xylosides in ethanol organosolv pretreatment

Typical operation conditions include 50-80% ethanol in water solvent with or without catalysts. Temperatures of 160-220ºC and times of 15-120 min are applied. Acid catalysts are much more commonly used, with concentrations ranging from 0 to 10%. Organic acids tend to be used close to the upper limit while mineral acids and salts fall to the lower limit (0-2%). Enzymatic digestibilities above 60% are usually obtained (Zhou et al. 2018).

Butanol

Butanol is a better solvent for lignin than ethanol and typically results in higher enzymatic digestibility of organosolv treated solids. Moreover, it benefits from limited solubility in water which allows for easy liquid-liquid separation of an aqueous phase enriched in sugars from a butanol organic phase enriched in phenolics/lignin (Zhao et al. 2017).

Phenol

Phenol as solvent reacts with lignin and facilitates its dissolution, particularly when small amounts of sulfuric or hydrochloric acid are used (Rodríguez et al. 2018). Phenol organosolv pretreatment can operate at low to mild temperatures of 100-170ºC and atmospheric pressure, reducing the amount of degradation products formed and without compromising the effectiveness of biomass fractionation. Typical phenol concentrations are around 40% with mineral acid concentrations between 0.01 and 1%. The hydrochloric acid concentration was found to be the most influential variable affecting solid yield and residual lignin in pretreated solids, followed by reaction time and phenol concentration. The produced phenol-lignin during pretreatment which incorporates phenol molecules in its structure via phenyl-oxygen ether linkages, can be used to produce good quality polyurethanes. It has been proposed that phenol-lignin can be submitted to pyrolysis to produce a mixture of substituted and non-substituted phenols, which can then be used as pretreatment solvent, potentially eliminating the need for solvent make-up. Alternatively, pretreated solids can be washed with methanol or water to remove phenol, which can be recovered by distillation, and then recycled after lignin precipitation by water or acid addition. Advantageously, phenol does not react with solubilized sugars. Phenol is used in the Battelle-Geneva

process proposed in 1983, which with only 100ºC of temperature can reach a solid yield of 40% for 40% phenol and 1% hydrochloric acid concentrations. Separation of sugars and lignin is facilitated by phase separation upon cooling. The process is described as having low investment costs and good economic viability, even for small productions. In general, phenol is more effective than other solvents, i.e. butanol, for organosolv pretreatment. However, its application is highly limited by its acute toxicity (Muurinen 2000).

1.3.1.2. Organic acids

Typically, formic (e.g. Formacell and Milox processes) and acetic (e.g. Acetocell and Acetosolv processes) acids are used. Organic acids are applied in organosolv pretreatment at a concentration of 60-98%. Treatment time varies between 30 minutes and 2 hours. Organic acid organosolv requires lower temperatures (80-110ºC) when compared to ethanol organosolv (160-220ºC) (Zhao et al. 2017). In fact, lignin solubility in organic acids is much higher than in ethanol, allowing for operation even below 100ºC and at atmospheric pressure. However, organic acid organosolv has received less attention than organosolv with alcohols due to the corrosion problems inherent to operation with concentrated organic acids. Organic acid organosolv also results in cellulose acylation (formylation for formic acid organosolv or acetylation for acetic acid organosolv), which is undesirable. Cellulose acylation is the substitution of cellulose hydroxyl groups by acyl groups (formyl or acetyl, for formic or acetic acid organosolv, respectively). This phenomenon inhibits the formation of productive binding between cellulose and cellulases, during enzymatic hydrolysis, lowering its yield. The increase in the diameter of the cellulose chain, increasing steric hindrance to enzymes also has a negative impact on enzymatic hydrolysis. Optimum operation conditions must then be applied to maximize delignification and polysaccharide solubilization, while minimizing cellulose acylation. Concentrations of organic acid was demonstrated to have a strong impact on all three variables, all of them increasing with increasing acid concentration. When organic acid concentration is high above 80% for formic acid or above 90% for acetic acid, acylation happens to a great extent compromising the enzymatic digestibility of pretreated solids and creating the need for a post-treatment of the solids with alkali to remove the acyl groups (Muurinen 2000).

1.3.1.3. Ketones

Acetone

Acetone can be applied alone with water and in mixtures with ethanol or formic acid (Rodríguez et al. 2018). Lignin has a higher solubility in acetone-water solvent than in aqueous ethanol, allowing for lower temperature operation (Zhao et al. 2017). Being an aprotic non-hydroxylic solvent, acetone does not undergo side-reactions with carbohydrates and lignin, like hydroxylic solvents, i.e. alcohols and organic acids. Because of the reduced side-reactions, the acetone organosolv affords more pure product streams, facilitating downstream processing of sugars and lignin to high value products. It also renders the subsequent enzymatic hydrolysis of pretreated solids more efficient. Even partial substitution of hydroxylic solvent for acetone is already beneficial to decrease the amount of side reactions. Additionally, acetone organosolv produces less sugar degradation products and less pseudo-lignin that ethanol organosolv, even for the same low temperatures, due to the lack of hydroxyl groups of the solvent (Smit, Huijgen, and Grisel 2015). In one patent (Smit, Huijgen, and Grisel 2015), acetone organosolv is preferably operated at 40-60% acetone between 130 and 150ºC for 60-150min with a LSR of 6:1 and 0.1-

1% acid sulfuric. Acetone performed better for solid solubilization, xylan solubilization and delignification of wheat straw, than ethanol and methanol, while performing similarly to butanol, for the same operation conditions (Sidiras and Salapa 2015). Acetone is easier to distill than most alcohols, representing a decreasing in operation costs for solvent recovery. However, acetone organosolv suffers from safety issues, due to high flammability of acetone.

Methyl isobutyl ketone

Methyl isobutyl ketone (MIBK) as an organosolv solvent allows for easier separation (liquid-liquid separation) of solubilized lignin from solubilized hemicelluloses as it originates two liquid fractions when mixed with water. MIBK organosolv is typically proposed with use of MIBK-water-alcohol mixture as solvent, which presents a single phase at treatment temperatures. After pretreatment, the liquid is mixed with water, resulting in phase separation and resolution of lignin-rich MIBK from aqueous alcohol containing hemicellulose/soluble sugars. A mineral salt, like NaCl, can be used to improve phase separation. MIBK is then recovered by distillation, which allows for MIBK re-use and recovery of lignin. Replacing ethanol with acetone appears to increase the performance for biomass fractionation. Negatively, MIBK presents risks in terms of operation safety while representing a health hazard (Zhang et al. 2016).

1.3.1.4. Amines

Organosolv using ammonia or amines results in high solid yields due to their limited effect on hemicellulose solubilization. Conversely, a good impact on delignification can be obtained. Typical solvents applied in this category are methylamine, ethylenediamine, 1,6-hexamethylenediamine, and ethanolamines (monoethanolamine proved more effective than diethanolamine and triethanolamine). However, organosolv with amines is typically avoided due to their general toxicity and limited effect on hemicelluloses. Also, amines are extensively incorporated into wood components, compromising their recyclability, as well as the upgrade of lignocellulosic structural macromolecules and therefore the economic validity of the whole process (Muurinen 2000; Rodríguez et al. 2018).

1.3.1.5. Dioxane

Dioxane organosolv has a good affinity for lignin solubilization, superior to other solvents such as methanol. However, it has been ignored in recent research due to its toxicity, high flammability and environmental impact. Moreover, it can form explosive peroxides when in content with air (Zhang et al. 2016; Muurinen 2000).

1.3.1.6. γ-valerolactone

γ-valerolactone (GVL) is a lactone, i.e. a cyclic ester, which is as green high-boiling solvent with the advantage of the fact that it can be synthesized from hexoses obtained from lignocellulosic biomass, via levulinic acid. Recently, this bio-derived solvent has received significant attention for organosolv treatment as it can achieve complete saccharification of biomass at low acid concentrations (below 0.1 wt% H2SO4). Values of 90 to 95% of total sugars were reported to be solubilized (including 10% of sugar degradation products) for 80% GVL organosolv treatment of multiple LM with 0.5wt H2SO4 at temperatures between 160 and 200ºC This approach eliminates the need for downstream enzymatic hydrolysis, simplifying the process design. Additionally, lignin can be precipitated to 95% recovery from GVL organosolv liquor through addition of water, making the process attractive for lignin valorization.

Biphasic separation by addition of sodium chloride or liquid carbon dioxide or solvent extraction methods have been proposed for efficient recycling of the solvent (Zhang et al. 2016).

1.3.1.7. Alkylene carbonates

Alkylene carbonates applied in organosolv fractionation of biomass include ethylene carbonate (EC), propylene carbonate (PC) and glycerol carbonate (GC), all of which are commercially available solvents with high dielectric constants that give them an increased potential for deconstruction of the lignocellulosic structure, enhancing lignin and hemicellulose solubilization by increased acid potential. Alkylene carbonates can be synthesized from their corresponding polyols, i.e. ethylene glycol, propylene glycol and glycerol, which makes their production from lignocellulosic feedstocks possible, making them sustainable solvents. Additionally, they are recognized as green safe solvents, as they are non-volatile, non-flammable and non-toxic (Zhang et al. 2016).

Ethylene carbonate has been used for deconstruction of woody biomass to produce hydroxyl rich liquors that can be applied for the synthesis of polymers. Complete biomass liquefaction in acidic EC occurs at low operation temperatures (below 150ºC). EC alone yields better results than its corresponding glycol, though the application of a mixture of EC-ethylene glycol is even more effective. Pretreatment of lignocellulose with acidified EC-EG mixtures (80%-20%) at only 90 °C for 30 min with 1.2% H2SO4 originated a delignification of 93% and enzymatic digestibility of 93% (Zhang et al. 2013), demonstrating the immense potential of these solvents. EC/EG pretreatments were demonstrated to be more effective than PC-propylene glycol (PG) and GC-glycerol mixtures for biomass pretreatment. Similarly, EC alone performed better than PG alone, which performed better than GC. The low temperatures associated with use of alkylene carbonates in organosolv pretreatment reduce operating costs and allow the reduced formation of undesirable degradation products. However, alkylene carbonates have the disadvantage of partially decomposing to their respective polyols and CO2 at pretreatment conditions (2-6% decomposition reported for the above conditions), and even though the formed polyols have a positive effect on biomass deconstruction, a make-up of alkylene carbonate solvents or reaction of respective polyols with carbon dioxide to reverse the decomposition reactions will become necessary, adding increased steps and therefore cost to the process. Additionally, the release of CO2 might dangerously increase the operating pressure, further increasing capital and operating costs (Zhang et al. 2016).

Table II. Summary of several organosolv works found in literature, in terms of solvent use, operation conditions and major performance results

Pretreatment	Feedstock	Particle size	Solvent	LSR	Catalyst	Temperature/ºC	Pressure/bar	Time/min	Solid yield/%	Cellulose recovery/%[1]	Hemicellulose solubilization/%	Delignification/%	Glucan digestibility/%	Ref.
Ethanol Organosolv	Eucalyptus	~2 cm	60% ethanol	6:1	-	180-200	-	30-120	52-58%	85-95	-	~70	65-77	(Muñoz et al. 2011)
Ethanol Organosolv	Eucalyptus	4-6 mm	60% ethanol	6:1	-	160	-	90	-	~100	~73	~50	48	(Mou and Wu 2016)
Acidified Ethanol Organo-solv	Wheat straw	10 mm	50% ethanol	10:1	H2SO4 0-0.3%	190-210	-	60-90	43-49%	~90	56.8-75.8	81.4-95.3	83.6-89.4	(Wildschut et al. 2013)
Acidified Ethanol Organo-solv	Eucalyp-tus; Bagasse	0.2 mm	75% ethanol	5:1	Acetic acid 1%	200-220	80	30-60	~21%	-	45.5	87.8	96.9	(Teramoto, Lee, and Endo 2008)
Acetone Organosolv	Wheat straw	< 2cm	50% acetone	14:1	-	205	25	60	48.7%	93	82	79	87	(Huijgen, Reith, and Uil 2010)
Acetone Organosolv	Pinus radi-ata	~2 cm	50% acetone	7:1	H2SO4 0.13%	183-197	~20	4-46	40-54.6%	~58-64	-	~54-58	45-71.8	(Araque et al. 2008)
Acetone organosolv	Sweet sor-ghum stalk	177-833 μm	50% acetone	10:1	H2SO4 0.1%	180	17	60	44.4%	71	81	69.4	94.2	(Jafari, Amiri, and Karimi 2016)

[1] In the remaining solid fraction.

Pretreatment	Feedstock	Particle size	Solvent	LSR	Catalyst	Temperature/ºC	Pressure/bar	Time/min	Solid yield/%	Cellulose recovery/%[1]	Hemicellulose solubilization/%	Delignification/%	Glucan digestibility/%	Ref.
Formic acid Organosolv	Bamboo culms	425-850 μm	88% formic acid-H2O	20:1	-	101	1	120	52.8	82.8	68.6	80.4	-	(Li et al. 2012)
Organic acid Organosolv	Wheat straw	5-15 cm	Acetic/formic acids 13:7, 85% in water	10:1	-	105	1	180	48	93	67	96	-	(Snelders et al. 2014)
Butanol Organosolv	Sorghum culms	2 mm	25% buta-nol- H2O	40:3	H2SO4 0.5%	200	-	60	45	95	90	76	97	(Teramura et al. 2018)
Glycerol Organosolv	Wheat straw	20 mm	70% glyc-erol- H2O	20:1	-	220	-	180	60	98	~70	~65	~90	(Sun and Chen 2008)
Glycerol Organosolv	Eucalyptus globulus	-	56% glyc-erol- H2O	10:1	-	200	-	69	54	99.7	96.6	65.2	98	(Romaní et al. 2016)
Glycerol Organosolv	Rice husk	-	90% glyc-erol-H2O	10:1	HCl 1.2%	130	-	60	66.7	88.2	47.4	21.2	69.7	(Ebrahimi et al. 2017)
Ethylene glycol Organo-solv	Sugarcane bagasse	0.25-0.5 mm	90% EG-H2O	10:1	H2SO4 1.2%	130	1	30	53.5	96	75	89	~95	(Zhang, O'Hara, and Doherty 2013)
Alkylene carbonate orga-nosolv	Sugarcane bagasse	0.25-0.5 mm	Ethyl car-bonate+eth-ylene glycol (4:1)	10:1	H2SO4 1.2%	90	1	30	54.9	96.5	75.8	87.8	93.4	(Zhang et al. 2013)

1.3.1.8. Polyhydric alcohols

Glycerol

Glycerol, for example, is a cheap industrial by-product from the biodiesel sector and a valuable green solvent with a promising potential for application in lignin fractionation (Romaní et al. 2016; Gu and Jérôme 2010; Sun et al. 2015; Jiang et al. 2017). In fact, a huge excessive amount of this solvent is available in the market which might compromise the sustainability of the biodiesel industry in the future. Hence, its application in the biorefinery context has the potential not only to improve the economic feasibility of such a plant but also solve that sustainability problem of the biodiesel industry (Sun et al. 2015). Besides, glycerol advantages include its non-toxicity, low vapor pressure (0.000187mmHg (Cammenga, Schuize, and Theuerl 1977) at 25ºC, compare to 58.7537mmHg for ethanol or 23.6864mmHg for water) which allows for operation at atmospheric pressure (even at high temperatures), low environmental impact, ability to penetrate into the fiber tissue of lignocellulosic materials creating an effective reaction medium for biomass delignification due to its highly polar polyol structure (Hundt et al. 2016). Sun et al. (Sun et al. 2015) claim that glycerol-water organosolv has an outstanding selectivity towards lignin and xylan solubilization (while maintaining the cellulosic fraction in the pre-treated solid), performing better than current leading pretreatments such as dilute acid, hot water, steam explosion and ammonia fiber expansion processes. These authors have found glycerol organosolv to be very similar to ethanol organosolv, in terms of fractionation yields and applicability, though more economically competitive due to glycerol lower market price. Various lignocellulosic materials have been shown to be susceptible to glycerol organosolv pretreatment, with pre-treated solids revealing an appealing digestibility when submitted to enzymatic hydrolysis (around 90% after 24 hours) (Sun et al. 2015).

The majority of reports using glycerol as a pretreatment solvent focus on organosolv processes in which biomass fractionation is performed with a mixture of glycerol-water in various proportions at temperatures between 170-230ºC. However, these processes, though allowing a relatively high delignification and a very high cellulose yield (in the pre-treated solids), also result in a relatively high hemicellulose loss (removed together with lignin), implying a further downstream processing of the liquid stream (typically membrane operations).

Romaní et al. (Romaní et al. 2013) performed batch Eucalyptus wood pretreatment in 40-80% glycerol-water solution with a 10:1 liquid-to-solid ratio at 180-200ºC for 40-90 min. They obtained an averaged delignification of 59% and an averaged xylan recovery in the pre-treated solid of 32% with cellulose losses in the liquid fraction lower than 9%. The maximum delignification of 77% was achieved with 80% glycerol-water at 200ºC for 65 min with 8% and 70% of cellulose and xylan losses respectively. At 180ºC for 65 min with the same solvent composition, they obtained only 40% delignification, but with a minimum xylan solubilization of 39%.

In other report (Romaní et al. 2016), the same team of researchers claims a feasible process for eucalyptus wood giving a pre-treated biomass susceptible to be substrate at high solid loadings on saccharification and fermentation. It consists of a glycerol organosolv (56%) in batch mode process, at 200ºC for 69 min with the same solid-to-liquid ratio, to obtain a delignification percentage of 65.2%. Also, recovered lignin presented similar features to commercial lignin while cellulose losses were only 0.3%

suggesting the significant potential of glycerol pretreatments. In this experiment however, the xylan removal was as high as 96.6%, implying an additional membrane separation downstream with the additional associated costs.

The influence of several parameters has been investigated in glycerol organosolv pretreatments (Domínguez et al. 2014). Higher temperatures (230ºC) and higher glycerol solvent content (80%) were proven to increase the delignification extent as well as the cellulose content of pretreated solids while smaller liquid-to-solid ratios had the opposite effect. Higher glycerol content also decreases the xylan removal. Pretreatment with glycerol-water solutions is ineffective after 2h of reaction as a result of cellulose degradation. Domínguez et al. (Domínguez et al. 2014) were able to recover 82.8% of lignin from *Acacea dealbata* with 80% glycerol at 230ºC in batch mode for 60 min at LSR of 10:1, proving the correlation between temperature and delignification percentage. The obtained cellulose and xylan losses were of 4.4% and 83.1%, respectively. This fact suggests the negative impact of increased temperature in increased xylan solubilization.

Several physicochemical changes of the lignocellulosic structure have been reported as a result of glycerol organosolv biomass pretreatment (Sun et al. 2015; Romaní et al. 2016). SEM analysis showed that the process induced the disaggregation and size reduction of the lignocellulosic fibers, suggesting the removal of lignocellulosic material. The fibrils became looser and thinner with increased surface roughness and surface area, resulting in higher accessibility to cellulose by the hydrolysis enzymes, thus increasing the posterior hydrolysis efficiency. Cutting points were found on edges or ends of the fibers – “chemical cutting” – while various holes were revealed on their surface. These irregular pores result from the perforation of aqueous glycerol on the fiber and dissolution on its composition since glycerol is a highly polar polyalcohol that easily penetrates into the fiber tissue. Such phenomenon only contributes to the increase of the surface area of the pre-treated fibers further improving hydrolysis yields. Atomic Force Macroscopy (AFM) analysis showed too that the surface structure becomes highly granular and rough after glycerol organosolv process. It also revealed the migration and formation of spherical lignin deposits onto the outer surface of fibers as a result of lignin re-condensation, which again increases enzyme accessibility. Through AFM analysis researchers also observed an increase of the hydrophilic character of the fibers surface which suggests the ability of the pretreatment to selectively remove lignin, hemicellulose and other extractives leaving a remaining hydrophilic cellulosic-rich substrate. FT-IR and ^{13}C NMR analysis results agree with this conclusion revealing an enrichment in cellulose as a result of a significant impoverishment in lignin and hemicellulose content of the glycerol-water pre-treated solids. These two analysis tools were also able to explain these morphological and compositional changes highlighting the effect that glycerol organosolv has on the biomass chemical structure: the pretreatment breaks down several chemical bonds of inter- and intra-molecules (e.g., hydrogen, β-ether and β-ester bonds) responsible for keeping the three biopolymers linked together. It is the breakage of these chemical bonds that allows the allomorphous transformation and the lignin re-localization that take place during pretreatment and that are responsible for good digestibility. Finally, XRD spectra revealed an increase in fibers crystallinity after pretreatment due to the removal of amorphous lignin and hemicellulose and the resulting enrichment in ordered crystalline cellulose. This phenomenon is often counteracted by swelling and dissolution of cellulosic structure during pretreatment, resulting in size

reduction of cellulose crystallites and a respective increase of amorphous region, with some authors obtaining even an overall decrease of substrate crystallinity.

Pure glycerol pretreatment has also been reported by Jiang et al.(Jiang et al. 2017) for corncobs prior to levoglucosan production by fast pyrolysis. A high selectivity for lignin and hemicellulose solubilization was observed, while the majority of cellulose remained in the solids increasing their crystallinity. The maximum delignification of 82% was obtained when pretreatment was performed at 240ºC for 3h with 5% solids loading. At these conditions 83% of total hemicellulose solubilized while all the cellulose remained in the pretreated solids. However, for a slightly lower temperature (220ºC) and residence time (0.5h) only 33% of total lignin was solubilized. For 3h at 220ºC, this value rose to 78%. As expected, delignification was proven to increase with increasing temperature, residence time and LSR. The researchers proposed that the recovered glycerol could be fermented, as it revealed a fermentability comparable to that of pure glycerol. Glycerol pretreatment enhanced levoglucosan yield in fast pyrolysis from 2.2% (without pretreatment) to 35.8% (with pretreatment).

Propylene Glycol (1,2-Propanediol)

Zhang et al. studied biomass fractionation in polyols, presented as safer options than ethanol or acetone due to their higher boiling points (Zhang, O'Hara, and Doherty 2013). Besides glycerol, ethylene and propylene glycol were studied at 90% in water with 1.2% sulfuric acid as catalyst, at 130ºC for 30 min for the pretreatment of sugarcane bagasse. EG and PG yielded 89% delignification whereas glycerol yielded only 57%. According to the authors, this was a result of lower solubility of lignin in glycerol. Conversely, xylan solubilization was higher in glycerol (77%) than in EG and PG (75%), presumably due to higher acidity of the former. With respect to enzymatic digestibility of the pre-treated solids, EG and PG performed better than glycerol (95% vs. 77%), which was attributed to their superior delignification capacity. It is worth noting that only 36% of total lignin was recovered from the hydrolysate via precipitation with water, which may threaten the recyclability of the solvent. A polydispersity of 2.48 of precipitated lignin was reported, indicating the need of further fractionation of the lignin fragments namely via size exclusion chromatography to obtain suitable fractions with desired molecular weight for downstream valorization. After this first assessment the authors proceeded to investigate different pretreatment conditions with ethylene glycol, based on the fact that it performed better than glycerol and that, though it was as effective as propylene glycol, it has a higher boiling point and cheaper price. Delignification was shown to increase when temperature increased from 110 to 130ºC and pretreatment time increased from 15 to 30 min. Additionally, increasing acid content from 0.2 to 1.2% also improved lignin recovery, while increasing water concentration from 2 to 30% had the opposite effect.

Uraki and Sano (Uraki and Sano 1999) evaluated acid catalyzed propylene and ethylene glycol pulping of some Japanese softwoods (larch, red cedar and fir). They verified that PG performed better than EG, while the addition of 5% water improved delignification results. Propylene glycol was the most effective at 95% concentration in water with 0.29% sulfuric acid (which gave a superior performance than hydrochloric acid) at 170ºC for 2h of cooking time. The resulting pulp had only 0.8% of lignin and presented properties similar to Kraft pulp. However, pulp yield was low (34%). Lignin recovered via precipitation reached 29%.

Propylene glycol has also been reported together with other glycols in liquefaction of chestnut wood with maleic acid anhydride and phosphoric acid as catalyst at 190ºC for 3-7 hours (Krzan, Kunaver, and Tisler 2005). A maximum liquefaction yield of 56% was obtained and the produced liquid presented a mixture of low molar mass oligomeric polyester molecules which can be used as feedstock for polymeric synthesis, e.g. for polyesters and polyurethanes.

1.3.2. Mechanism of organosolv pretreatment

Two main mechanisms are present in organosolv pulping, namely hydrolysis of internal lignin bonds and 4-O-methylglucuronic bonds between lignin and hemicellulose; and hydrolysis of glyosidic bonds in hemicellulose and partially in cellulose (Kucharska et al. 2018). As such, a biphasic model of two parallel pseudo first-order reactions is usually employed to describe the kinetic behaviors of delignification and polysaccharide solubilization (Zhao et al. 2017). The organic solvent in organosolv pretreatment has mainly two effects: improving the selectivity of delignification by increasing the solubility of lignin and reducing the hydrolyzing power of the liquor and therefore sugar degradation. It also improves the transfer and adsorption of reagents in the wood (Muurinen 2000).

The mechanism of ethanol delignification (ethanolysis) involves depolymerization and polymerization reactions. Distillable oils are formed by cleavage of high molecular lignin aggregates, while simultaneous polymerization reactions yield a complex ethanol-insoluble polymer. Ethanolysis is associated with cleavage of lignin-lignin and/or lignin-carbohydrate bonds and is catalyzed by the presence of hydrogen or hydroxyl ions in the pretreatment liquor. Increasing pretreatment temperature accelerates those reactions, while the dissolution of lignin is facilitated by the presence of an appropriate solvent, generally an organic solvent, as lignin is insoluble in water and acidic media. Ethanol organosolv is proven effective for treatment of both hard- and softwoods, even though delignification rates are considerably higher for the former case. Ethanol organosolv without catalysts enhances delignification rates with minor sugar degradation. Some degradation may still occur as pH decreases from 7 to about 3-4, due to liberation of acetic acid into solution by cleavage of acetyl groups of hemicelluloses during pretreatment. Optimum degradation occurs for ethanol contents between 50 and 70%. Alcohol penetration into the fibrous structure is easy, resulting in uniform delignification of the LM. Ethanol decreases surface tension of pulping liquor which improves the diffusion of other pulping chemicals, for example, alkali. Ethanol acts as a scavenger for the free radicals formed during pulping and reduces the extent of lignin condensation. Ethanol is also not consumed significantly in the process, with reported losses below 1% on wood mass. Linear correlations were obtained between solid yield and pulping time and between residual lignin and pulping time, indicating approximately first-order kinetics.

The kinetics of wood delignification by organosolv involves three different mechanisms all of first order kinetics, independently of feedstock composition and organosolv solvent (even though, reaction rates vary with these two variables): 1) fast initial delignification, 2) relatively fast bulk delignification and 3) slow residual delignification (Kim and Holtzapple 2006). During the initial delignification stage phenolic α-O-4-linkages in lignin and some β-O-4-linkages are cleaved, while during bulk delignification cleavage β-O-4-linkages predominates. During residual delignification, breakdown of carbon–carbon linkages occurs. Since C-C linkages are much more stable than C-O-C bonds, residual delignification is considerably slower than the first two stages. The bulk delignification step corresponds to breakdown of high

molecular lignin and solubilization of its breakdown products, and is composed of two first-order kinetics steps. Lignin solubilization mainly occurs through cleavage of beta ether linkages, resulting in an isolated lignin with a phenolic hydroxyl content more than twice as high as the lignin of LM feedstocks. Some side chain rearrangement and ethoxylation of benzyl alcohol groups also is reported. These reactions prevent the lignin molecule from recondensing during the organosolv process. Diffusion seems to play an important role in delignification mechanism, while removal of wood moisture reduces the rate constant of delignification (Muurinen 2000).

During organosolv, acid-catalyzed cleavage of α- and β-ether linkages originates lignin fragments with smaller molecular weights that become soluble whereas higher ethanol concentrations increase solubilization of the lignin without so much fragmentation. Cleavage of β-O-4 ether linkages is the major step for lignin depolymerization, as they represent the most common type of bonds in lignin structure. However, α-ether linkages are weaker than the β-ether linkages, and so are faster to breakdown, as the energy activation of cleavage of β-ether linkages is considerably higher.

1.3.3. Towards commercialization

The basic concept of an organosolv process to be implemented industrially follows the scheme represented in Figure 7. The LM are mixed with the organosolv solvent at appropriate process conditions. After the reaction, the pretreated solid enriched in cellulose is separated from the black liquor – enriched in lignin and hemicelluloses –, by a regular solid-liquid separation as pressure filtration or centrifugation. The pretreated solid is then subjected to enzymatic hydrolysis and fermentation (separate or simultaneously) or chemical processing to produce ethanol and other products (e.g. butanol, glycerol, lactic acid, succinic acid, xylitol, sorbitol, furfural, while the black liquor is treated for solvent recovery. Typically, lignin is removed by precipitation with water or acid (usually sulfuric acid) followed by solid-liquid separation. Lignin can be upgraded to many commercial products. Thereafter, the solvent is separated from the aqueous solution containing the hemicelluloses, which can undergo enzymatic hydrolysis and fermentation together with cellulose or be converted to other products, such as xylitol and furfural. In the case of using the hydrolysates to fermentation, a detoxification step is necessary to remove fermentation inhibitors (acids, furans, phenolics). Lignin can be divided into two streams: one, that serves as fuel for power generation that fuels the processes' energy requirements and the other, that can be upgraded to high-quality lignin and then used for multiple high-value applications, such as phenolic resins, polyurethane and polyisocyanurate foams, and epoxy resins. Solvent separation would typically occur by distillation for solvents more volatile than water, with the associated high costs. For high-boiling solvents, alternative separation methods must be proposed (Zhao, Cheng, and Liu 2009).

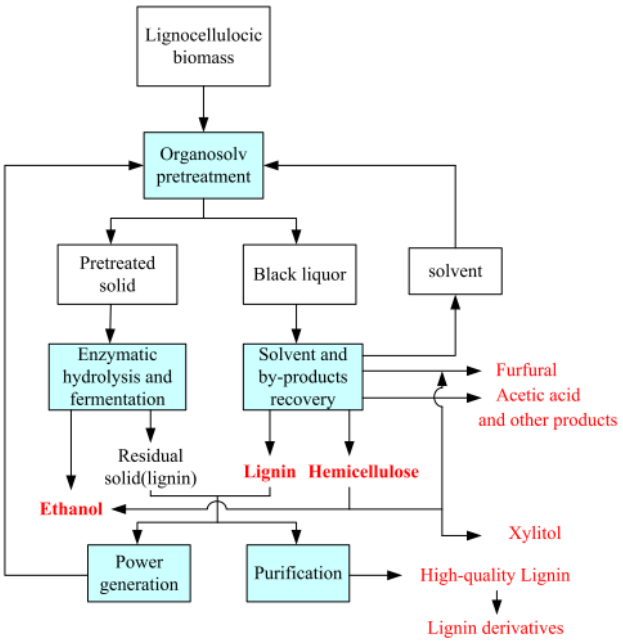

Figure 7. Conceptual design of organosolv process as described by (Zhao, Cheng, and Liu 2009)

Full industrial application of the organosolv process does not yet exist. However, some pilot and demonstration plants are in operation:

- Lignol Innovations Inc. developed a biorefinery technology, called the Lignol process, which uses an ethanol-based organosolv to treat woody biomass. A pilot plant was implemented in 2009 by the same company with this process, in Burnaby, British Columbia, Canada, which treats 1 ton/day of a mixture of hard- and softwood to generate 30 ton/year of cellulosic ethanol and high-purity lignin. Temperatures of 180–195°C for 30–90 min with an ethanol concentration of 35%–70% (w/w) at a liquor to solids ratio of from 4:1 to 10:1 (w/w) are applied (Zhou et al. 2018).
- Compagnie Industrielle de la Matiere Vegetale (CIMV) has a pilot plant in operation in Reimns, France since 2007, that treats any type of lignocellulosic feedstock to produce 89% purity cellulose pulp, pentose sugar syrup and 91% pure, low molecular weight and high-quality lignin. Minerals and proteins are also extracted which can be used as fertilizers, and silica is obtained when wheat and rice straws are used as feedstocks. The pilot plant processes 60 kg/h of biomass. The CIMV process involves cooking of lignocellulose at 95 to 110ºC under atmospheric pressure in a mixture of 55% formic acid, 30% acetic acid and 15% water. Lignin is recovered by precipitation with water and the acids are recycled via distillation (Delmas 2008; Delmas and Mlayah 2013).
- Chempolis Ltd. Operates a demonstration plant since 2008 in Finland that processes 25 000 ton/year of non-woody biomass (wheat and rice straw, bagasse, corn stalks) to produce 5 000 ton/year of bioethanol. The Chempolis process uses formic acid organosolv at 110-125ºC and atmospheric pressure for 20-40 minutes. Acid recovery occurs by distillation as for the CIMV process (*Pulp production and processing : From papermaking to high-tech products* 2013).

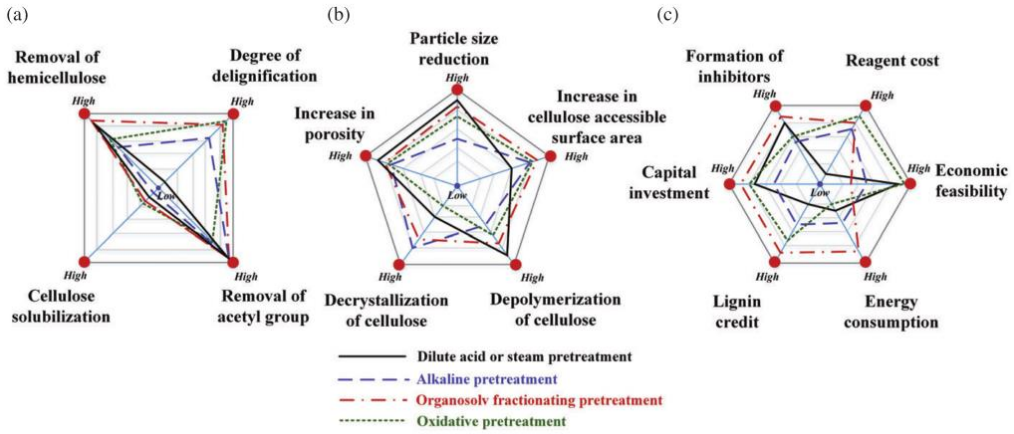

Figure 8. Qualitative comparison of organosolv with other pretreatment processes for a) removal of lignocellulose constituents, b) impact on lignocellulose structure and c) relevant economic aspects (Zhao et al. 2017)

While presenting superior performance than other pretreatment processes for hemicellulose and lignin solubilization, as well as significant impact in cellulose accessibility and crystallinity, and biomass particle size and porosity, all while preserving the cellulose fraction, full industrialization of organosolv pretreatment still faces some limitations (Figure 8) (Zhao et al. 2017):

- Technical limitations: pretreated substrates cannot be directly washed with water, and should be washed with organic solvent to avoid re-precipitation of dissolved lignin, resulting in cumbersome washing configurations; lignin is not fully solubilized, with negative impact on enzymatic hydrolysis;
- Inhibitors for downstream processes: most organic solvents may inhibit the enzymatic hydrolysis and subsequent fermentation process; high temperatures applied during organosolv lead to considerable formation inhibitors;
- High energy consumption for solvent recovery: necessity to recover solvents which are expensive and toxic to enzyme and microorganisms;
- Environment concerns: volatile organic solvents are typically hazardous to the environment and human health; also, solvent washes originate liquid effluents potentially harmful to the environment.

1.4. Objectives

The main goal of this work was the investigation of an alternative organosolv pretreatment that could mitigate the common limitations of this kind of processes (see above). Particularly, propylene glycol was selected as a greener and safer option than more conventional solvents. Focus was then given to the study of the effect of process conditions, including temperature, time and solvent composition on the performance of the organosolv pretreatment for two different types of feedstocks. The evaluation of such performance was conducted on the basis of pretreatment effectiveness for solubilization of lignocellulose structural macromolecules as determined by characterization of resulting solid and liquid fractions and for enhancement of enzymatic digestibility of pretreated biomass. Pretreatment was investigated with and without catalysts and in both batch and continuous operation.

2. Materials and methods

2.1. Feedstock and reagents

The model feedstocks used in this work were Eucalyptus residues (ER), a hardwood forestry residue, and wheat straw (WS), an agricultural residue. WS were collected in the Netherlands. The eucalyptus residues (ER) were kindly provided by The Navigator Company from their paper mill in Cacia, Portugal. The feedstock was grounded with a knife mill IKA® WERKE, MF 10 basic (Germany) to particles smaller than 0.5 mm, homogenized in a defined lot, and stored in plastic containers at room temperature, before use. The humidity of ER and WS was evaluated at 4.9 and 6.8%, respectively. Composition of feedstocks is presented in Supplementary material A.

Granulometric mass distribution of used feedstocks was determined as represented in Figure 9. Sauter mean diameter of ER and WS particles were calculated from the granulometric curves as 0.817 and 0.56 mm, respectively.

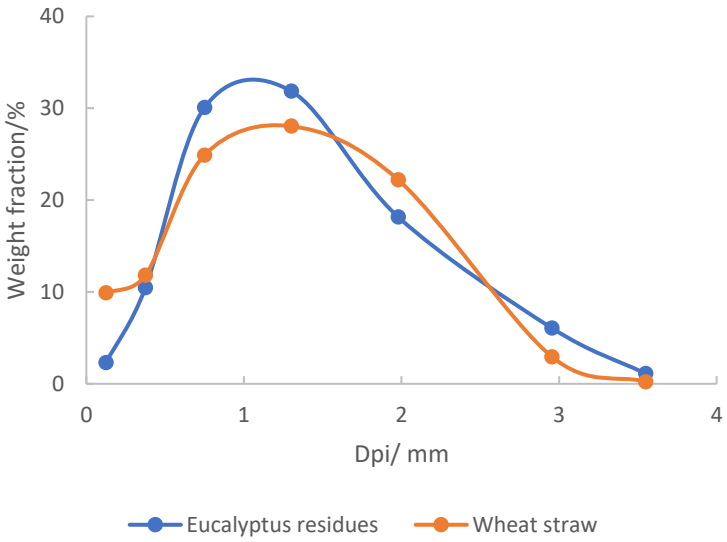

Figure 9. Granulometric curves of feedstocks

In terms of reagents, the following were used: 1,2-propanediol (PG; >99.5%, Merck, Germany), distilled water (17 M Ω cm-1) produced by the PURELAB Classic Elga system (ELGA LabWater, UK), glycerol (99.0-101.0%, Honeywell, Malaysia), Baysilone oil M300 (Bayer, Germany), and Polyethylene glycol 2000 (Merck, Germany), olive oil (commercial brand) and sunflower frying oil (commercial brand).

Sulfuric acid (96 wt.%, Panreac, Spain) and sodium hydroxide (eka, Portugal) were used as catalysts. Glucose (≥98 wt.%, Merck, Germany), xylose (≥98 wt.%, Merck, Germany), arabinose (≥ 98 wt.%, Merck, Germany), furfural (99 wt.%, Sigma-Aldrich, Germany), 5-hydroxymethylfurfural (99 wt.%, Sigma-Aldrich, Germany), formic acid (98%, Panreac, Spain) and acetic acid (glacial, 99.8 wt.% Merck, Germany) were used as standards for HPLC analysis. Sulfuric acid (96 wt.%, Panreac, Spain) was used to prepare mobile phase for HPLC analyses (5 mM sulfuric acid).

For the enzymatic hydrolysis assays it was used sodium citrate buffer prepared from citric acid monohydrate (99.5-100.5%, Merck, Germany), tri-sodium citrate dihydrate (99.7%, VWR, Belgium), and

sodium azide (Merck, Germany). Celli®CTec2 was kindly provided by Novozymes Europe (Denmark) with 199.9 FPU/mL.

2.2. Analytical procedures

The following procedures have been applied in this work. Detailed explanation of performed calculations and the used equations can be consulted in Supplementary material B.

2.2.1. Physical characterization – granulometric analysis

Particle size characterization was performed using a test sieve shaker (EVS1, Endecotts, England) and seven sieves (ASTM E11, Retsch, Germany) with different pore sizes arranged in crescent series according to the pore diameter: 0.25, 0.50, 1.00, 1.60, 2.36 and 3.55 mm. To carry out the analysis, 3 samples (approximately 100 g each), were screened for 20 min, after which the pre-weighed sieves containing the biomass were weighed in a precision balance (N2B110 Navigator, OHAUS, Switzerland).

2.2.2. Determination of moisture content

Moisture content of solid samples was determined according to standard NREL laboratory procedure NREL/TP-510-42621 (Sluiter et al. 2008). Nickel dishes oven-dried at 100ºC for at least 12 hours, then cooled down in a desiccator to room temperature and weighed on an analytical balance (Mettler 160 HK, Switzerland) to the nearest 0.1 mg. Thereafter, 0.5 g of sample were weighed to the nearest 0.1 mg in the previously weighed dishes, which were then placed in an oven at 100ºC for a minimum of 12 h. The dishes containing the dried samples were then cooled down in a desiccator and reweighed. The determination of the moisture samples was performed in duplicate and reported as mean values.

2.2.3. Determination of ash content

Ash content was determined according to NREL/TP-510-42622 protocol (Sluiter et al. 2005). Porcelain crucibles were dried to constant weight in a muffle furnace at 550ºC for at least 5 h and weighed to the nearest 0.1 mg on an analytical balance (Mettler 160 HK, Switzerland), after cooling in a desiccator. The solid samples (0.5±0.0001 g) were loaded into the tared crucibles, burned with a heating plate and then placed in the muffle at 550ºC for at least 18 h. The crucibles with the remaining ash were cooled to room temperature in a desiccator and then weighed. Ash content was determined calculating the difference between the final weight of the crucibles and its tare. Determinations were performed in duplicate and reported as mass percentage of initial samples on a dry basis (mean values).

2.2.4. Determination of carbohydrates, acetyl groups and Klason lignin

Original and pretreated solids were subjected to a process of quantitative acid hydrolysis following NREL/TP-510-42618 protocol (Sluiter et al. 2012). Solid samples placed into test tubes were rigorously weighed (200.0 ± 10.0 mg) to the nearest 0.1 mg on an analytical balance (Mettler 160 HK, Switzerland). Sulfuric acid 72% w/w (2 mL) was added to test tubes containing the solid sample, which were kept for 1 h at 30ºC in a Memmert (Schwabach, Germany) W350 water bath, manually stirred every 10 minutes with a glass stirring rod, to ensure proper contact between the samples and the acid enabling complete dissolution of the polysacharides. The content of the tubes was then transferred to 250 mL Duran™ Schott™ borosilicate glass flasks with screw caps, diluted with distilled water to a concentration

of 4% (w/w) of H_2SO_4 and placed inside an autoclave (Uniclave, Portugal) at 121ºC for 1 h, to ensure complete hydrolysis of sugars to monosaccharides and acetyl groups. After cooling down, the mixture was filtered through previously dried (in a muffle furnace) and weighted sintered glass crucibles (#3 porosity). The solid remaining in the crucibles (corresponding to Klason lignin and ash) was washed with 100 mL of distilled water and dried in an oven at 100ºC to constant weight and then burned in a muffle furnace for gravimetric determination of Klason lignin (with ash correction). An aliquot of the obtained liquid phase was filtered through 0.45μm nylon filters and analysed via HPLC for monomeric sugars and acetic acid. A factor of 1.04 was considered for hexoses and 1.09 for pentoses, for taking into account sugar degradation during the hydrolysis step. Mass concentrations of monomeric sugars and acetic acid were converted into polysaccharides and acetyl groups concentrations considering a respective factor, which accounts for the gain of a water molecule per each monomer molecule during acid hydrolysis of the polymers. For each sample, the procedure was performed in duplicate and results were reported as mean values on a dry basis.

2.2.5. Enzymatic hydrolysis

Enzymatic digestibility of pretreated solids and original biomass was evaluated based on standard NREL/TP-510-42629 laboratory procedure (Selig, Weiss, and Ji 2008). Specifically, 0.2000 g of sample biomass were dispersed in small capped-plastic 50 mL vials within a 4 mL total volume aqueous solution (5% w/w solids, dry basis) of 50 mM sodium citrate of 5 pH buffer, 40 µL of 2 g/L sodium azide solution (0.02% w/v), as anti-microbial agent, and 10% (w/w cellulose) of Celli®CTec^2 enzyme (enzyme activity of 199.9 FPU/mL; density of 1058 mg/mL). The volume of biomass was taken into account for the calculations of the amount of distilled water to be used in order to obtain the desired final volume (4 mL), considering that the biomass has a density of 1 g/mL.

All assays were performed, at least, in duplicate. Blank assays were also prepared to account both the sugars that may arise from the enzyme solution (carried out without added biomass) and the sugars derived from non-enzymatically hydrolysis of the biomass (carried out without added enzymes).

Thereafter, the vials containing the sample mixtures were placed in an orbital incubator (Opticivymen® system, Spain), at 50ºC under orbital shaking of 180 rpm. After 72 h, the samples were removed from the incubator and immersed in a boiling water bath, for 10 min, to inactivate the enzymes, after which they were filtrated using nylon filters (pore size of 0.45 µm) and analyzed by HPLC for released sugars. The enzymatic digestibility was determined by the ratio of released glucose and xylose to the maximum glucose and xylose that could be obtained from total glucan and xylan contained in biomass prior to enzymatic hydrolysis, considering the factors of (162/180) and (132/150) to account for the hydrolysis glucan and xylan to their monomeric forms, respectively.

2.2.6. Quantification of sugar monomers and oligomers and degradation products

Analysis of pretreatment liquors was performed according to NREL/TP-510-42623 laboratory procedure (Sluiter et al. 2006). Pretreatment liquors were directly analyzed for quantification of sugar monomers (hexoses and pentoses), aliphatic acids and furans via HPLC. Oligomers were indirectly quantified after submitting the liquors to quantitative acid hydrolysis followed by HPLC analyis, as the difference between the concentration of monomers after and before hydrolysis (with the correction factors for degradation and water uptake). The hydrolysis step was performed by adding concentrated

sulfuric acid (72% (w/w)) to the liquid samples (10 g to the nearest 0.1 mg) in the appropriate amount to achieve an acid concentration of 4% w/w, taking into account the pH of the liquid sample. The resulting solution was placed in an autoclave at 121ºC for 1 h. After completion of the autoclave cycle, the hydrolysates were slowly cooled down to room temperature, with an aliquot filtered through 0.45 µm membranes (Millipore®) and analyzed by HPLC. This procedure was always performed in duplicate.

2.2.7. HPLC analysis

Quantification of sugars (glucose, xylose and arabinose), aliphatic acids (acetic and formic acids) and furans (HMF and furfural) in the liquors obtained from the pretreatments, the hydrolysates from quantitative acid hydrolysis and the enzymatic digests was performed using an Agilent 1100 Series HPLC system (Waldbronn, Germany), equipped with an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA), with a 7.8 mm diameter and 300 mm length. Analyses were performed at 50ºC, using 5 mM H_2SO_4 as mobile phase at a flow rate of 0.6 mL/min, with an injection volume of 5 µL. All samples were filtered using nylon filters with pore size of 0.45 µm before being injected in the HPLC system. The detection was performed using a RID (refractive index) detector at 50ºC for monosaccharides (glucose, xylose and arabinose) and aliphatic acids quantification, and a DAD UV/Vis photodiode detector set at 280 nm for furans (furfural and 5-hydroxymethylfurfural). The quantification was performed by external calibration using standard solutions of the measured compounds (HPLC grade). The calibration curves were plotted as the area of the compound peak obtained in the respective chromatogram versus the compound concentration, varying between 0.01-0.1 g/L and 0.5-20 g/L. The appropriate range selected for quantification was always the one that included the concentration to be determined. The analysis of the obtained chromatograms, including the identification of compound peaks (by their elution time) and their integration (area determination for quantification) was performed using Agilent™ ChemStation™ for LC 3D systems (Agilent Technologies, 2001-2005, USA).

2.2.8. Quantification of soluble phenolics and acid soluble lignin

Pretreatment liquors and hydrolysates from quantitative acid hydrolysis were analyzed for soluble lignin/phenolic content following NREL/TP-510-42618 protocol (Sluiter et al. 2012). A background of the same solvent of the analyzed sample was run in a Jasco 7800 UV-vis spectrophotometer (Jasco Inc., Japan). The absorbances of the liquid samples were measured at 320 nm as recommended (Hyman et al. 2007), after dilution with distilled water as appropriate to obtain absorbance values below 1.0. Absorbance values were then converted to phenolic content through the Lambert-Beer Law, considering an extinction coefficient of 30 $Lg^{-1}cm^{-1}$ for ER (Sluiter et al. 2012) and 16 $Lg^{-1}cm^{-1}$ for WS (Xu et al. 2006), while accounting for the performed dilution. Each sample was analyzed in triplicate and the average values reported.

3. Propylene Glycol (1,2-propanediol) -based organosolv

3.1. Introduction

Currently the existing biorefineries focus on the upgrading of the cellulose and hemicellulose fractions, which can yield ethanol used as biofuel or chemical feedstock after being hydrolyzed to fermentable monosaccharides. Moreover, lignin is treated as a low value by-product used for energy or fuel production (to recover inorganic chemicals resultant from the fractionation process and for heat/power demands of bioethanol production) due to its low purity and high content of carbohydrates, microorganisms, proteins and salts from the enzymatic hydrolysis and fermentation stages (Renders et al. 2017). However, the economic viability of the biorefinery can be significantly increased if the valorization of the lignin fraction, the largest renewable source of aromatics, is considered. Such endeavor should be attained following a “lignin-first” approach, as lignin recovered in the early stages of biomass fractionation (before enzymatic hydrolysis and fermentation steps) meets the high purity levels required for its valorization (Nitsos, Rova, and Christakopoulos 2017; Renders et al. 2017).

In accordance with this line of thought, the organosolv process has gained significant emphasis due to its ability to solubilize lignin as well as hemicelluloses, originating a soluble lignin fraction with suitable properties for high-value applications. Based on the potential presented by organosolv pretreatments, it was the main focus of this work to select a solvent that could improve existing biomass deconstruction results as well as the operation safety and sustainability and economic viability of the organosolv process as a whole.

A solvent with potential application in the biorefinery context must prove itself to be effective for biomass deconstruction. In order to understand which solvent performs better, it is relevant to understand the main mechanism of organosolv pulping. This is admitted as the combination of three types of chemical reactions: 1) lignin degradation resulting from cleavage of α-aryl (for lower severities) and β-aryl (only for harsh conditions) ether bonds, 2) breakdown of glycosidic bonds of hemicellulose and to a less extent cellulose to oligosaccharides and monosaccharides, and 3) degradation of those sugars to their dehydration products, i.e. furfural (from pentoses) and HMF (from hexoses), which then degrade to formic and levulinic acids, respectively (Zhang, Pei, and Wang 2016; Sannigrahi and Ragauskas 2013). All these reactions are known to be acid catalyzed, for which the acid character of the selected solvent becomes a relevant aspect in predicting its effectiveness for biomass deconstruction. The acid potential of the solvents depends on their dielectric constant (in fact the dielectric constant of the mixture of solvent with water), with the higher acidity corresponding to the higher dielectric constant. Typically, this property is measured by solubility and solvatochromic parameters. Such parameters also consider other properties relevant for determining if a solvent is effective for the removal of lignocellulose structural components, particularly its ability to solubilize those components. Such properties include measures of intensity of intermolecular forces – van der Waals, hydrogen, polar and London interactions –, measures of acidity, basicity, polarity, polarizability- all of which influence the solvent solubilization capacity. Particularly, polar solvents are more effective for penetration into the lignocellulosic structure, with the resulting enhanced deconstruction effect, due to the hydrogen bonding interactions that prevail between the solvent and the hydroxyl and carboxylic groups of lignocellulose (Demirbas 2008). The

most commonly used solubility parameter is the Hildebrand solubility parameter, though more complex ones exist, that consider more of the mentioned properties, such as the Kamlet-Taft empirical polarity and E_T empirical polarity. Additional, photochromic parameters also include viscosity and molecular size (molar volume) of solvent as these macroscopic variables determine the ability of the solvent to penetrate into the porous structure of biomass and the effect of cellulose swelling, both also very important to guarantee the solvent effectiveness (Zhang et al. 2016).

Similar values of the Hildebrand solubility parameter of solute and solvent indicate good solubility of that solute in that solvent, while the opposite it is also verifiable. For example, lignin has a Hildebrand solubility parameter of 22.5 $MPa^{1/2}$, which is similar to that of many organosolv solvents, particularly for ethanol (26.5), butanol (23.1), ethylene glycol (32.9), glycerol (36.1), propylene glycol (30.7) acetic acid (21.4), acetone (20.0) making them good solvents for delignification during pretreatment (Zhang et al. 2016).

The above-mentioned E_T empirical polarity combines polarity and acidity, besides the solubility compatibility of the Hildebrand parameter. Higher values of E_T should therefore indicate better potential for biomass deconstruction. The main organosolv solvents, in decreasing order of their E_T values, are glycerol (0.817 kcal/mol), ethylene glycol (0.79), methanol (0.762), propylene glycol (0.72), ethanol (0.654), acetic acid (0.648), butanol (0.586) and acetone (0.355) (Zhang et al. 2016).

More values and a deeper discussion of solubility and photochromic parameters can be found in (Zhang et al. 2016) and (Ratajzak and Orville-Thomas 1982). From the data above, it can be expected that glycerol, ethylene glycol, methanol and propylene glycol are the best alternatives for lignocellulose fractionation, and in fact alcohols are generally recognized as good biomass solvents being the main focus of organosolv research (Demirbas 1998; Jin et al. 2011; Liu et al. 2018; Romaní et al. 2016; Guo 2013; Jia et al. 2015; Zhao, Cheng, and Liu 2009).

Besides being effective for biomass deconstruction, any solvent to be selected should be a green solvent, following the ideas of sustainability and environmental responsibility. For the comparison of different available solvents typically used in organosolv processes, several solvent selection guides exist online that classify each solvent in several relevant categories: safety, health, flammability, environment, waste, reactivity and lifecycle. The safety category considers both the flammability and reactivity categories. For the flammability, boiling point, flash point, autoignition point, electrical conductivity and vapor pressure of solvent are considered. For the reactivity category, peroxide formation, self-reactivity, acidity or basicity and special hazards of the solvent are include. The health category considers occupational exposure limit, EU risk phrases and vapor hazard. The environment category includes the VOC status (vapor pressure and boiling point), the water impact (acute toxicity, log K_{ow} and biodegradation) and the air impact (rate of photolysis, photochemical ozone creation potential and odor threshold). In the waste category, three subcategories are included: incineration (heat of combustion, air pollution and water solubility), recovery (boiling point, ease of drying, chemical risks such as peroxides, reactivity and water solubility) and biological treatment (treatability in aeration basins, prevention of air releases and aqueous burden) of the solvent. Lifecycle analysis focus on the production and disposal impacts of the solvent, particularly the materials used, energy required, greenhouse gas emissions,

fossil resource depletion, acidification potential, eutrophication potential, photochemical ozone creation potential and total organic carbon before waste treatment (Byrne et al. 2016).

A combination from ACS GCI Pharmaceutical Roundtable Solvent Selection Guide and GSK Solvent Selection Guide were used (Henderson et al. 2011; Alder et al. 2016; Byrne et al. 2016). The results for the most common organic solvents used in organosolv pretreatments are illustrated in Figure 10. The classification scale used goes for each category goes from 1 (the best result) to 9 (the worst result). As such solvents for whose lines are more on the interior of the heptagon of Figure 10 are the greener ones, i.e. the ones that are preferable to be chosen for any industrial application. As such, methyl isobutyl ketone and methanol are definitely to be excluded, mainly due to their toxicity and safety issues, as well as other solvents that are also poorly classified (not represented), such as dioxane and phenol, despite being relatively common choices for organosolv studies. Solvents such as ethanol and acetone represent great risks of flammability and explosion and as such of safety as well, due to their volatility. The solvents that seem to score better overall are glycerol and propylene glycol as they are not flammable, non-toxic, safe, stable, and environmentally friendly. Propylene glycol is reported as having a poor lifecycle score, however it is assumed that this is a result of the fact that its production from fossil fuels is being considered. It is believed that if the production from biomass, already demonstrated as feasible in several reports (Cortright, Sanchez-Castillo, and Dumesic 2002; Guo et al. 2015; Clark et al. 2015), is assumed, then that lifecycle score will improve a lot, making PG a suitable green solvent.

Solvents that are more commonly studied for organosolv pretreatment, such as ethanol and acetone, are less recommended due to their high flammability and high vapor pressures (at pretreatment conditions), which pose a threat in terms of safety of operation. Methanol and MIBK are excluded due to their toxicity and negative impact on health.

The low vapor-pressures of high-boiling alcohols, as glycerol, ethylene glycol and propylene glycol, allowing for operation at near atmospheric pressure operation, present the additional advantages of lower capital costs (cheaper equipment and reactors) as well as lower operation costs (reduced pumping costs and energy requirements relating to vapor processing).

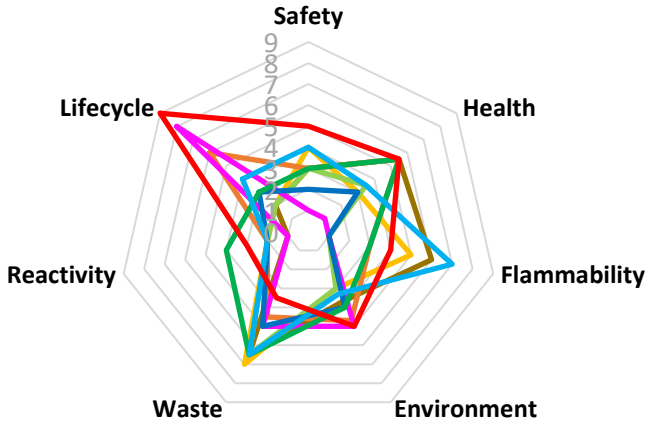

Figure 10. Solvent comparison according to green solvent selection guidelines. Higher values imply worse performance. Solvents: — methanol, — ethanol, — 1-butanol, — ethylene glycol, — propylene glycol, — glycerol, — acetic acid, — acetone, — MIBK

The use of a propylene glycol (PG)-based organosolv process has the advantage of its lower vapor pressure (as opposed to ethanol or acetone organosolv processes) (Aspen Technology 2016) that allows for operation at, cheaper, near atmospheric pressure, even at moderately high temperatures. Additionally, PG is a non-toxic solvent, less flammable and hazardous than most commonly solvents in organosolv processes, e.g. ethanol and butanol (Henderson et al. 2011). Furthermore, PG can be synthetized from lignocellulosic materials (Clark et al. 2015), turning it into a green sustainable solvent in agreement with the circular bio-based economy strategy. The use of an organosolv process allows the simultaneous solubilization of lignin and hemicelluloses, yielding a remaining cellulosic fraction highly susceptible of undergoing enzymatic hydrolysis. Propylene glycol has a considerably lower viscosity than glycerol (0.581 against 954cP), which makes its industrial application easier. PG also has the advantage of a lower vapor pressure when compared to more traditional ethanol or butanol organosolv (0.13mmHg vs. 59.3 for ethanol and 7.0 for butanol, at 25ºC), which allows for operation at cheaper near atmospheric pressure. PG has also been considerably less studied than glycerol and many other organosolv solvents, making its study an interesting endeavor. For preliminary experiments with other solvents, that further justify the solvent selection in the present work, consult Supplementary materials D and E.

In this chapter, a PG-based organosolv pretreatment was evaluated for the deconstruction of eucalyptus residues (ER) and wheat straw (WS) – widely available biomass materials suitable for potential valorization in the context of a biorefinery concept. Pretreatment conditions and their effect on the lignocellulosic composition of the feedstock is assessed. The enzymatic digestibility of pretreated solids is also discussed.

3.2. Materials & Methods

3.2.1. Batch operation

Pretreatment experiments were carried out in pressure tubes (25 mL) with Teflon screw caps (ACE Glass Inc., USA), using 1 g of dry biomass and a liquid-to-solid ratio of 10 (dry basis). The tubes were placed in a silicone-oil bath previously preheated to the prescribed temperature. Homogenization was assured by magnetic stirring. Bath temperature was kept constant at 140 or 160ºC (±1ºC) by means of a temperature controller (IKA C-MAG HS7). Pretreatments were carried out for 1 or 3h with different solvents and aqueous solutions of propylene glycol of 0-100% on weight. Once the reaction was finished, the contents of the pressure tubes were allowed to naturally cool down to room temperature, after which they were filtered through previously oven-dried weighted VWR glass microfibers filters (pore size of 1.2 μm). Aliquots of the recovered liquid hydrolysates were filtered through 0.45μm nylon filters and analyzed by HPLC and for soluble phenolic content via UV spectrophotometry. The hydrolysates were also analyzed by UV spectrophotometry for solubilized phenolic content determination. The recovered solid fractions were washed with 20 mL of fresh solvent, and then extensively washed again with distilled water. The washed solids were oven-dried at 100ºC overnight, for pretreatment solid yield determination. This was evaluated as the ratio of the weight of the recovered dried solids to the initial weighted mass (dry-basis). The dried solids were chemical characterized for determination of their structural composition and also subjected to enzymatic hydrolysis to assess the digestibility of treated solids.

3.3. Results and discussion

3.3.1. Pretreatment of eucalyptus residues

3.3.1.1. Effect of propylene glycol pretreatment on the biomass composition

Figure 11 shows the solubilized biomass during pretreatment of eucalyptus residues (milled to 0.5 mm) with aqueous solutions of propylene glycol at 140ºC and 160ºC per initial dry feedstock (obtained as the difference between calculated solid yield and 100%, corresponding to no-treatment), in mass percentage, as a function of both the weight composition of PG in the solvent (PG wt%) and the severity factor, log Ro (see Equation 1 in item 1.2).

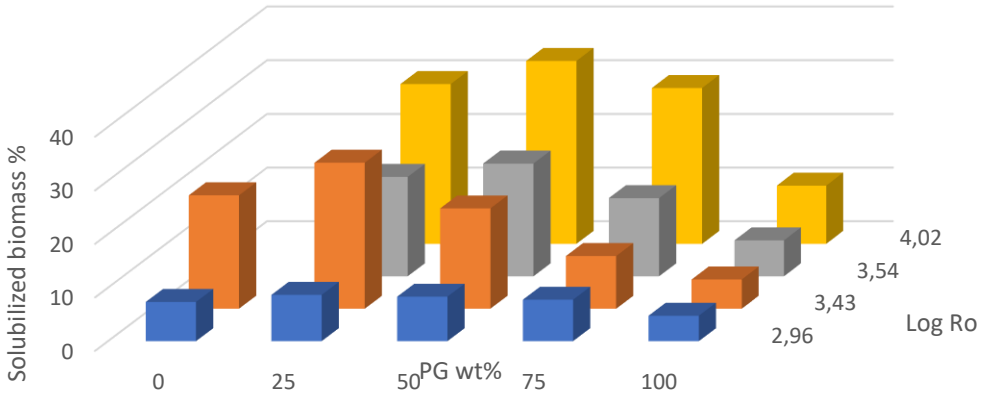

Figure 11. Effect of PG content and the pretreatment severity factor (■ 2.96, ■ 3.43, ■ 3.54, ■ 4.02) on ER solubilization (% of initial dry feedstock)

Figure 12 further discriminates the results of solid yield in terms of the recoveries of glucan, xylan and lignin in the solid fraction remaining after pretreatment (also as a function PG content and severity). These results are based on the chemical composition analysis (quantitative acid hydrolysis (Sluiter et al. 2012)) performed for the dried pretreated solids.

According to Figure 11, the solubilized biomass increased with pretreatment severity (log Ro), as a result of higher reaction temperature and time with consequently higher levels of breakdown of macroconstituent molecules. Mixtures of the organic solvent and water, in different proportions, performed always better than both individual solvents. Treatment for a severity factor of 4.02 (160ºC, for 3h) with PG 50% (w/w) gave the maximum solubilized mass of 34%. Generally, optimum deconstruction was obtained for pretreatment solvents containing 50% propylene glycol, for which the xylan and lignin solubilization were the highest (see below).

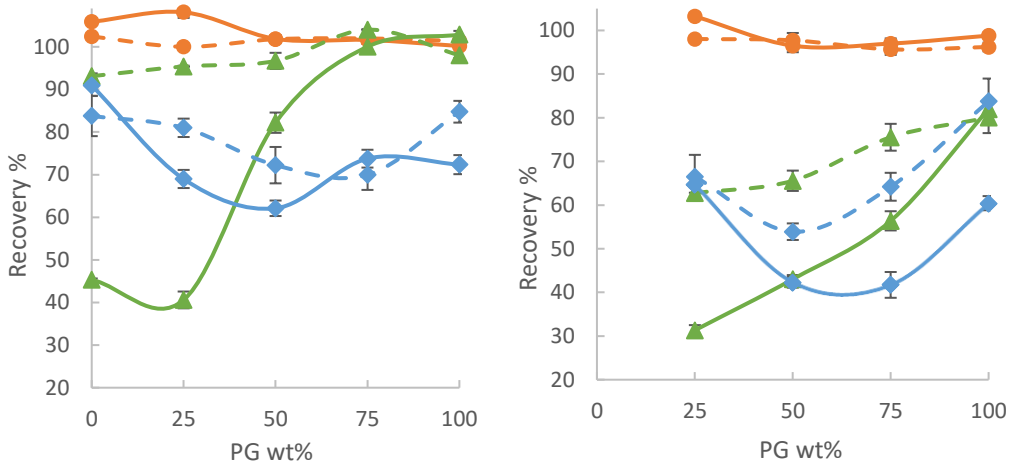

Figure 12. Recoveries of glucan (●), xylan (▲) and lignin (♦) in the solid fraction remaining after ER pretreatment of for **1 (- - -)** and **3h (—)** with aqueous solutions of propylene glycol at **140ºC (left)** and **160ºC (right)**

For every tested condition, all the glucan was preserved in the solid fraction (Figure 12), while hemicellulose and lignin were partially solubilized, in accordance with other reported organosolv experiments at such mild temperatures (Muñoz et al. 2011), which prevent degradation of the cellulosic fraction. However, at 140ºC, xylan solubilization is so slow that the 1h treatment led to almost no xylan removal at this temperature. At 160ºC, the reaction rates are enhanced, so that even pretreatment time of 1h already led to significant xylan solubilization. Furthermore, increasing water of the pretreatment solution led to an extensive increase in xylan solubilization, particularly for 3 hours of pretreatment, due to increased acidic potential of treatment solvent which catalyzes the reactions of lignin and xylan breakdown. Delignification was also improved as a result of higher water content in treatment solvent up to 50%. Above this value, delignification values decreased again, due to lignin's lower solubility in water when compared to PG. As such, PG content around 50% seemed to be the most effective for biomass deconstruction, as a result of synergistic effect of both xylan and lignin solubilization. This effect is very well known in organosolv literature, and has been observed for multiple organosolv pretreatments based on other organic solvents, including acetone (Huijgen, Reith, and Uil 2010), ethanol (Mou and Wu 2016) and glycerol (Romaní et al. 2013).

The increase of pretreatment temperature from 140 to 160ºC significatively increased xylan and lignin solubilization whilst glucose remained in the solid fraction. For example, delignification increased from 38 to 58% for the experiment with PG 50% for 3h when temperature was raised from 140º to 160ºC, meanwhile xylan solubilization was enhanced from 18 to 57%.

At 140ºC (Figure 12, left), xylan solubilization reached values as high as 60% for treatment with propylene glycol at 25% (3 hours), while maximum delignification was only 38% for treatment with the same solvent at 50% (also 3 hours). Solid yield reached its lowest value of 73% for the same conditions as the maximum xylan solubilization. As temperature was increased to 160ºC (Figure 12, right), minimum solid yield achieved was 66% for pretreatment with PG 50% for 3 hours, conditions that led to a maximum for delignification close to 60% (58%), accompanied by a similar xylan removal (57%). Maximum delignification obtained is similar to the value of 55% reported for organosolv pretreatment of the same feedstock with 50% ethanol at 160ºC and 3 hours (Oliet et al. 2000), though xylan solubilization

is lower than the reported 73% for organosolv with ethanol 60% at the same conditions (Mou and Wu 2016). The maximum xylan removal of 69% was obtained at 160ºC for PG content of 25% during 3-hour pretreatment, which is closer to the literature. However, delignification reached only 35.3% at those conditions.

Arabinan solubilization was always complete (data not shown). A reason for this could be the fact that, independently of the treatment conditions, arabinan is easily solubilized, as it exists on the side-branches of the hemicellulosic structure.

Figure 13 represents the lignin and xylan solubilization obtained for each batch assay. The experimental points represented by orange dots in Figure 13 do not follow the general tendency, for which they were not considered for the regression. Those points correspond to the treatments that utilized solutions with PG contents equal or below 25%, which explains their apparently unexpected small values of lignin solubilization (and deviation to the trendline in Figure 13), as lignin has a low solubility in solutions with predominantly aqueous character.

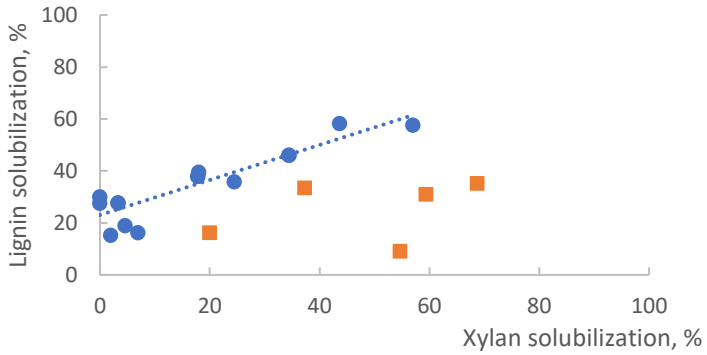

Figure 13. Correlation between lignin and xylan recovery in solid fraction obtained from pretreatment of ER with PG (r=0.91). **Orange dots** correspond to pretreatments with PG content equal or below 25%.

A positive linear correlation (Figure 13) was obtained between xylan and lignin solubilization for the pretreatment experiments. Such observation is in agreement with previously published papers, that report an interdependent mechanism of xylan and lignin solubilization (Archambault-Leger, Shao, and Lynd 2012). The positive intercept of the line in Figure 13 emphasizes that delignification occurred before the beginning of xylan solubilization. This observation implies that lignin solubilization is only dependent on xylan solubilization for delignification values above a specific value (26%). This result suggests the existence of two lignin fractions in ER: one that is solubilized before the attack to xylan and the other that is more strongly bonded to xylan because it is only removed when xylan is solubilized. Such result is in agreement with the mechanism of delignification proposed in literature by several articles (Zhao et al. 2017), that mention the existence of three different lignin fractions – initial, bulk and residual lignin – all with different reaction rates. In the case of the present work, the lignin fraction that is easily removed should correspond to the initial lignin, while the other fraction would be the bulk lignin. Interestingly, the value obtained in this work for the percentage of initial lignin in eucalyptus (25%) is considerably superior to the one (9%) reported for ethanol organosolv of the same feedstock by (Oliet et al. 2000), but it is however close to the value generally presented for woody biomass of 20% (Shatalov and Pereira 2005). Given the low severities applied, the residual lignin, the slowest to solubilize, and

part of the bulk lignin should still remain in the pretreated solids, in agreement with the delignification yields obtained.

The relation between the solid yield and the solubilization of xylan and lignin is shown Figure 14. As expected, good negative correlations were obtained as a higher solubilization of the structural macromolecules implies fewer remaining solids by the end of the pretreatment.

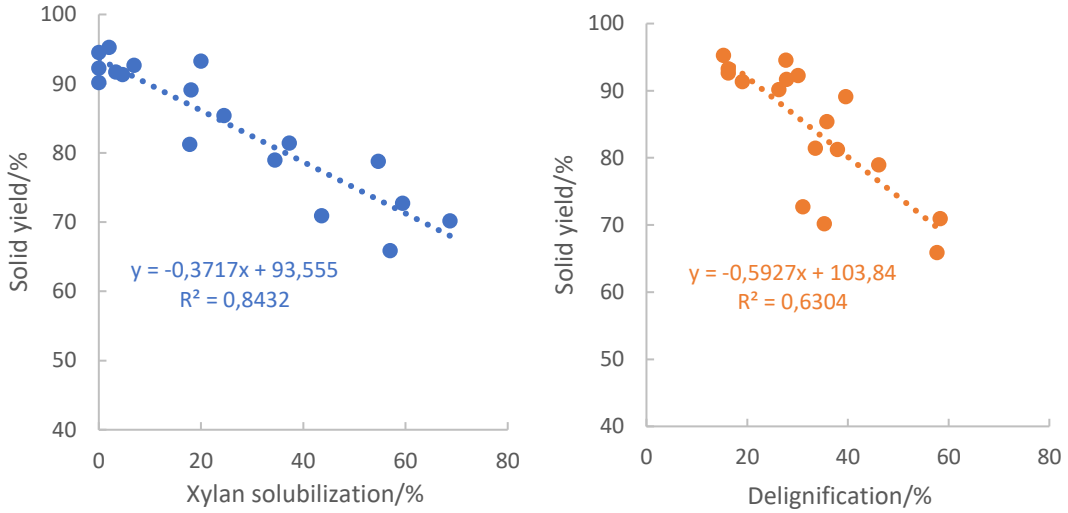

Figure 14. Correlation of fraction of solubilized xylan (left) and lignin (right), weight percentage, with solid yield for PG-organosolv pretreatment of ER

Interestingly, ER delignification seems to have an impact on solid yield that is approximately the double of that of xylan solubilization, as evidenced by the slopes of Figure 14. Moreover, when either xylan or lignin solubilization is null, solid yield is close to 100%, meaning that solubilization of other components is insignificant. Deviations to this value are most likely a result of error propagation during experiments.

3.3.1.2. Effect of propylene glycol pretreatment on the enzymatic digestibility of biomass

Glucan and xylan digestibility (72h) of the solid fractions remaining after pretreatment were evaluated (Figure 15), whereas untreated ER revealed extremely low glucan and xylan digestibilities of 3.5% and 1.3%, respectively. As seen, a similar behavior was obtained for the digestibility of both polysaccharides and the 3h pretreatments resulted always in higher digestibility than pretreatments for 1 hour, in agreement with more extensive lignin and xylan solubilization. It was also observed that pretreatment of eucalyptus at 140ºC was practically ineffective for a reaction time of one hour, whilst being mildly effective for three hours. In fact, glucan digestibility reached a maximum of 39% for the experiment with propylene glycol 25% at 140ºC for 3 hours, which is in accordance with the more extensive removal of xylan and lignin observed in this pretreatment (Figure 12).

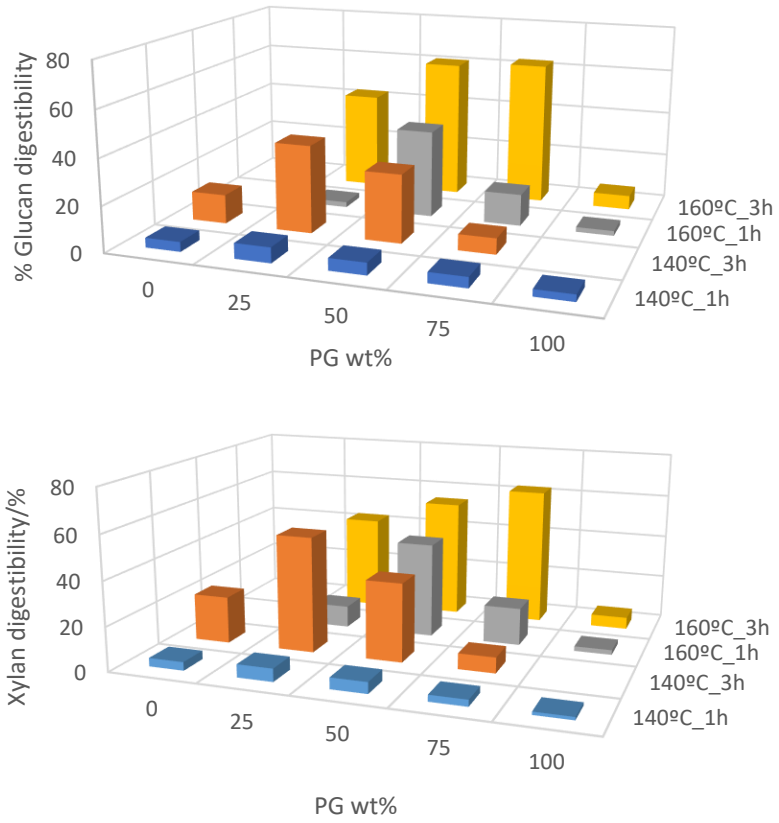

Figure 15. Effect of PG content of solvent and pretreatment conditions on glucan and xylan digestibility (72h) of ER

When pretreatment temperature was raised to 160ºC, the digestibility of eucalyptus increased considerably (Figure 15), even for reaction times of only one hour. This is correlated with the increased efficiency of the pretreatment at this temperature (Figure 12), i.e. lower solid yields and higher lignin and xylan solubilization. Glucan digestibility reached its highest of 63% for the 3-hour treatment with PG 75%. This value reveals already some potential of the proposed pretreatment for application in the biorefinery. Additionally, the maximum digestibility values obtained are similar to others reported in literature for other uncatalyzed organosolv processes, namely ethanol organosolv (Muñoz et al. 2011; Teramoto, Lee, and Endo 2008), while presenting the advantages of a lower operation temperature and pressure and no-use of catalysts.

The dependence between digestibility and xylan and lignin recoveries was properly modelled by a linear multivariable equation (Equation 2), visually represented in Figure 16, where x_1 corresponds to the xylan recovery and x_2 to the lignin recovery. This model was selected, as opposed to one with less (i.e. without interaction term) or more terms (i.e., quadratic factors), since it was the model with the least terms that was statistically significant. In fact, the use the minimum terms is desirable to prevent over-fitting and worse performance for external validation due to the inclusion of noise in the model (Braz et al. 2019). This model refinement was done by performing ANOVA analysis (Microsoft Excel, 2016) to obtain statistically significant F value, taking into account the coefficient of multiple correlation (r) and the coefficient of determination (R^2), as well as the significance levels of the model coefficients, all to be maximized.

$$\%\ \text{Glucan digestibility} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 \quad \text{(Equation 2)}$$

Table III. Coefficients of the multivariable regression of glucan digestibility as a function of xylan (x1) and lignin (x2) recoveries in the solid fraction after PG organosolv

	Coefficients	Confidence level (%)
β_0	200 ± 30	>95%
β_1	-1.8 ± 0.4	>95%
β_2	-2.1 ± 0.5	>95%
β_{12}	0.020 ± 0.006	>95%

The mathematical regression was obtained with both significant r (0.92) and R^2 (0.85) values. Furthermore, model parameters, β_i, were statistically significant at a 95% confidence level (Table III). A negative correlation was found between digestibility and both lignin and xylan recoveries, i.e. the solubilization of those fractions improves the digestibility of biomass, as expected (Zhao et al. 2017; Yang and Wyman 2004). The model highlights the importance of the synergistic effect of both xylan and lignin solubilization in enhancing the digestibility of treated ER. In fact, these variables alone did not have a significant impact on the digestibility.

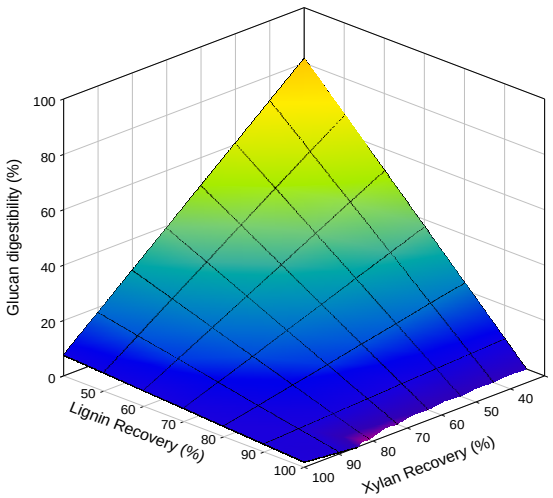

Figure 16. Glucan digestibility (%) as a function of xylan and lignin recovery in the ER pretreated solids (multivariable regression modelling)

It should be noted that the empirical model is only useful for predicting the digestibility values of ER treated with PG-based organosolv in the range of studied conditions when lignin and xylan recoveries are known and for identifying the type of relation between those three parameters. In fact, the model is purely mathematical and does not allow for an interpretation of the mechanism subjacent to enzymatic hydrolysis.

Glucan digestibility of treated ER is also well correlated to the solid yield, in agreement with its correlation with xylan and lignin recoveries. A quadratic correlation was fitted to the experimental results, as represented in Figure 17 (R^2=0.86), demonstrating that not only the digestibility of ER increases when pretreatment solid yield is decreased (i.e. when biomass deconstruction is more prevalent), but also that

that increase is steeper as more and more biomass is solubilized. In effect, significant increase in the digestibility of the feedstock occurs only when solid yield drops down to at least 80%.

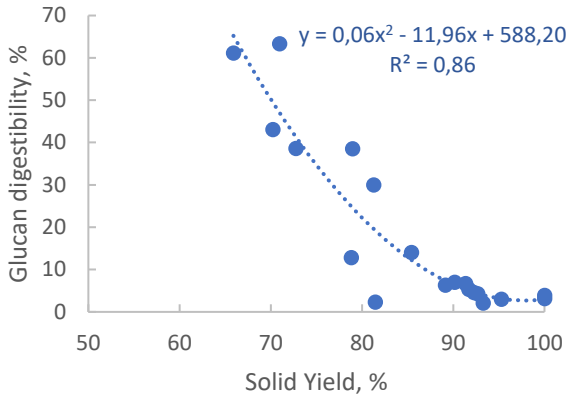

Figure 17. The impact of pretreatment solid yield on glucan digestibility of ER

3.3.1.3. Effect of propylene glycol pretreatment on the hydrolysate composition

The hydrolysates resulting from PG pretreatment were analyzed by HPLC for sugar and degradation products and by UV spectrophotometry for soluble lignin/phenolic compounds.

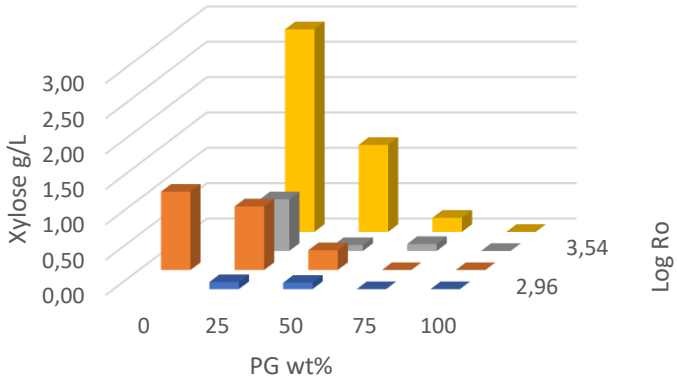

Figure 18. Xylose concentration in hydrolysate as a function of PG weight percentage and severity factor of pretreatment

The results showed that no glucose nor HMF were found in the hydrolysates, in agreement with the results presented above that showed almost complete glucan preservation in the solid fraction. Conversely, significant amounts of xylose, arabinose, acetic acid, and furfural were identified. Xylose concentrations measured in the hydrolysates are reported in Figure 18. Generally, as expected, an increase in xylose concentration was observed as pretreatment severity and the solvent water content were increased. Furthermore, no xylose was detected in the experiments using pure PG, since xylan solubilization and hydrolysis only occurs in the presence of water. These results are also in agreement with the xylan recovery trend in the treated solids (see above).

Additionally, acetic acid and furfural concentrations in the hydrolysates followed similar behaviors to xylose (data not shown). However, furfural was only found for pretreatment experiments at harsher conditions (160ºC, 3h). Arabinose was found in all experiments, except for 100% PG, at similar concentrations, as it is easily removed from hemicellulose side-branches.

An analysis of the calculations of the distribution of the pentoses and acetic acid between the solids and the hydrolysates indicated the production of oligosaccharides, but also the potential conversion into other products like PG-xylosides and PG-acetates (see Supplementary material F).

According to Figure 19, phenolic content in PG hydrolysates reached its maximum value in the solutions of 50% PG for all severities. The increase of the treatment severity increased the phenolic content of the hydrolysate, as a result of the higher delignification values. The combination of water and PG in equal parts revealed an important synergistic effect on lignin solubilization and phenolic compounds production.

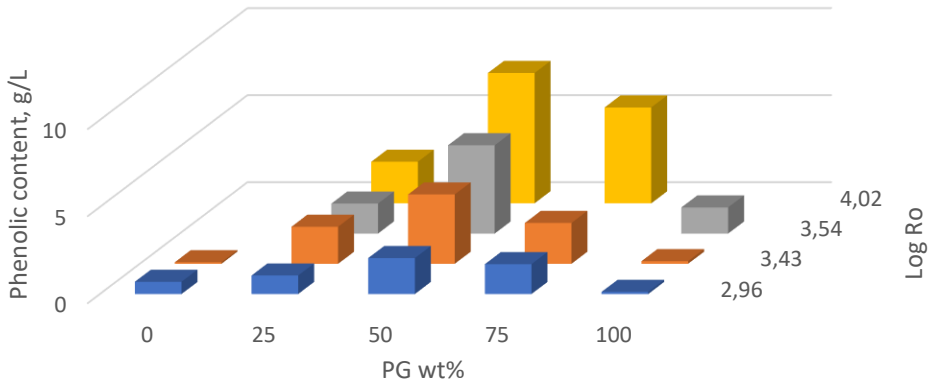

Figure 19. Phenolic content of hydrolysates as determined by UV-spectrophotometry

When compared with the delignification values determined from quantitative acid hydrolysis of treated solids (Figure 12), the values obtained from UV spectrophotometry were significantly lower. In fact, the delignification values derived from optical methods were 3 times lower than the values determined from the solid composition (Figure 20). This might indicate that the extinction coefficient used for ER, 30 $Lg^{-1}cm^{-1}$, (Sluiter et al. 2012) was not the most accurate. However, a value of 10 $Lg^{-1}cm^{-1}$ (3 times lower), so different from the literature, is also not expected. Hence, it is possible that some phenolic compounds reacted with PG to form other compounds that do not absorb, or absorb less, in the UV region, which leads to an underestimation of the delignification yield. Further, some of the products of lignin breakdown during organosolv pretreatment may not absorb or have lower extinction coefficients, contributing to the that underestimation of the phenolic content in hydrolysate.

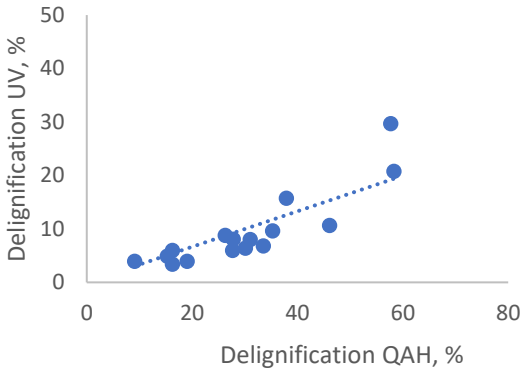

Figure 20. Correlation between delignification values calculated by two different methods: quantitative acid hydrolysis and UV-spectrophotometry. Regression equation: y=0.33x (R^2=0.72)

3.3.2. Pretreatment of wheat straw

3.3.2.1. Effect of propylene glycol pretreatment on the biomass composition

Solubilized biomass during propylene glycol organosolv treatment of wheat straw (Figure 21) showed a similar trend to ER, though with lower values. Maximum solubilization was only 27% for PG 25% pretreatment at a severity of 4.02 (160ºC for 3h) – compare to 34% for ER. Additionally, the increase of solubilized biomass with severity was a lot less steep than for ER. In fact, only the experiment at 160ºC for 3h induced a significant increase on that parameter, which was nearly constant for the other three conditions. Other authors also found wheat straw to be resistant to pretreatment at the mild conditions applied in this work. Wildschut et al. obtained a solid yield of 86.5%, for ethanol-organosolv of wheat straw at 160ºC for 1h with 60% ethanol content (Wildschut et al. 2013), while Huijgen at al. reported a 82.8% value for 50% acetone organosolv at the same conditions (Huijgen, Reith, and Uil 2010), both very similar to this work's value of 86.7%.

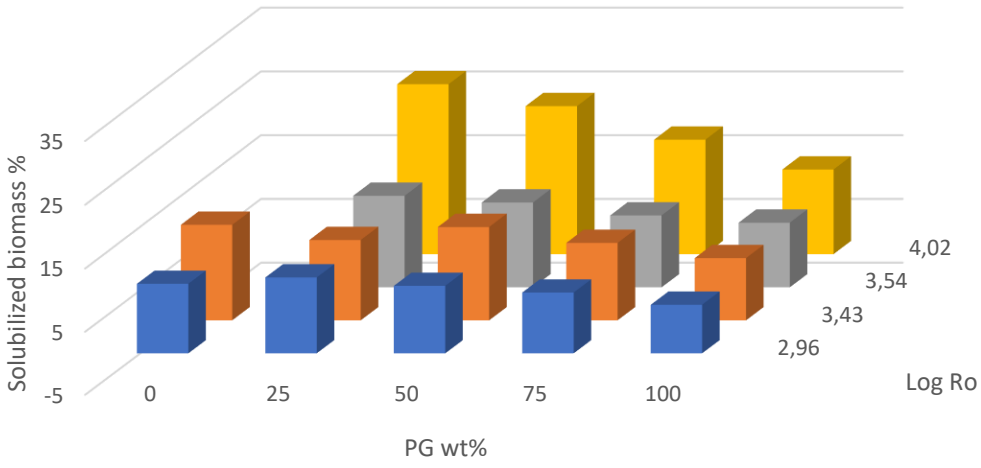

Figure 21. Solubilized WS biomass (% of initial dry feedstock) as a function of PG content and severity factor of pretreatment

Figure 22 indicates that, comparing with ER, the pretreatment was considerably less effective for the deconstruction of WS, particularly when the water content of the solvent was higher than 50%. However, the tendency for the preservation of the cellulosic fraction and for the removal of xylan and

lignin was similar. Even arabinan, normally the easiest constituent to be solubilized as discussed already, was not completely removed during any conditions tested on WS. Actually, all the pretreatments at 140ºC had very little impact on WS, because no xylan solubilization occurred and delignification was only around 20% (Figure 22). The increase of residence time from 1 to 3 hours added no additional improvement to the solubilization extent of WS constituents at 140ºC, as wheat straw was very recalcitrant to PG organosolv pretreatment at that temperature.

Figure 22 also indicates that at 160ºC, xylan and lignin solubilization increased slightly, particularly for treatment during 3 hours. Delignification reached a maximum value of only 37% (58% for ER) for PG 50% and xylan solubilization was only 37.7% (68.7% for ER) for a PG content of 25%, both at 160ºC and 3h. From these results, it is clear that wheat straw is much more resistant to propylene glycol-based pretreatment than eucalyptus. Despite this finding, it is interesting to note that the profiles of structural components recoveries are very similar for both the studied feedstocks (Figure 12 and Figure 22), indicative of an identical underlying mechanism of propylene glycol organosolv pretreatment. Pretreatment of wheat straw with ethanol organosolv at 160ºC for 1hour was reported to result in 4.7% delignification and 5.0% xylan solubilization, while preserving 96% of the cellulosic fraction (Wildschut et al. 2013). Though those values of xylan solubilization and glucan recovery are similar to the ones obtained in this work, at the same conditions, PG-organosolv resulted in a considerably higher delignification of 27.6%, which, while still low, suggests the superior performance of propylene glycol to delignify wheat straw when compared to ethanol. PG organosolv also delignified more the LM than acetone-based organosolv, which reportedly solubilized only 11.5% of total lignin for the same conditions (Huijgen, Reith, and Uil 2010). Xylan solubilization was however higher in this case – 14.5% compared to 4.7% in the present work.

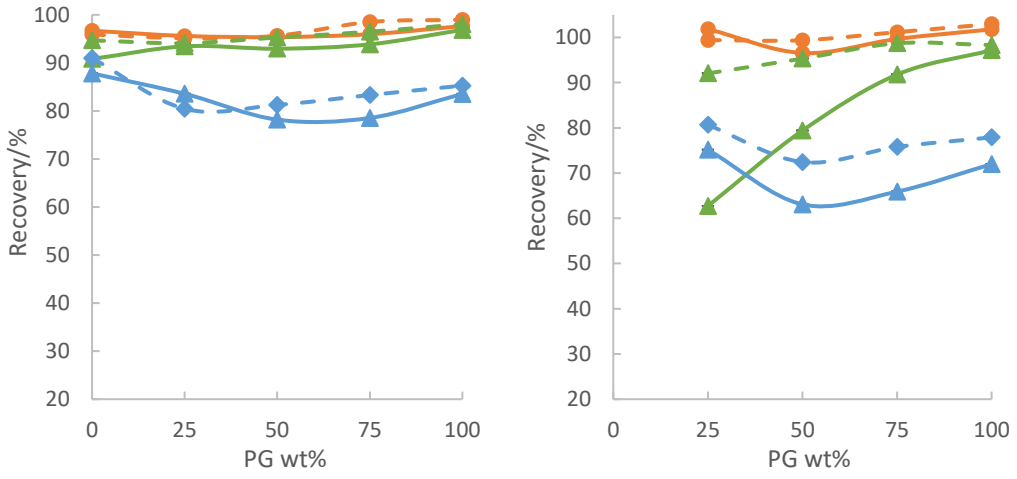

Figure 22. Recoveries of glucan (●), xylan (▲) and lignin (♦) in the solid fraction remaining after pretreatment of WS for **1 (- - -)** and **3h (—)** with aqueous solutions of propylene glycol at **140ºC (left)** and **160ºC (right)**

The negative correlations between the solubilization of both xylan and lignin structural compounds and solid yield for WS presented in Figure 23 are similar to those of ER. However, for WS, xylan solubilization had more impact on solid yield than delignification, as evidenced by the slopes of lines in

Figure 23. Again, as discussed for ER, the graphic of xylan solubilization had a y-intercept more distant to 100% than the one of delignification.

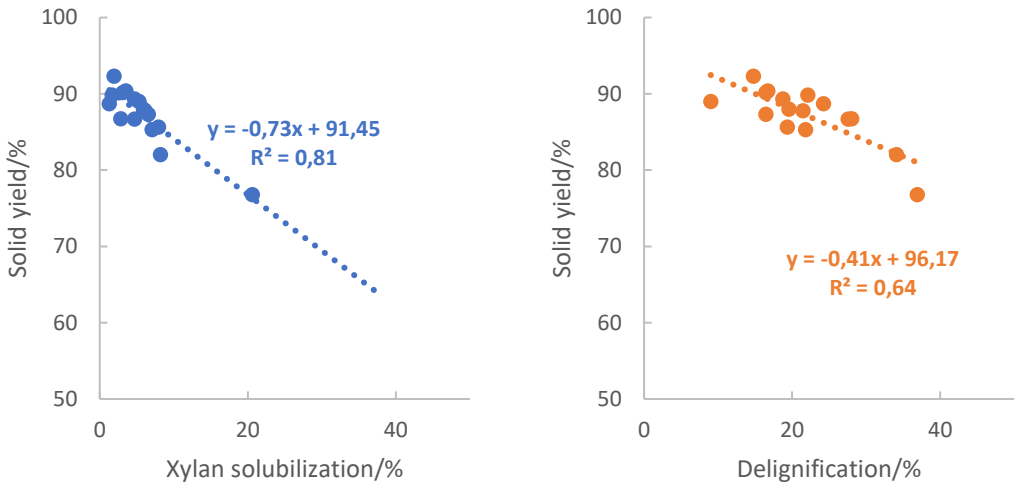

Figure 23. Correlation of fraction of solubilized xylan (left) and lignin (right), weight percentage, with solid yield for PG-organosolv pretreatment of WS

3.3.2.2. Effect of propylene glycol pretreatment on the enzymatic digestibility of biomass

In what concerns the digestibilities, untreated WS originated glucan and xylan digestibilities of 17.7% and 8.5%, respectively, higher than the corresponding 3.5% and 1.3% of untreated ER. This difference is probably a result of the higher lignin content of untreated ER (25.4% vs. 17.2%), which inhibits the action of cellulolytic enzymes (Herbaut et al. 2018). Digestibility of untreated WS was similar to that reported in literature, 20.7% in (Wildschut et al. 2013) and 16% in (Huijgen, Reith, and Uil 2010). After pretreatment, WS digestibility (Figure 24) improved, obviously, but with lower values than those of ER. This is certainly related with the lower effectiveness of PG pretreatment to deconstruct WS, as enlightened above (Figure 22). In fact, WS pretreatment removed lesser xylan and lignin than the pretreatment of ER, and so glucan was less accessible in treated WS, hindering enzymatic digestibility (Herbaut et al. 2018).

Exceptions to this were the treatment conditions that had very limited impact on feedstock composition and digestibility. Those include experiments at 140ºC for 1h and the ones using pure water or pure PG. In these cases, WS digestibilities were superior than the ones of ER, since they approached the values of initial unreacted feedstocks.

Considering Figure 24, a similar behavior was obtained for the digestibility of both glucan and xylan of WS, as pretreatment affected both digestibilities in the same way. As found for ER, the increase of the severity favored the digestibility. However, WS digestibility was only significantly enhanced for pretreatments at 160ºC during 3 hours. In fact, glucan digestibility of wheat straw reached a maximum of 56.1% (compared to 63% of ER) for the experiment with propylene glycol 25% at 160ºC for 3 hours because these conditions correspond to more extensive removal of xylan and lignin (Figure 22). The obtained digestibility of WS pretreated with PG50% at 160ºC for 1hour (32.9%) was very close to

the ones reported for ethanol organosolv and acetone organosolv at the same conditions – 30.5% (Wildschut et al. 2013) and 32% (Huijgen, Reith, and Uil 2010), respectively.

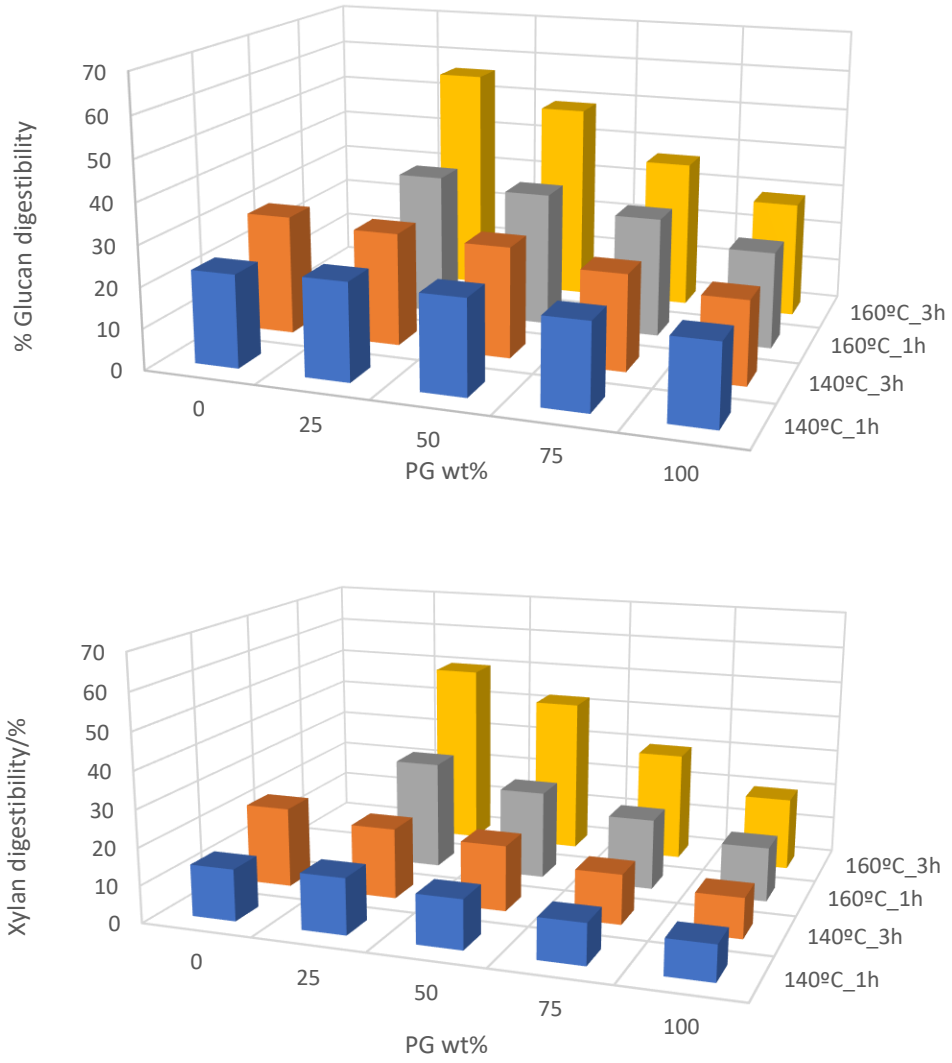

Figure 24. Effect of PG content of solvent and pretreatment conditions on glucan and xylan digestibility (72h) of WS

Adjustment of WS digestibility as a function of xylan and lignin recoveries following a linear multivariable equation such as the one used for ER (Equation 2) did not result in statistically significant model parameters. As such, a linear correlation without the interaction term was tested (Equation 3), for which statistical significance was then obtained. The model parameters, β_i, presented in Table IV were obtained with a 95% confidence level and the correlation parameters yielded significant values of 0.95 and 0.91, for r and R^2, respectively. Visual representation of the model is represented in Figure 25.

$$\%\ \text{Glucan digestibility} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 \quad \text{(Equation 3)}$$

Table IV. Coefficients of the multivariable regression of glucan digestibility as a function of xylan (x1) and lignin (x2) recoveries in the solid fraction after PG organosolv

	Coefficients	Confidence level (%)
β_0	140 ± 9	>95%
β_1	-0.87 ± 0.10	>95%
β_2	-0.38 ± 0.9	>95%

Table IV confirms that, as expected, a negative correlation was found between glucan digestibility and both lignin and xylan recoveries. The similarity of WS digestibility model with that of ER is rather evident with the importance of the synergistic effect of both xylan and lignin solubilization in enhancing the digestibility of WS (Figure 25). Moreover, for the same recovery values, WS digestibility is always higher than for ER, implying the more recalcitrant nature of latter for enzymatic hydrolysis when compared to WS. This is likely a consequence of the lower lignin content of WS (treated and untreated), for the same solid recoveries of xylan and lignin. Since WS has higher xylan content (26.3 vs. 22.3%) and lower lignin content (17.2 vs. 25.4%) than ER, this also explains why xylan recovery has more than a two-fold impact (patent in Figure 25, as the surface slope is a lot steeper along xylan recovery axis than along the lignin recovery axis, and indicated by a corresponding model coefficient, -0.87, that is more than double the one of lignin recovery, -0.38) on digestibility than lignin recovery for WS, while for ER the impacts of both variables are similar (similar values for corresponding model coefficients, -1.8 vs. -2.1). It is worth mentioning the fact that $\beta 1$ and $\beta 2$ values are superior for ER, which should be more of an inherent consequence of the selected model, originated by a lower initial digestibility of untreated ER when compared to WS, than an actual stronger impact of xylan and lignin solubilization on the digestibility of that feedstock. Therefore, it can be concluded that the reduced digestibility of WS when compared to ER presented above was a consequence of the former's resistance to PG organosolv pretreatment, rather than of it being more recalcitrant. Hence, if deconstruction of wheat straw is enhanced, the glucose yields obtained by enzymatic hydrolysis can potentially achieve higher values than for ER, unlike what seems to be suggested by the low yields obtained in these experiments.

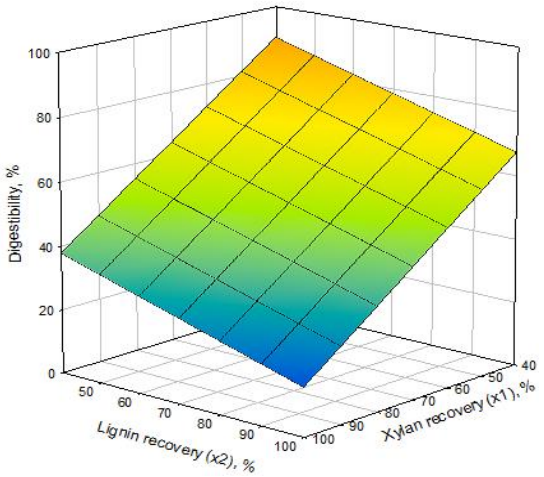

Figure 25. Glucan digestibility (%) as a function of xylan and lignin recovery in the WS pretreated solids (multivariable regression modelling)

Like for ER, glucan digestibility of treated WS adjusted properly to a quadratic trend-line (Figure 26), with a high correlation coefficient (R^2=0.93). In agreement with the previous discussion, for the same solid yield, WS digestibility is higher than that of ER. Digestibility values of wheat straw are always higher than those eucalyptus, as the former is less recalcitrant to enzymatic hydrolysis.

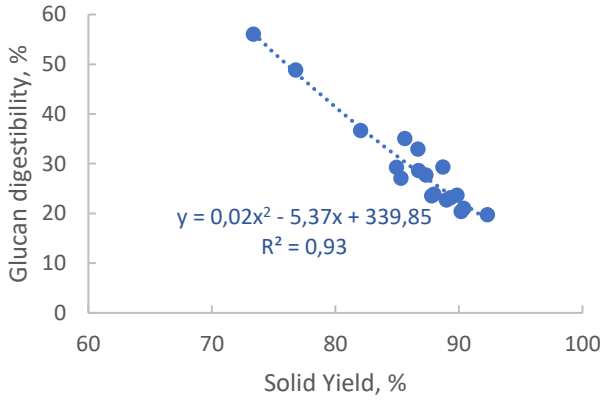

Figure 26. The impact of pretreatment solid yield on glucan digestibility of WS treated with PG-organosolv

3.3.2.3. Effect of propylene glycol pretreatment on the hydrolysate composition

Sugar content of pretreatment hydrolysates from WS treated at 160ºC (1-3 hours) was assessed (Figure 27). Results agree with the sugar recoveries reported previously (Figure 22) for those conditions, with well-closed mass balances. Increasing water content increased the concentration of free and oligomeric sugars as higher acid potential enhanced sugar hydrolysis. Increasing pretreatment time from 1 to 3 hours also increased the solubilization of sugars, particularly for XOS sugars, since these are the ones that are removed at this temperature range (glucan is not solubilized at 160ºC and arabinan is very easily solubilized even for small reaction times, so time does not barely affect their solubilization). Additionally, treatment at 160ºC was not harsh enough to hydrolyze the solubilized xylo-oligosaccharides to xylose. Sugar content in treatment liquors of WS were lower than for ER, due to the less extensive sugar solubilization of the former during PG organosolv, as shown previously.

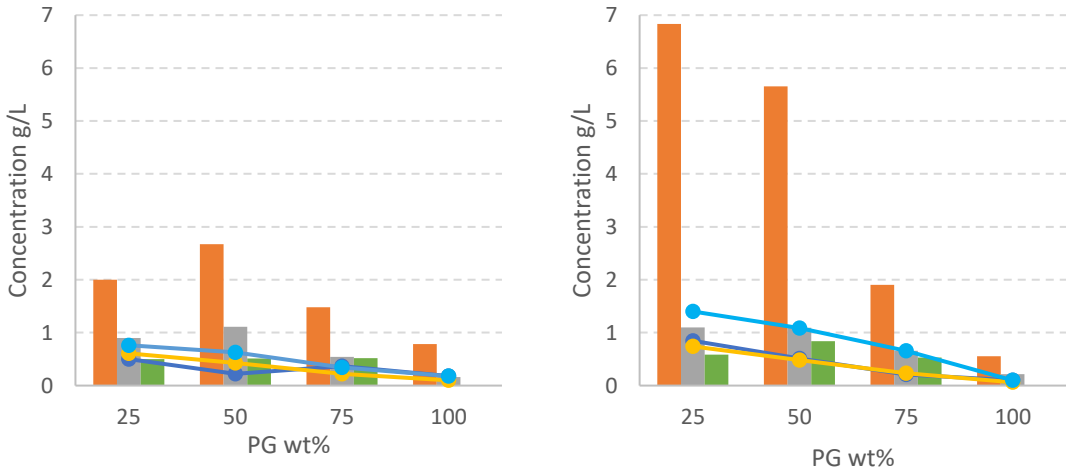

Figure 27. Concentration of oligosaccharides (■ GOS, ■ XOS and ■ AOS) and free sugars (● glucose, ● xylose and ● arabinose) in liquors resulting from PG organosolv of WS at 160ºC for 1 hour (left) and 3 hours (right)

No furfural nor HMF were detected in the hydrolysates, implying that no sugar degradation occurred. Compared to ER, higher amounts of acetic acid were observed (above 1 g/L), increasing with treatment time and solvent's water content.

Phenolic content of WS hydrolysates as determined by UV-spectrophotometry was represented as a function of pretreatment conditions (Figure 28). Determination of phenolic content was done using the Lambert-Beer equation as for ER, but considering an extinction coefficient reported for wheat straw of 16 $Lg^{-1}cm^{-1}$. (Xu et al. 2006) The obtained profile was generally similar to the one obtained for ER and agreed with the one obtained for the delignification values of straw (Figure 29), with phenolic content increasing with temperature and time and reaching improved values for PG/water ratios closer to 1. However, the values obtained for delignification calculated from the measured phenolic content for WS are higher than the ones measured from quantitative acid hydrolysis (Figure 22), on average 1.2 times superior, as indicated by the slope of the fitted line in Figure 29. Such fact suggests that the admitted extinction coefficient used for phenolic content calculation was too low and that the value of 19 $Lg^{-1}cm^{-1}$ (1.2 times superior) would be appropriate instead. This value is still in the range of values reported for wheat straw in literature, (Xu et al. 2006) for which it was concluded that it is in fact the value of the extinction coefficient for wheat straw lignin extracted with propylene glycol organosolv.

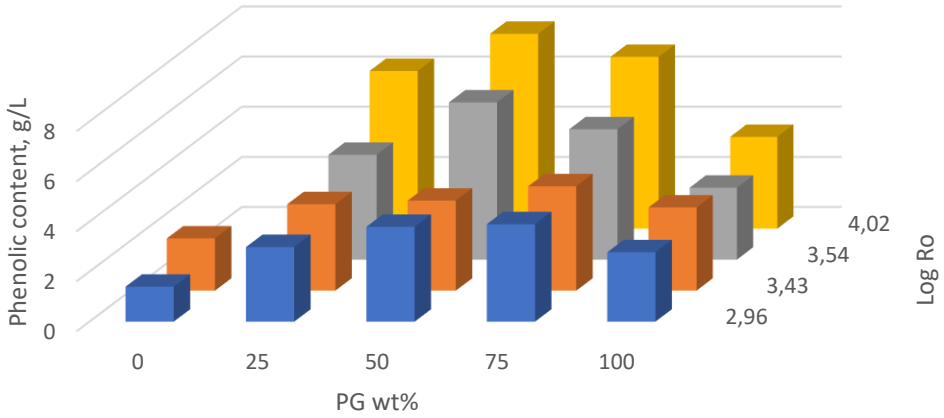

Figure 28. Phenolic content of hydrolysates from WS pretreatment as determined by UV-spectrophotometry

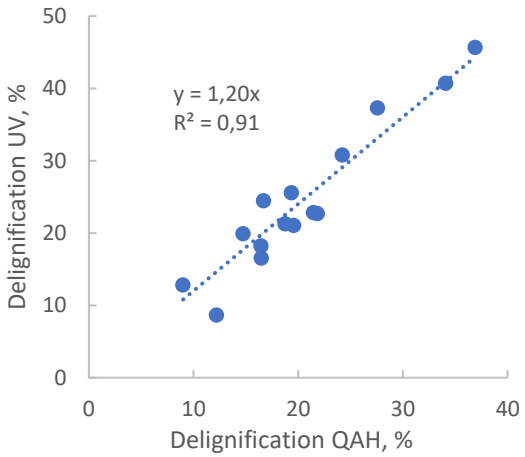

Figure 29. Correlation between delignification values calculated by two different methods: quantitative acid hydrolysis and UV-spectrophotometry

3.4. Conclusions

Propylene glycol was demonstrated to be a potential solvent for the organosolv fractionation of ER and WS, in the context of the biorefinery. Deconstruction of ER was more effective than for WS, but results were comparable to other organosolv processes. The synergistic effect of combined PG and water enabled hemicellulose and lignin removal close to 60% for ER, while preserving the cellulosic fraction in the pretreated solids. This glucan enriched fraction can posteriorly undergo enzymatic hydrolysis to release glucose, which can then be upgraded by fermentation or chemical pathways into multiple products. Enzymatic digestibility of treated ER and WS reached values of 63 and 56%, respectively, similar to other organosolv methods. However, PG consists of a greener, less volatile and safer solvent, which can be operated at near atmospheric pressure, with the respective gains in process competitiveness.

4. Catalyzed propylene Glycol (1,2-propanediol) -based organosolv

4.1. Introduction

The introduction of catalysts, either acid or alkaline, into the organosolv process has been connotated with a positive increase in biomass deconstruction, and yield of subsequent enzymatic hydrolysis. Organosolv pretreatment under acidic conditions results in higher hydrolysis and removal of hemicelluloses, while superior delignification are obtained under alkaline conditions (Zhao et al. 2017).

The use of catalysts during organosolv pretreatment enhances reaction and depolymerization of lignin. Acid catalysts strongly enhance carbohydrate hydrolysis, leading to low pulp yields enriched in precipitated lignin which remains undissolved in acid medium. Moreover, lignin condensation is observed in acid-catalyzed organosolv, as well as for organic acid organosolv, and is also a first order reaction, like lignin solubilization (Zhao et al. 2017). Under such conditions, increasing pretreatment severity increases dehydration of the side chains and condensation of lignin. Although highly-condensed lignin is formed during acid-catalyzed organosolv, delignification efficiency is not impaired and it has been suggested that condensed lignin with an high content of phenols and carboxylic acids, and low aliphatic carbon content may have be used as antioxidants or other added-value products (Zhang et al. 2016). Additionally, if harsh conditions are applied to acidic organosolv to achieve complete hydrolysis, it is even possible to obtain a fermentable sugar solution, dismissing the enzymatic hydrolysis step (Muurinen 2000).

To combine the effect of acid catalyst concentration with other operation conditions, i.e., time and temperature, in one parameter, an extension of the regular severity factor is usually applied – the combined severity factor, CS (Duarte et al. 2004). This parameter is defined in Equation 4, where pH refers to the pretreatment solvent.

$$\mathrm{CS} = \log\left[\mathrm{texp}\left(\frac{\mathrm{T}-\mathrm{T_{ref}}}{14.75}\right)\right] - \mathrm{pH} \quad \text{(Equation 4)}$$

The optimal CS, that yields high cellulose recovery and digestibility, depends on the type of feedstock and solvent applied, but is typically in the range of 1–2.5 (Zhao et al. 2017). Increasing the CS enhances sugar and lignin solubilization, though for high values undesirable cellulose removal and sugar degradation become predominant. A disadvantage of the combined severity factor is not accounting for the effect of the concentration of the organic solvent, which has obviously a relevant impact on the fractionation results.

As acid catalyst, sulfuric acid is the most common option (Zhao et al. 2017), though other inorganic acids (hydrochloric, phosphoric) have been used, as well as organic acids (acetic, formic, oxalic, acetylsalicylic, and salicylic) and metallic salts ($MgCl_2$, $FeCl_3$, $AlCl_3$, and $Al_2(SO_4)_3$). Generally, inorganic acids have a superior performance than organic acids and metallic ionic compounds.

Huijgen et al. verified a decrease in solid yield for ethanol organosolv of wheat straw from 78% to about 50%, when H_2SO_4 and HCl were applied at 190ºC. Correspondingly, xylan and lignin solubilization values raised from 20 and 30% to more than 80% and 70%, respectively. As a consequence, the enzymatic digestibility of the feedstock more than doubled, reaching values close to 99% (Huijgen et al. 2011). When $MgCl_2$ was used solubilization values were only increased to about 60%, while digestibility still reached the 90% value, indicating the superior performance of inorganic acids where compared to Lewis acids. Teramoto et al. reported an increase in cellulose digestibility of eucalyptus chips from 68 to 97% when 1% acetic acid was applied to ethanol organosolv at 200ºC (Teramoto, Lee, and Endo 2008).

In terms of alkaline organosolv, the application of alkali catalysts improves delignification, as it increases lignin solubility in pretreatment liquor, while strongly limiting sugar hydrolysis and degradation reactions, which are acid-catalyzed. However, very high temperatures in alkaline organosolv can reduce delignification yields be inducing re-lignification.

For alkaline organosolv, metal hydroxides are predominantly used, particularly NaOH, and to lesser extension KOH and $Ca(OH)_2$, though ammonia and amines can also be used. In general, sodium and potassium hydroxides perform better than $Ca(OH)_2$, and the latter performs better than ammonia and amines. Studies of alkaline organosolv are more unusual than acidic organosolv, due to the limited effect of alkali catalysts on the hemicellulosic fraction (Zhao, Cheng, and Liu 2009). For example, Hundt et al. achieved 89% delignification of beech wood with glycerol organosolv catalyzed with 8% KOH at 200ºC for 20 minutes, but hemicellulose solubilization was only at about 20% (Hundt et al. 2016). Even though digestibility of pretreated solids yielded more than 90% glucose, such high temperatures are not favored for industrialization. However, other authors have proposed alkaline organosolv pretreatments at mild conditions. For example, Raita et al. applied an acetone organosolv with 5% NaOH at 80ºC for only 5 minutes to rice straw, which increased its digestibility in a six-fold manner (to 93%) when compared to no catalyst (Raita et al. 2017). The same authors also teste the use of amines, for which the digestibility suffered only a two-fold increase. In other report, corn stover was delignified to 55% extent, while preserving 98 and 84% of cellulose and xylan, by using methanol with 10% NaOH at 80ºC for 1 hour, and resulting in 97% digestibility (Yuan et al. 2018). Alkaline ethanol organosolv has also been reported, e.g. 50% ethanol with 2% sodium hydroxide pretreatment of pich pine at 190ºC for 20 minutes, removed 75% of total lignin (25% more than without catalyst), while increasing the digestibility from 15% (without catalyst) to 57% (Park et al. 2010).

The study of propylene glycol organosolv as presented in the previous chapter yielded modest results for deconstruction of lignocellulosic materials and associated enzymatic digestibility. As discussed above, the use of catalysts in organosolv pretreatment has been proposed and extensively applied as a way to enhance the kinetics of hemicellulose and lignin solubilization, for a specific treatment severity (temperature and time) and, as a consequence, enzymatic hydrolysis yields of treated biomass have also been improved. Therefore, the possibility of inclusion of catalysts, either acid or alkali, in PG-organosolv should be considered.

In this chapter, acid and alkali-catalyzed PG-organosolv pretreatment is tested for fractionation of lignocellulosic eucalyptus. Results are compared with the ones of autocatalyzed pretreatment of the

previous chapter. Particularly, the effect on biomass composition and enzymatic digestibility after the pretreatments is discussed. The extensive impact obtained for acid-catalyzed PG organosolv on sugar solubilization justifies the focus on optimization of hydrolysate composition that follows.

4.2. Materials and Methods

4.2.1. Catalyzed batch operation

Batch pretreatments were performed as described in the previous chapter, except that with the introduction of acid (sulfuric acid) or alkaline catalyst (sodium hydroxide) in the solvent medium with a concentration of 0-1% w/w.

4.2.1. Optimization of liquor composition resulting from acid-catalyzed batch operation

A Plackett-Burman experimental design (Greasham and Herber 1997) with 8 trials and 4 variables was used to evaluate the effect of pretreatment conditions (time, temperature, PG content and acid concentration) during sulfuric acid-catalyzed PG organosolv. The selected studied ranges are reported in Table V, according to previous results (see chapter three). Exceptionally, for the time variable, the range 15-60 minutes was selected, since preliminary experiments with acidic PG-organosolv for 3h resulted in very high formation of degradation products (see item 4.3.1). The results obtained for the 8 experiments were used to devise novel operation conditions to be tested, namely by exploring intermediary conditions within the studied range, an extension beyond the traditional Plackett-Burman experimental design that only evaluates two levels for each variable.

Table V. Plackett-Burman experimental design for acid-catalyzed PG organosolv

Variables	Time (X1)	Temperature (X2)	PG content (X3)	H_2SO_4 concentration (X4)
Dimensions	Min	ºC	%w/w	%w/w
Basal Level	15	140	25	0.5
High Level	60	160	75	1

A multivariable regression model was applied to fit the experimental data following Equation 5, in order to better understand the impact of the individual operational conditions on the process (Silva-Fernandes et al. 2015).

$$Y = \beta_0 + \beta_{time}\text{Time} + \beta_{temperature}\text{Temperature} + \beta_{PG}\%PG + \beta_{H_2SO_4}\%H_2SO_4 \qquad \text{(Equation 5)}$$

Where, Y is any relevant studied response, β_0 is the regression coefficient at center point, and the remaining β_i are the coefficients of the respective variables. The linear regression and its analysis of variance (ANOVA) were carried out using Microsoft Excel 2016 regression tool pack. This approach also expands the traditional Plackett-Burman experimental data analysis, as it enables the quantification of the impact of the variables and not only its qualitative evaluation.

4.3. Results and discussion

4.3.1. Catalyst selection for PG-based organosolv

Eucalyptus residues were subjected to propylene glycol organosolv catalyzed by sodium hydroxide and sulfuric acid at 1% concentration on solvent mass at 160ºc for 3 hours. Figure 30 shows that alkaline catalysis did not result in an improvement in solid deconstruction, as solid yields remained practically identical to the ones of uncatalyzed treatments. However, it resulted in an enhanced selectivity, since the recovery of xylan was increased, while lignin was more extensively solubilized, particularly for PG 25%. Such result was expected given that lignin is more soluble in alkaline solutions and that the reactions for xylan solubilization are acid catalyzed (Zhao et al. 2017; Zhou et al. 2018).

Acid catalyzed PG-organosolv (with sulfuric acid 1%) had a superior performance for ER deconstruction than the alkaline pretreatment, significantly reducing experimental solid yields (when compared to uncatalyzed treatments) – from 68.4 to 50.0% for PG 25% and from 70.9 to 27.2% for PG 75%. These results are a direct result of the extensive solubilization of sugars, with complete removal of xylan and more than 50% solubilization of glucan, for both concentrations of PG. Acid catalyzed treatment with PG 75% remove 80% of total glucan present in the feedstock, which represents a very effective deconstruction of the lignocellulosic structure. Recoveries of lignin in the remaining solids were always higher than those of uncatalyzed experiments – 100% for PG 25% and 68.8% for PG 75% –, since lignin is very insoluble in acidic solutions. Nonetheless, decreasing solvent's water content improves the solubility of this macroconstituent, which justifies the lower solid recovery for PG 75%. Like the alkaline pretreatment, the acid catalyzed PG-organosolv increased the selectivity of uncatalyzed pretreatments, but by enhancing sugar solubilization (as opposed to lignin solubilization, enhanced for alkaline organosolv).

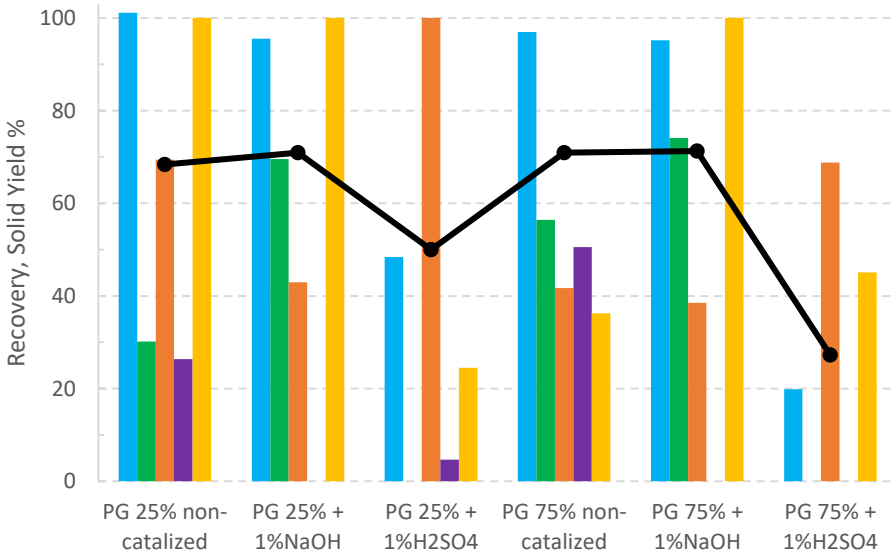

Figure 30. Solid yield (●) and recoveries of glucan (▪), xylan (▪), lignin (▪), acetyl groups (▪) and ash (▪) in the solid fraction remaining after autocatalyzed and acid/alkali-catalyzed PG-organosolv pretreatment of ER

Acetyl groups were almost completely solubilized for either acid or alkaline treatments, while ash was only removed in the acid catalyzed experiments, since alkaline media generally results in ash precipitation.

Comparing the enzymatic hydrolysis digestibility of the catalyzed and uncatalyzed treatments presented in Figure 31, it can be concluded that neither of the catalyzed treatments enhanced the digestibility obtained for the uncatalyzed experiments, which still remained somewhat low. Increased lignin removal appeared to improve ER digestibility for alkaline treatment with PG 25%, though only slightly to 57.4%, while complete recovery of the same compound in remaining solid fraction increased ER recalcitrance for acid catalyzed treatment with PG 25%, to 37.7%. In spite of these observations, as seen in Figure 30, acid catalyzed treatment with PG75% was able to remove a large fraction of cellulose. And so, enzymatic hydrolysis may not be a required step, as acid catalyzed PG organosolv could potentially perform simultaneous biomass fractionation and hydrolysis of sugars (pentoses and hexoses, simultaneously).

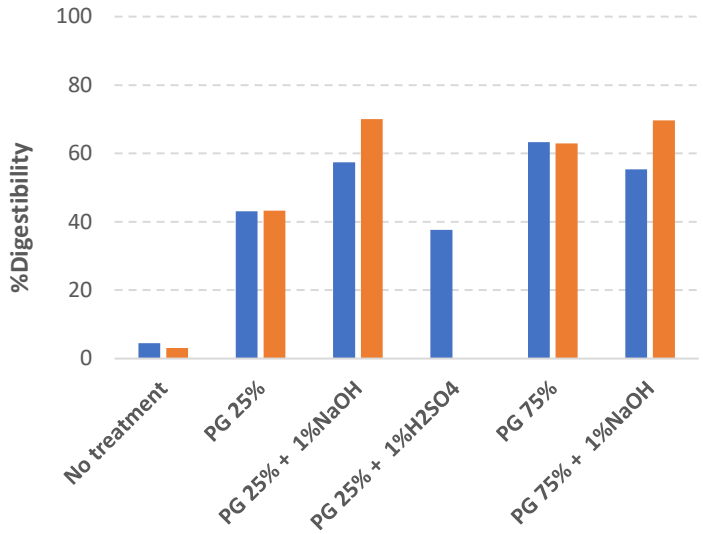

Figure 31. Glucan (▪) and xylan digestibility (▪) (72h) of pretreated acid catalyzed PG organosolv of ER

To evaluate that idea, the analysis of the composition of pretreatment liquors is very important. Results are presented in Figure 32. Even though glucose concentrations reached high values for the acid catalyzed treatments, 12 and 8 g/L for PG 25 and 75%, respectively, so did the concentrations of sugar degradation products, particularly formic acid (4.3 and 5.8 g/L) and furfural (3.4 g/L). Moreover, no XOS were detected in the liquors. From mass balances, the fraction of solubilized sugars that went over degradation reactions was between 50 and 70%, which represents a very high value of sugar losses. These results revealed that the conditions of the pretreatments were too harsh, meaning that the process could benefit from a reduction of acid concentration, pretreatment time and/or temperature. Additionally, other acids than sulfuric could be tested.

Noteworthy, in the acid pretreatments between 16 to 37% of total initial sugars were not quantified, suggesting the existence of sugar-PG side-reactions during treatment (see Supplementary material F).

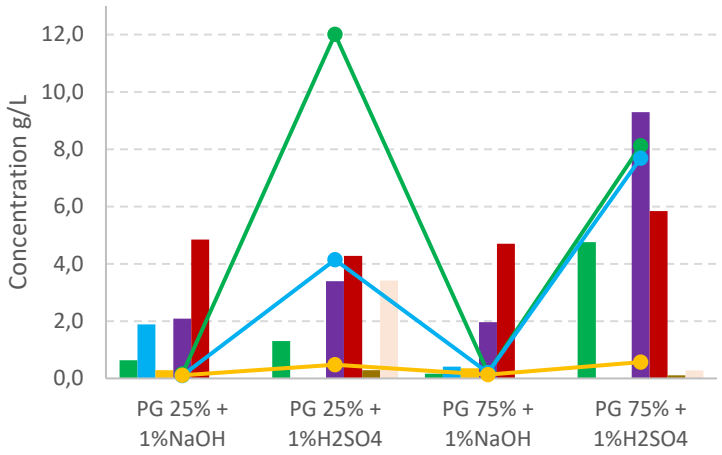

Figure 32. Concentration of sugars (● glucose, ● xylose, ● arabinose, ▪ GOS, ▪ XOS, ▪ AOS) and sugar degradation products (▪ formic acid, ▪ acetic acid, ▪ HMF, ▪ furfural) in alkaline and acid catalyzed PG-organosolv pretreatment liquor of ER

Regarding the alkaline pretreatment liquors, sugar concentrations were all very low, in agreement with the recovery results presented above (Figure 30). Alkaline ethanol organosolv of poplar wood, for example, also yielded much lower concentrations of sugars and inhibitors such as furfural than the acid-catalyzed pretreatment (Koo et al. 2011).

Based on this results, sulfuric acid-catalyzed treatment was further investigated, in the next section. Particularly, the optimization of the pretreatment conditions was pursued, in order to minimize the harsh amounts of sugar degradation products obtained in the treatment liquors.

4.3.2. Optimization of sulfuric acid catalyzed PG-based organosolv

The results of acid-catalyzed pretreatments with PG concentrations of 25 and 75% are represented in Figure 33, in terms of total xylose and XOS (xylose basis), total glucose and GOS (glucose basis), total sugars (mono- and oligomers), total sugar monomers, total degradation products (formic acid, HMF and furfural), phenolic content and TS-TDP, and as a function of the combined pretreatment severity factor (Equation 4). All the conditions of time, temperature, and PG and acid content tested, and corresponding CS, as well as the full set of results obtained can be consulted in Supplementary material G (Table XV).

At studied conditions, total xylose+XOS presented a maximum value for both graphics (at CS of 2 for PG25% and 3.4 for PG75%), resulting from the counteracting effects of xylan solubilization (positive effect) and degradation of pentoses to furfural and other products (negative effect). Glucose+GOS were present in pretreatment liquors at much lower concentrations than xylose+XOS, due to the slowest hydrolysis of cellulose, when compared to hemicellulose. Nonetheless, for the harshest severities, glucan solubilization occurs significantly as suggested by the levels of hexose concentration obtained. The expected peak for glucose+GOS similar to that of xylose+XOS was not observed, indicating that, advantageously, degradation of hexoses (in this case to HMF) was not significant for the severities applied. This agrees with the very low concentrations of HMF detected in pretreatment liquors. The concentrations of pentoses and hexoses were comparable for the experiments with PG content of

25% and 75%, with xylose/XOS concentrations around the maximum value (maximum of 16.6 vs. 14.9 g/L).

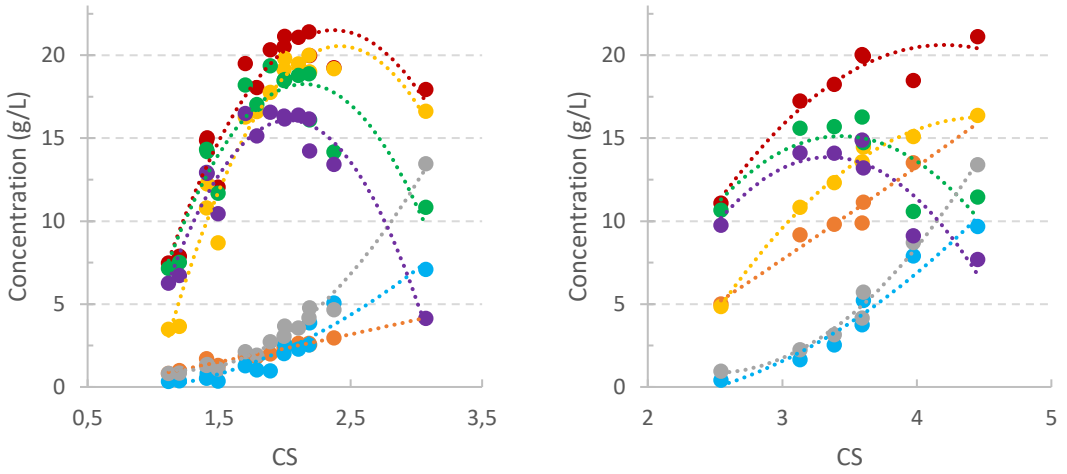

Figure 33. Composition of pretreatment liquors (● Xylose+XOS, ● Glucose+GOS, ● Total sugars, ● Total sugar monomers, ● Total degradation products, ● (Total sugars-Total degradation products), ● Phenolic content) from sulfuric acid-catalyzed PG-organosolv vs. combined severity factor of pretreatment (PG25% on the left, PG75% on the right)

Total sugars also had a maximum value (21.4 and 21.1 g/L for PG 25% and 75%, respectively) for similar reasons to that of total xylose+XOS. However, these maxima were displaced towards harsher severity factors due to the additional positive effect of glucan solubilization. The same applied for monomeric sugars. The difference between total and monomeric sugars, associated with the existing soluble oligomers, is considerably larger in the experiments with PG75%, while being almost null for PG25%. From this, it was concluded that the soluble sugars in the pretreatments with lower PG content were almost entirely in monomeric forms, while for the experiments with higher PG content a significant fraction was in oligomeric forms, varying between 25-50% of total sugars (c.a. 5 g/L).

Figure 33 also shows that higher severity conditions led to an increase of total degradation products, as expected, that became most predominant for the more extreme conditions, particularly the experiments at 160ºC for long times (more than 1 hour). Additionally, concentration of degradation products was higher for the experiments with propylene glycol content of 75% PG than 25% (9.7vs. 7.1 g/L of degradation products at highest severity). This result suggests that, disadvantageously, PG acts as a catalyst of degradation reactions. As a result, the difference between total sugars and total degradation products (TS-TDP) was also superior for PG25% than for PG75% (19.4 vs. 16.3 g/L). This could suggest that a 25% PG content is preferably. However, as discussed in the previous chapters, the capacity of the pretreatment to delignify the biomass is also important to enhance the yields of enzymatic hydrolysis. Additionally, from Figure 33, the phenolic content in pretreatment with PG75% is much higher than the that of PG25%. In fact, phenolic content in the first case was between 5 and 17 g/L, while for the latter it never surpassed the 5 g/L level. It is worth noting that the phenolic content increased as a linear function of the combined severity factor of pretreatments, unlike the remaining variables.

Even though the conditions with 50% PG were not represented to avoid overloading this chapter (see Supplementary material G), it was observed that those experiments followed the same trends discussed above, being located somewhere in middle of the plotted conditions.

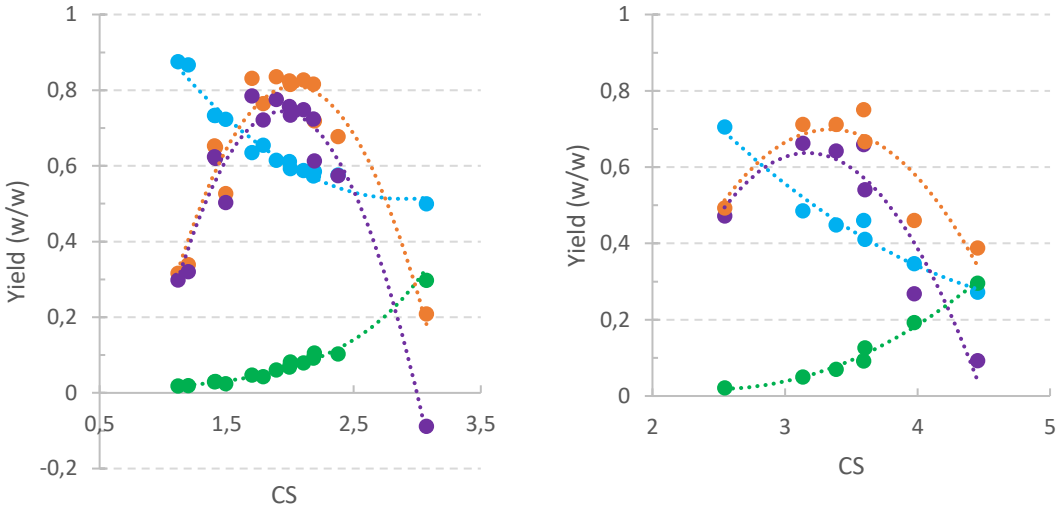

Figure 34. Solid yield (●), glucose yield in hydrolysate (●), xylose yield in hydrolysate (●) and the difference between xylose and glucose yield in hydrolysate (●) as target variable for optimization vs. combined severity factor of acid catalyzed PG-based organosolv (25% PG on the left, 75% PG on the right)

Since relevant concentrations of glucose+GOS could not be obtained without high concentrations of degradation products in liquors from sulfuric acid-catalyzed PG organosolv (Figure 33), optimization of operation conditions should then focus on maximizing xylose+XOS concentration, while preserving the cellulosic fraction for enzymatic hydrolysis. As such, the results from Figure 33 were plotted in Figure 34 as glucose and xylose yields. These were calculated as the ratio of total solubilized glucose+GOS (in glucose basis) and xylose+XOS (in xylose basis) to the maximum amount of the corresponding sugars that can be solubilized, which corresponds to the total amounts of glucan and xylan existing in the untreated feedstock. The difference between xylose and glucose yields was also represented as the target variable, as well as solid yield which serves as an indicator of the extent of biomass deconstruction obtained.

Given the objective is to have xylose yield as high as possible (close to 1), while keeping glucose yield as low as possible (close to 0), the target variable (difference between the previous two) should be as close to 1 as possible. This value was never obtained due to xylose degradation and/or some glucose solubilization, but a maximum of 0.78 and 0.66 were observed for PG 25 and 75% respectively. Higher xylose yield and less extensive degradation in PG 25% experiments presented above justify the difference between the two values. As indicated in Figure 34, considerable maximum xylose yields of 84 and 75% were obtained in treatment liquors of 25 and 75% PG content, respectively, while only degrading around 10% of initial cellulose. These optimum values were obtained around a combined severity factor of 2 and 3.5, respectively for each PG content. In spite of performing better for sugar recovery, the optimum conditions of PG 25% gave solid yields around 60% as represented, which is still a very high value, since glucan accounts for only 45.7% of ER. This means that PG25% in somewhat ineffective in

delignifying lignocellulose, in agreement with the phenolic content results of the treatment liquors presented above. However, for PG75% the optimum sugar recovery corresponded already to a solid yield of about 45%, revealing a much better performance for biomass deconstruction, particularly for lignin removal. Nevertheless, the use of PG75% resulted in more degradation products. Therefore, it could be concluded that PG contents between 25 and 75%, most likely around 50% result in the optimum tradeoff between all these factors. The operation conditions that correspond to the optimum CS are: short residence times of 15 minutes, temperatures of 150-160ºC and acid concentrations of 0.5-2%. Preferably, when the lowest temperature is applied, the highest acid concentration should be used and vice-versa.

The proposed multivariable regression models were fitted to the obtained results (see Supplementary material G) in order to better understand the impact of the individual operational conditions on the process. The resulting model coefficients (β_i), as well as the obtained coefficients of determination are summarized in Table VI for the selected studied variables, that only presents the statistically significant model coefficients (at the confidence level of at least 95%) with the model parameters left blank being found not to be statistically significant. Pentose content respects to free xylose and arabinose, while oligo-pentose content considers XOS and AOS. Additionally, hemicellulose solubilization (in g/L) includes both free and oligomeric pentoses, as well as acetyl groups and the respective degradation products (furfural), all in oligomer basis.

Table VI. Coefficient of determination and β coefficients obtained for multivariable regression models applied to acid-catalyzed PG organosolv (model equation 5)

Response variables	R^2	β_0	β_{time}	$\beta_{temperature}$	β_{PG}	$\beta_{H_2SO_4}$
Solid yield (%)	0,93	63,84	-6,83	-14,01	-13,97	-6,27
Hemicellulose solubilization* (g/L)	0,86	17,14	2,12	6,29		3,42
Pentose content* (g/L)	0,82	9,64		4,75	-3,73	2,54
Oligo-pentose content* (g/L)	0,95	4,11	-1,37	-1,35	2,65	
Phenolic content* (g/L)	0,89	4,28	1,21	2,58	5,70	

* In organosolv liquor

The R^2 values were all superior to 0.8 (superior to 0.9 for solid yield and oligo-pentose content), implying that the models obtained are able to effectively describe the reported data. However, for degradation products (DP) content this value was slightly inferior (0.73). Statistically significant coefficients are associated with process conditions that influence the response variables, while the magnitude of the same coefficients indicates the relative impact of each independent variable on the response (only for each model).

Table VI shows that all variables had a significant impact on solid yield. Furthermore, all of them have a negative coefficient, which means that the increase of the operational values will lead to lower solid yields, as expected, with temperature and PG content presenting a considerably higher impact. Specifically, hemicellulose solubilization was particularly enhanced by temperature, as well as by time

and acid concentration, with PG content showing no impact. However, the PG content has a strong impact on lignin solubility (increasing phenolic content). PG content also positively affected the concentration of solubilized oligomers, while negatively impacting pentose concentration. These results indicate that the solvent concentration only influences the level of hydrolysis of solubilized oligomers to pentoses, rather than the solubilization extent of hemicelluloses. Time and temperature presented a negative impact on oligomers content, due to their effect on enhancing hydrolysis reactions. Additionally, time was not significant for pentose content, putatively due to similar rates of hemicellulose hydrolysis and pentose degradation reactions. The concentration of sulfuric acid had no significant impact on oligo-pentose content under the studied range, as the minimum studied sulfuric acid concentration is enough to effectively catalyze oligosaccharides hydrolysis. This result is supported by the positive impact of the acid content on the pentose content. The amount of soluble phenolic compounds was not influenced by sulfuric content in the organosolv solvent, likely because of lignin re-condensation and precipitation on the surface of lignocellulose in acid environment.

Although this model is useful to understand the mechanism involved in biomass fraction, it has a limited capability to be used to derive optimal operation conditions, for which a more complete model (e.g. a quadratic model, encompassing interaction terms) would be needed. Experimental ongoing work will allow the derivation of these models in the future.

Analysis of pretreated solids resulting from three selected experiments carried out at conditions in the optimum range of Figure 34 was performed. Results are summarized in Table VII. Cellulose recoveries above 90% were always obtained, which is convenient for posterior enzymatic saccharification of the pretreated solids. Pretreatment at 160ºC with 75% PG and 0.5% sulfuric acid for only 15 minutes gave the best results for xylan and lignin solubilization, 82.5 and 83.9%, respectively, with the most interesting solid yield of 48.5%. The analysis of the sugars in this solution revealed a 17.3 g/L total sugars content, a 71% xylose+XOS yield, a 5% glucose+GOS yield and 1.6 g/L of degradation products, i.e. the best compromise between high sugar yields and the lowest concentration of inhibitors and hexose sugars.

Table VII shows that pretreatments at 150ºC and 50% PG using the same time resulted in less extensive delignification (64.5 and 68.2% for 1 and 2% acid concentration, respectively), due to lower PG content. However, the one with 2% sulfuric acid was able to completely remove hemicelluloses, resulting in a solid yield of 65.5%, not too distant from the experiment at 160ºC. Pretreatment at 150ºC with 50% PG and 2% acid for 15 minutes corresponded to a hydrolysate with 21.9 g/L of total sugars, 86.2% of xylose+XOS yield, 2 g/L degradation products, 7.8% glucose+GOS yield and a value of 0.78 for the target variable introduced above. These represent the highest values obtained pentose yield while preserving the cellulosic fraction and also maintaining a relatively low concentration of inhibitors. However, delignification is still somewhat low, and so the option of 160ºC pretreatment seems to be the most interesting one.

Table VII. Solid yield, composition of pretreated solids and recovery of structural components for the optimized experiments of acid-catalyzed PG-based organosolv of ER

Experiment				Solid Yield%	Solid composition of pre-treated ER %			Cellulose recovery in pretreated ER (%)	Liquid recovery/ solubilization (%)	
PG (%)	H_2SO_4 (%)	T (ºC)	Time (min)		Glucan	Xylan	Lignin		Xylan	Lignin
75	**0.5**	**160**	**15**	48.5	84.8	8.1	8.5	90.0	82.5	83.9
50	**1.0**	**150**	**15**	54.6	76.8	6.9	16.5	91.8	83.2	64.5
50	**2.0**	**150**	**15**	50.3	87.2	0.0	16.1	96.0	100.0	68.2

It is worth noting that, comparing with the results of Chapter three, the introduction of small amounts of sulfuric acid in PG organosolv allowed to reduce operation times from 3h to 15 minutes reducing operation costs and increasing production rate, while enhancing xylan solubilization and delignification values from about 60% to more than 80% each, at 160ºC. Moreover, solid yield was reduced from 65.9 to 48.5%, accordingly. Even though cellulose recovery in solid decreased from about 100 to 90% in the acid-catalyzed process, this is still an interesting value from an economical perspective, also because the solubilized 10% glucan did not suffer degradation, but were instead recovered in the liquor as free and oligomeric glucose.

Furthermore, the results of this work compare well with literature. For example, the acid catalyzed PG organosolv performed similarly to ethanol organosolv of poplar wood with 50% ethanol, 1.25% sulfuric acid at 180ºC for 60 minutes (Pan et al. 2006). In fact, the results of the present work were close to the ones reported by Pan et al. (solid yield of 52.7%, while xylan removal was 81% and delignification 72%), while allowing for a reduction of operational temperature, time and acid concentration, with the respective cost benefits. The same applies when comparing to 75% ethanol organosolv of eucalyptus with 1% acetic acid at 200ºC for 60 min, which reportedly yielded 46 and 89% xylan and lignin solubilization, respectively (Teramoto, Lee, and Endo 2008). Zhang et al. reported 74% xylan solubilization and 89% delignification of sugarcane bagasse with propylene glycol at 90% concentration with 1.2% sulfuric acid for 130ºC and 30 min (Zhang, O'Hara, and Doherty 2013). The results obtained in terms of the hydrolysate composition from ER treatment (Figure 33) in conditions similar to those reported by Zhang et al. (2013) indicates a solubilization of only about 60% of xylan and lignin with a solid yield of 54.2%. However, apart from the use of more favorable conditions than those used in the present work, sugarcane bagasse is expected to be much more easily deconstructed than ER studied in this case, which is a hardwood.

The application to the acid catalyzed experiments of Table VII of glucan digestibility as a function of xylan and lignin recovery developed in Chapter three (Equation 2) to the acid catalyzed experiments of Table VII, would allow the estimation of the digestibility that the acid-catalyzed pretreatments would originate. However, since the recoveries of the acid catalyzed experiments were lower than the ones originally used in the model, the direct application of the latter would result in significant extrapolation errors. As such, the model was recalculated to include the results of xylan and lignin recovery obtained in flow-through PG organosolv of ER (see Chapter 6), which allowed to extend the range of the independent variables to values that would include the ones obtained in the acid-catalyzed experiments. With this updated model, the digestibilities of these experiments were then predicted, as presented in Table VIII. The results allowed to conclude that it would be possible to obtain enzymatic digestibilities

of at least 80%, much superior than the maximum values of 63% presented in Chapter 3 (Figure 15) for the uncatalyzed PG organosolv.

Table VIII. Predicted glucan digestibility of optimum acid-catalyzed PG organosolv pretreatment of ER (model equation 2; standard deviation=9)

Experiment	Predicted cellulose digestibility (%)
PG75%, 0.5%H2SO4, 160ºC, 15min	89
PG50%, 1%H2SO4, 150ºC, 15min	73
PG50%, 2%H2SO4, 150ºC, 15min	87

In conclusion, mild temperatures (150-160ºC) and short residence times (15 min) can be applied in a propylene glycol-based organosolv when catalyzed with small amounts of sulfuric acid to result in extensive biomass fractionation, also enabling high glucose yields of the pretreated solids when subjected to enzymatic saccharification.

4.4. Conclusions

Sulfuric acid catalyzed PG-organosolv was able to effectively deconstruct the lignocellulosic structure of eucalyptus residues at 160ºC using 75% PG and 1% acid concentration after 15 minutes of reaction, resulting in 82.5 and 83.9% xylan and lignin solubilization, respectively. Cellulose was almost completely (90%) preserved in the remaining solid fraction and it can be predicted to result in 89% glucose yield when submitted to enzymatic saccharification. Moreover, the use of acid catalyst into PG organosolv process resulted in economically interesting yields similar or higher than the ones obtained in other autocatalyzed and acid-catalyzed organosolv processes described in literature, but using mild conditions. Alkali-catalyzed PG pretreatment did not result in enhanced biomass deconstruction, but merely in a slight superior selectivity for delignification.

5. Transition to continuous operation mode: Autohydrolysis of lignocellulosic biomass under diverse operation regimes

5.1. Introduction

Once good results are obtained in batch essays, the next natural step is the transition to a continuous configuration, as it has several advantages over batch mode: higher conversion, higher throughput, no time losses in heating, filling, cooling and emptying of reactor which have a negative impact on productivity, superior energy efficiency, lower CAPEX and OPEX indexes, easier controllability of the process variables, particularly with biomass as raw-material (Hundt et al. 2016). Additionally, in flow-through pretreatment the ratio of liquid to solid residence times is smaller than one (in batch operation is 1), meaning that the liquid leaves the reactor without allowing the degradation of polysaccharides and the solubilized lignin and hemicellulose to reprecipitate on the surface of the remaining solid. Therefore, continuous operation is able to achieve higher lignin and xylan removal, higher solids digestibility and less sugar degradation than batch systems for the same reaction conditions. Moreover, continuous operation allows lower liquid-to-solid ratio than batch mode while preventing dilution of product streams, decreasing costs associated with decreased solvent amounts and lower energy consumption of downstream purification steps (Archambault-Leger, Shao, and Lynd 2012).

Commercially available flow-through systems for biomass fractionation and investigation in the area focus mainly on liquid hot water and dilute acid pretreatments (Bhagia et al. 2016; Archambault-Leger, Shao, and Lynd 2012). Bhagia et al. reported a successful hot water continuous system at 300 L scale and a partial flow-through commercially available for wood chip (Bhagia et al. 2016). Continuous operation has been proved to enhance lignin and hemicellulose removal while batch mode removes mostly only the latter. The superior results can be due to more favorable mass transfer effects, prevention of lignin reprecipitation (as there is not enough time for it to happen), lignin-xylan complexes removal before they break down to more insoluble products, among others (Archambault-Leger, Shao, and Lynd 2012; Yang and Wyman 2008). Hence, flow-through allows operation at lower temperatures than batch pretreatment, which represents a decrease in capital and operation costs. As a downside, the dilution effect of the liquid stream in continuous processing makes downstream separations more costly as it increases energy requirements and equipment volume. Bhagia et al. (Bhagia et al. 2016) achieved delignification levels of 63-69% for flow-through LHW and dilute acid pretreatment (as opposed to 20-33% for batch) in a stainless steel tubular reactor at 350-400psig for 140ºC during 192min and for 180ºC during 12min. The obtained solid yield was c.a. 50%. 20-30% higher than for batch and the solids lignin content was 18-20% as opposed to 28-30% for batch mode. Xylan removal was measured at 77-94% for flow-through, higher than the 32-68% for batch. The solids pre-treated in flow-through mode achieved a hydrolysis yield of 94% revealing the efficacy of this operation mode. The authors found that flow-through can increase mass transfer coefficients by 90% when compared to batch operation, which contributes to increased lignin and xylan removal. They also verified that combining high velocities (15.8cm/min) with lower temperatures proved to be as effective as combining low velocities with higher temperatures. an important conclusion in terms of energy-related costs. In another paper (Yang and Wyman 2004), 85% delignification was achieved at $\log R_o$=4.8, 50% higher than for batch. Lignin and

xylan removal were observed to increase with increasing flow rate and acid concentration. Similarly, Archambault-Leger et al. (Archambault-Leger, Shao, and Lynd 2012) reported a 30% increase in lignin removal (up to 68% for poplar wood; 78% for corn stover), a 20% increase in glucan conversion and xylan yields higher than 90% for flow-throughhot water (at 220ºC by 12min) comparative to batch. Yang et al. (Yang and Wyman 2008) found similar results.

In this chapter, a flow-through operation mode is applied to autohydrolysis of a model lignocellulosic feedstock (energy crop) – miscanthus, to evaluate the potential of this configuration to increase biomass deconstruction. Results are compared to the ones of batch operation for the same biomass with respect to treated solids composition and enzymatic digestibility and hydrolysates composition, as usual. Flow-through operation was studied as a trade-off between batch and continuous operation, as it enables the study of potential continuous operation traits at lab-scale, for which continuous studies suffer from technical limitations.

It is important to refer that the batch mode experiments presented in this chapter were carried out by another researcher of LNEG (Sampaio et al. 2019). However, to allow the comparison between the two operation modes, it was decided to present these results in this dissertation, while highlighting their authorship.

5.2. Materials and Methods

5.2.1. Feedstock

Mischanthus pellets (8.9 mm dia. x 13 mm length) were kindly provided by CERTH (Greece), they were stored at room temperature and use as is without any milling. Composition of feedstock is presented in Supplementary material A.

5.2.2. Batch operation

Batch autohydrolysis was performed as described elsewhere (Alves-Ferreira et al. 2019). Refer to Table IX for specific operation conditions.

5.2.3. Flow-through operation

Flow-through pretreatment experiments were carried out in a Dionex™ ASE™ 150 Accelerated Solvent Extractor (Figure 35) from ThermoFisher Scientific, USA, with a modified approach, in that the system was used to process the biomass on the structural level, as opposed to the conventional use for extraction of free components. A rigorously weighted amount of air-dried feedstock was mixed with 420 stainless steel spheres for support and loaded into a 100 mL stainless steel cell. A layer of spheres and glass fiber filters (1.2 μm, VWR) was placed at the top and bottom of the cell to prevent clogging of the cell seals. The cell was placed into the oven of the ASE system, previously pre-heated to the pretreatment temperature, in a vertical position. The selected pretreatment solvent was placed in the pressure-resistant glass solvent bottle which was admitted to the extraction cell in a vertical top to bottom flow configuration. The system performed a series of 5 cycles, each consisting of 1) quick filling of the extraction cell by a 70mL/min pump, 2) followed by a static period of several minutes (selected according to the desired pretreatment time) while biomass and solvent were kept at constant pretreatment temperature and around 100 bar, 3) after which the cell was decompressed and the liquid released to the

collection 250 mL glass bottle (previously weighted for determination of liquor recovery). Refer to Table IX for specific operation conditions.

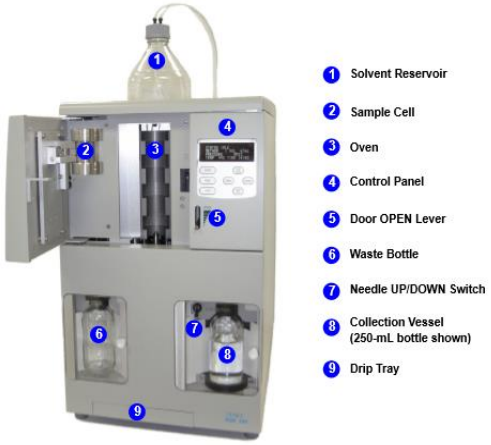

Figure 35. Dionex™ ASE™ 150 system used for flow-through pretreatment of lignocellulosics

After the completion of pretreatment, extraction cell and collection bottle were allowed to naturally cool down to room temperature. The extraction cell content was unloaded and extensively washed with distilled water by vacuum filtration with a rapid filtration quantitative filter paper (20-25 µm. Filter-Lab), previously oven-dried and weighted for solid yield determination. The spheres were separated from the treated solids, which were weighed and analyzed for water content for solid yield determination, and then frozen and stored for posterior analysis of structural composition and enzymatic digestibility. The recovered liquor was centrifuged at 9600 rpm for 30 minutes to recover the precipitated solid lignin. The liquid supernatant was stored at 4ºC for posterior HPLC and UV spectrophotometry analysis. The solid lignin was washed four times with 40 mL deionized water and, then, centrifuged and dried in an oven at 45ºC overnight. Finally, the dried lignin was weighed to determine its mass recovery.

Table IX. Operation conditions during miscanthus autohydrolysis for batch and flow-through modes

	Batch	Flow-through
Temperature/ºC	190	150-190
Pressure/bar	up to 11	80-120
LSR	6	6-7

5.3. Results and discussion

5.3.1. Effect of autohydrolysis operation mode on the biomass composition

At lower severities, the batch reactor performed similarly to the flow-through operation (Figure 36), presenting limited solubilization of the main macromolecular components. However, as severity increased, flow-through operation induced a higher glucan content as a result of increased xylan and lignin solubilization. In fact, lignin solubilization was only achieved in flow-through configuration, as in batch reactor all lignin remained in the solid fraction, putatively, partially re-condensed as pseudo-lignin.

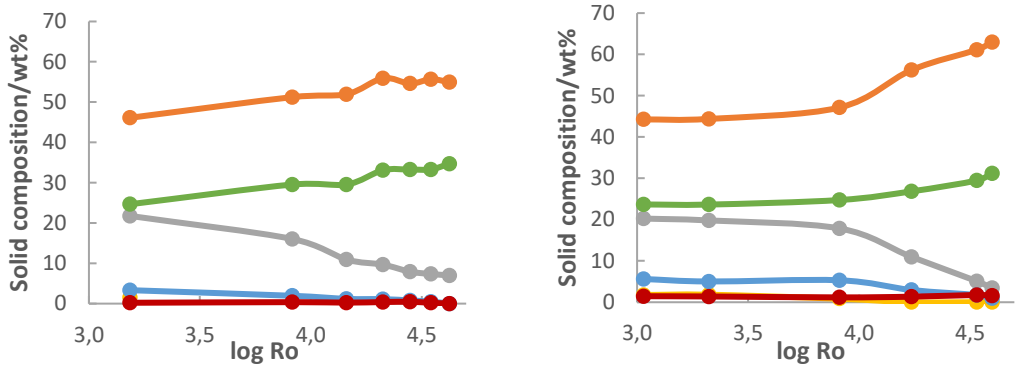

Figure 36. Composition of miscanthus (dry weight basis) subjected to batch (left) and flow-through (right) autohydrolysis (● glucan, ● xylan, ● arabinan, ● lignin, ● acetyl groups and ● ash)

5.3.2. Effect of autohydrolysis operation mode on the enzymatic digestibility of biomass

As can be seen in Figure 37, digestibility of miscanthus treated in batch operation was higher than the one of flow-through configuration for lower severities, in agreement with the superior glucan content of the former and more extensive xylan solubilization. Glucan digestibility of flow-through configuration became superior for higher severity values, as a consequence of higher lignin and xylan solubilization. The digestibility of batch-treated miscanthus presented a maximum value, decreasing for harsher severity factors, presumably as a result of the formation of pseudo-lignin which limits the accessibility to cellulose by hydrolytic enzymes. Increasing pretreatment severity might further increase the digestibility of flow-through treated miscanthus, as suggested by the tendency represented in Figure 37 (right).

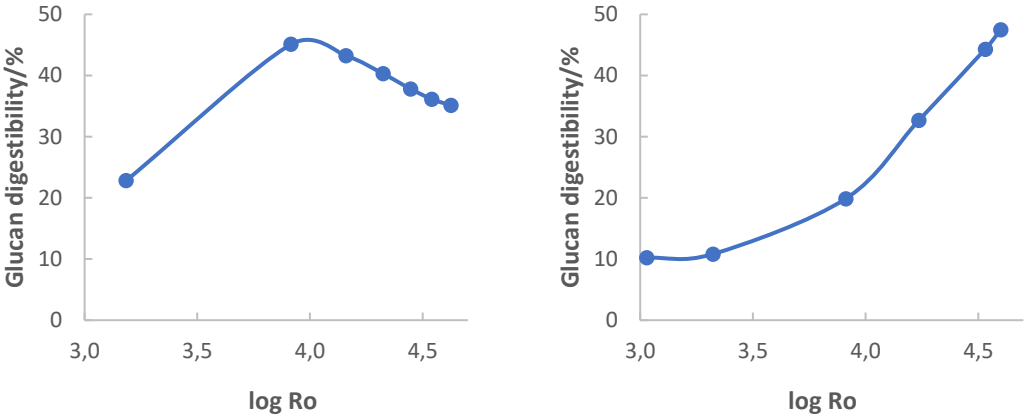

Figure 37. Glucose yield after enzymatic hydrolysis of batch (left) and flow-through (right) autohydrolysis miscanthus

5.3.3. Effect of autohydrolysis operation mode on the hydrolysate composition

Composition of hydrolysates from batch and flow-through operation were compared (Figure 38). For lower severities, batch configuration yielded more XOS and free sugars, while for higher severities flow-through configuration had a superior performance. Increasing treatment time in batch operation strongly reduced the amount of XOS and free xylose in hydrolysate, as opposed to what happens in flow-through operation for which XOS hydrolysis and sugar degradation are prevented. The amount of sugar degradation products is higher in batch configuration, as the continuous removal of solvent from

the reactor in the flow-through system reduced the amount of time that the solubilized sugars were subjected to high temperatures, even though the liquid/solid ratio is similar. The flow-through system presents the advantage of easy separation of solubilized lignin, which precipitated as the hydrolysate was being removed (and cooled) from the reactor. This phenomenon is in line with similar observations found in literature, namely Kilpeläinen et al. observed the formation of a precipitate in the cooled hydrolysate resultant from flow-through autohydrolysis of birch sawdust. Characterization of the same precipitate by pirolysis-GC revealed a predominant composition of lignin-derivate compounds, with a minor fraction of sugars of up to 12% (Kilpeläinen et al. 2012). For batch system, no free lignin was recovered, as expected, due to re-condensation into the pretreated solids.

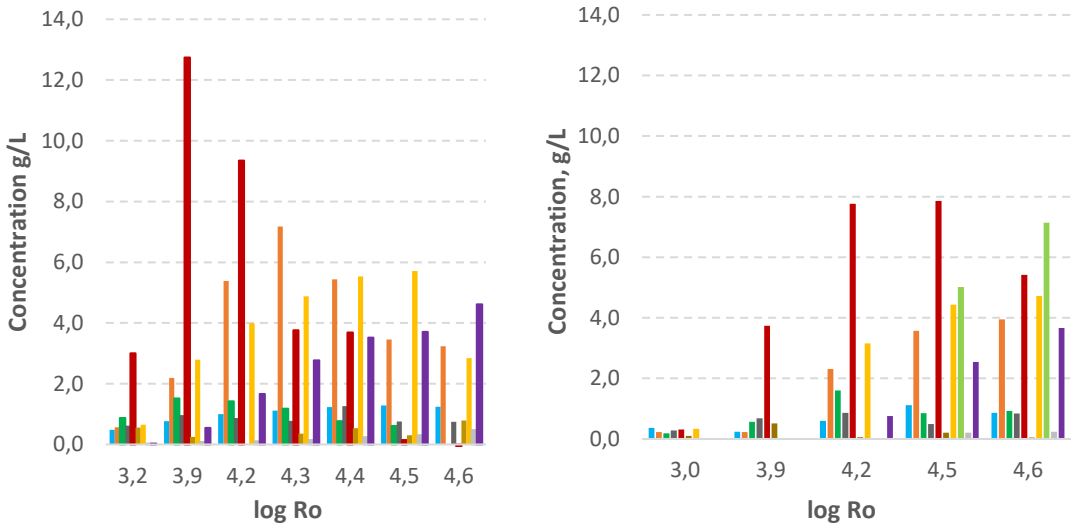

Figure 38. Composition of hydrolysates resulting from batch (left) and flow-through (right) autohydrolysis of miscanthus (■ glucose, ■ xylose, ■ arabinose, ■ GOS, ■ XOS, ■ AOS, ■ acetic acid, ■ HMF, ■ furfural, ■ precipitated lignin)

5.4. Conclusions

Operation regime was demonstrated to strongly impact the product distribution resulting from biomass autohydrolysis, as batch and flow-through operation modes have very distinct effects upon biomass components. When compared to batch operation, flow-through autohydrolysis reduces the hydrolysis of XOS and also the degradation of sugars due to the its continuous removal from the reaction media. Moreover, it enables the direct lignin recovery from the solid biomass, without the need of organic solvents, with a simplified downstream processing, as it precipitates, spontaneously, from the hydrolysate, upon cooling. Batch operation favors selectivity, but flow through operation enables faster processes and the recovery of potential added-value compounds from lignin.

6. PG-based organosolv under flow-through operation mode

6.1. Introduction

The benefits of continuous operation highlighted for liquid hot water and dilute acid pretreatments of biomass can potentially be extended to the organosolv process, increasing even more the solubilization of hemicelluloses and lignin, which are usually already superior for the latter process. Besides the enhanced delignification rate and selectivity, flow-through organosolv should allow the recovery of more unmodified lignin due to reduced secondary/degradation reactions (Pan et al. 1992).

Despite the above, however, few research works have been conducted on this topic yet. Nonetheless, the available results suggest flow-through fractionation of lignocellulosic biomass as a promising route for biorefinery processing.

Park et al., for example, reported the treatment of corn stover with ethanol organosolv in a flow-through column reactor with ethanol concentrations of 20-100% (w/w) at temperature between 150 and 190ºC, with a total liquid throughput of 80, 200 and 320 mL to 20 g of biomass (LSR of 4, 10 and 16), for 40 minutes. Flowrate was kept constant at 2-8 mL and pressure at 23 bar. Optimum results were obtained at 190ºC, with 60% ethanol in total throughput of 320 mL, for which 84.9% delignification and 58.9% xylan solubilization. Cellulose yield was around 90% with a digestibility of 81%. Interestingly, the authors also verified a good capacity of the solvent to be reused, with minor reduction of product yields, a relevant aspect to compensate the higher LSR of flow-through operation when compared to batch (Park, Kim, and Kim 2018).

In another report (Lohre, Kleinert, and Barth 2017), using a similar flow-through apparatus, Norway spruce (softwood) was delignified to 83% extent giving a solid yield of about 25%. In this case, acid-catalyzed ethanol-organosolv with 0.06% sulfuric acid was employed at 175ºC for 10 hours, with a flowrate of 1.5 mL/min and a pressure of 20 bar. It is also suggested the use of multiple parallel flow-through reactors, which would operate alternatively to maintain a continuous operation, for future scale-up of the process.

Pan et al. demonstrated a flow-through ethanol organosolv with 0.1M acetic acid to achieve 68% delignification from spruce wood, at 175ºC for 5 hours. A flowrate of 24mL/min was applied, with a mean residence time in the tubular reactor of 4 minutes. From the solubilized lignin, 74% was recovered as solid precipitate, after ethanol evaporation, with very high purity and with a low-level of modification, with very high preservation of β-aryl bonds (Pan et al. 1992).

Finally, Tirtowidjojo et al. studied the delignification of cottonwood in both batch and flow-trough acidified methanol organosolv and concluded that the delignification rate is superior for the flow-through configuration, while also leading to less degradation of cellulose and to a more intact lignin structure with superior valorization potential (Tirtowidjojo et al. 1988).

Based upon the positive effects of transition to flow-through configuration of autohydrolysis pretreatment discussed in the previous chapter, particularly for reduced sugar degradation and enhanced delignification coupled with easier lignin recovery from hydrolysate liquids, and considering the success-

ful application to other organosolv processes, flow-through operation can potentially benefit the propylene glycol organosolv pretreatment studied in chapter three, alternative or complementary to the introduction of an acid catalyst, as discussed in chapter four. Therefore, in this chapter, flow-through PG organosolv pretreatment of lignocellulose was tested. Comparison with results obtained in batch configuration is highlighted, focusing on the pros and cons of both operation modes.

6.2. Materials and Methods

6.2.1. Flow-through operation

Flow-through experiments were performed as described in the previous chapter, and again as a trade-off between batch and continuous operation. Experiments were conducted between 140 and 190ºC for 1 to 3 hours, for WS and ER with 50%PG aqueous solutions. For comparison, ER was also submitted to flow-through autohydrolysis in the same conditions. Temperature and time conditions were combined in the severity factor as presented in Table X for easier systematization of results.

Table X. Severity factor, temperature and time conditions applied to flow-through pretreatment experiments of ER and WS

Log Ro	T/ºC	time/h
3.0	140	1
3.6	160	1
4.1	160	3
4.2	180	1
4.5	190	1

6.3. Results and discussion

As illustrated in Figure 39, PG-based organosolv flow-through pretreatment resulted in more extensive solubilization of xylan, lignin and acetyl groups, percentagewise, for ER than for WS (10-20% more xylan/lignin removal and 20-30% more acetyl removal), similarly to the batch pretreatment for which ER was also more readily deconstructed. The solid yields obtained for ER and WS follow this same trend. Glucan recoveries were similar for both feedstocks, as well as for flow-through autohydrolysis of ER, all in the range of 85-90%, an interesting value for post enzymatic hydrolysis. Autohydrolysis of ER in ASE system for 160ºC 1 hour resulted in superior delignification and xylan removal (20 and 64.2%) than results reported in literature (12 and 32%, respectively) for the same severity factor (Tunc and Heiningen 2008). This is probably due to the simplistic character of the severity factor, which does not distinguish between high temperatures at short times and low temperatures and long times, even though both conditions are probably different. In this case, the temperature of the present work (160ºC) is higher than the one of the reported work (150ºC), which might explain the higher values obtained in the first situation. For eucalyptus residues, flow-through autohydrolysis was able to remove more xylan and acetyl than the organosolv pretreatment due to increased acid potential of the solvent. The opposite was observed for lignin solubilization, given the higher solubility of lignin in a solution of propylene glycol when compared to water for itself. However, while xylan solubilization became closer to similar values

for both pretreatments with increased temperatures, particularly above 180ºC for which the difference is smaller than 10% (85 vs 76% xylan removal), the same was not verified for lignin as autohydrolysis yielded a maximum delignification plateau of 20% independently of the temperature applied. For flow-through organosolv though delignification increased with increasing temperature, reaching a very significant maximum of 85% for 190ºC 1h operation. The solid yield obtained for autohydrolysis and organosolv plotted against the severity factor results from these observations, i.e. for lower severities, solid yield is inferior for autohydrolysis due to much more extensive xylan solubilization in that case, while for higher severities, solid yield of PG-organosolv became inferior since delignification became much more extensive than for autohydrolysis while xylan removal became similar for both pretreatments. Therefore, PG-organosolv has the potential to benefit from the combined effects of extensive xylan solubilization of autohydrolysis and extensive delignification associated with the presence of propylene glycol.

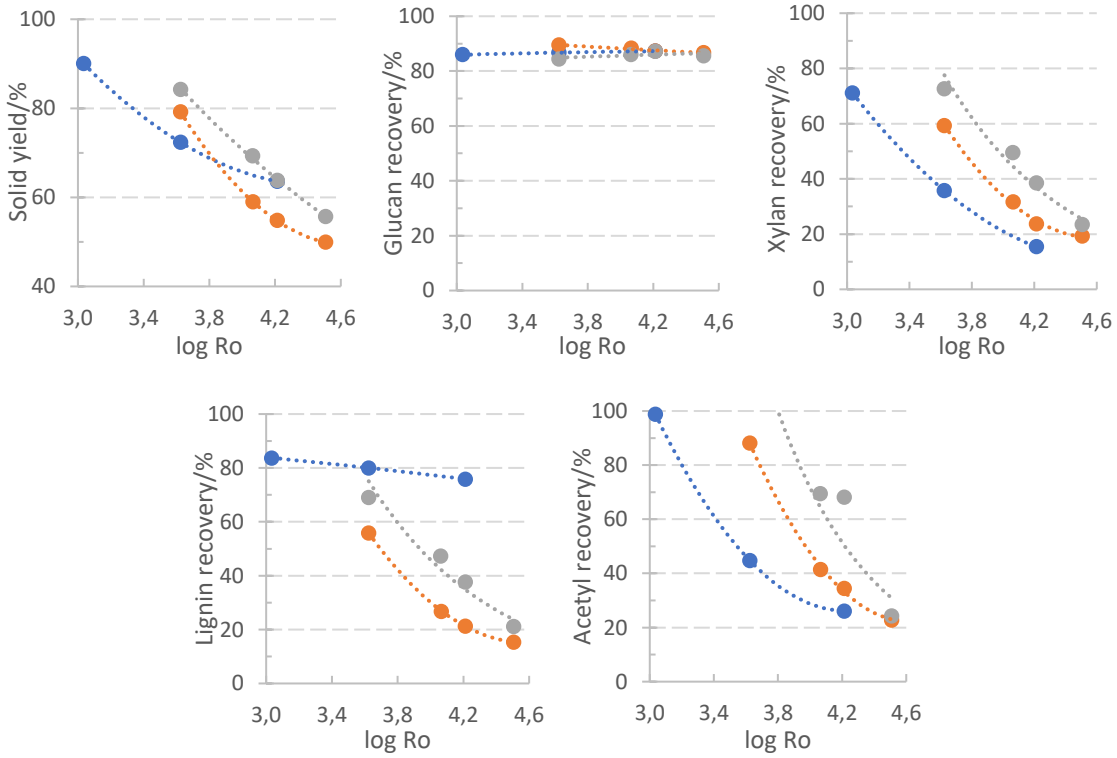

Figure 39. Solid yield and recovery percentages of total components of feedstock obtained in the solid fraction remaining after flow-through autohydrolysis of ER (●) and flow-through 50%PG organosolv of ER (●) and WS (●)

In one work, flow-through pretreatment of corn stover at 190ºC with 60:40 ethanol/water ratio originated a xylan and lignin solubilization of 47.1 and 76.8%, respectively, while preserving 90% of cellulose in the pretreated solids (Park, Kim, and Kim 2018). These results were similar to the ones of this work for wheat straw flow-through PG-organosolv (78.8% delignification and 85.6% cellulose recovery), except for xylan solubilization for which PG pretreatment considerably performed better (76.5% solubilization). In another report, Norway spruce (softwood) was delignified to 83% extent giving a solid yield of about 25% in a flow-through reactor with 63% ethanol-organosolv including 0.06% sulfuric acid as catalyst at 175ºC for 10 hours (Lohre, Kleinert, and Barth 2017). In this work, a delignification of 73.2%, not so far from the value reported for Norway spruce, was obtained for ER at lower temperature

(160ºC), shorter time (3 hours) and without the addition of catalyst. Furthermore, solid yield was of 59% instead of 25%, representing a preservation of cellulose of 88.3% against the reported value of only 67%. This comparison suggests the potential of the flow-through PG organosolv process for biomass deconstruction.

Figure 40 shows the solid lignin recoveries (as weight percentage of total initial lignin) obtained from the centrifugation of hydrolysates of the flow-through pretreatments. The recovered solid lignin was found to increase with pretreatment severity, particularly above 160ºC. It was also considerably higher for organosolv than for autohydrolysis pretreatment, in line with the solid recoveries reported above. About 60% lignin was recovered as spontaneous precipitate in organosolv pretreatments at 180ºC, and for WS this reached 80% at 190ºC. Such are very interesting values with potential to make costly and intricate post-treatment separation steps obsolete, reducing CAPEX and OPEX costs, and mitigating another major disadvantage of conventional organosolv processes, the downstream processing (see item 1.3.3). However, before that, the composition of the precipitated lignin must be evaluated to assess if its purity is high enough for commercialization. Particularly, the precipitated solid might be more of a so-called pseudo-lignin, a result of condensation reaction between lignin and sugar degradation products that form a lignin-like precipitate during pretreatment (Sannigrahi et al. 2011). The formation of pseudo-lignins is therefore favored at higher temperatures, for which sugar degradation reactions occur. This fact might explain the high lignin yields obtained in this work for the more extreme temperatures. Particularly, total mass balance of lignin for the organosolv pretreatment of WS at 190ºC closed at more than 100%, suggesting exactly the possible incorporation of other products into the precipitated lignin. Flow-through operation is suggested however to be able to reduce degradation reactions (of sugars and lignin) by constantly removing sugars and lignin from the reaction medium before they can undergo further reactions, which can have a positive impact in preventing the formation of pseudo-lignins (Schutyser et al. 2018). This idea is supported by the observation of low concentrations of degradation products in this work, i.e. furfural concentration was always below 1 g/L and HMF was not even detected. Additionally, propylene glycol may also be responsible for increasing lignin yields, due to PG reaction with hydroxyl groups from lignin structure, as incorporation of other alcohols into lignin structure during organosolv pretreatment has been reported, i.e. for ethanol (Wildschut et al. 2013), ethylene glycol (Kubo et al. 2007) and 1,4-butanediol (Kishimoto and Sano 2002). The introduction of alcohols into the phenolic structure alters lignin solubility and reactivity, allegedly making it interesting for production of polymers ang gels (Kubo et al. 2007), with a positive impact on its valorization potential.

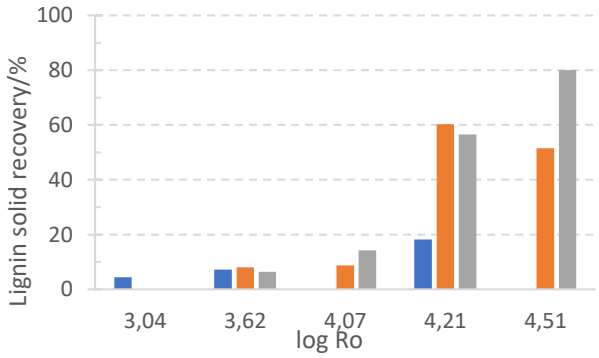

Figure 40. Recovered solid lignin (%w/w of lignin content in feedstock) as a function of pretreatment severity factor for flow-through autohydrolysis of ER (■) and flow-through 50%PG organosolv pretreatment of ER (■) and WS (■)

Table XI evidences the comparison of flow-through results (autohydrolysis and PG organosolv of ER and WS) with the ones of batch operation presented in chapter three. Whereas for all batch experiments, the cellulose was preserved completely (more than 96%), for the flow-through experiments, cellulose recovery was always between 84 and 90%, which in spite of still being an interesting range, suggests that flow-through mode already induces some undesirable glucan removal. Advantageously, this effect appeared to be independent of pretreatment severity (as well as solvent), which allows the increase of selectivity for hemicellulose and lignin solubilization by increasing the former, as pretreatment severity still influenced these variables.

Table XI. Comparison between batch and flow-through operation (FT) with respect to solid yield and recovery of lignocellulosic components in autohydrolysis and PG-based organosolv of ER and WS

Pretreatment				Solid Yield (%)		Recovery in pretreated solids					
						Glucan (%)		Xylan (%)		Lignin (%)	
Biomass	Solvent	T (ºC)	Time (h)	Batch	FT	Batch	FT	Batch	FT	Batch	FT
ER	H_2O	140	1	92.7	90.0	100.0	86.0	93.1	71.1	83.8	83.7
ER	PG 50%	160	1	79.0	79.2	97.7	89.5	65.6	59.3	53.9	55.8
ER	PG 50%	160	3	65.9	59.0	96.5	88.3	43.0	31.6	42.3	26.8
WS	PG 50%	160	1	86.7	84.2	99.4	84.4	95.3	72.6	72.4	69.1
WS	PG 50%	160	3	76.8	69.3	96.5	86.0	79.4	49.4	63.1	47.3

For xylan and lignin, flow-through operation was found to enhance the solubilization of both components in comparison to batch operation for the same conditions. Flow-through autohydrolysis removed more 22% of total initial xylan in ER though not affecting its delignification. Flow-through PG-based organosolv of ER was more modest in increasing xylan solubilization (only 6 and 11% additional xylan solubilization for 160ºC 1 and 3 hours, respectively), but while enhancing delignification in 16% for 160ºC 3h. For WS, flow-through PG-based organosolv resulted in a similar enhancement of delignification, nevertheless having a stronger impact on xylan solubilization, with an additional 23 and 30% removal of total xylan of feedstock for 160ºC 1 and 3 hours, respectively.

The apparent superior improvement of the 3-hour experiments is obviously related to the higher liquid-to-solid ratio applied (in the range of 15-16), which results from running a continuous operation for longer times (if the liquid flow is to be kept), and not necessarily from the specific dynamics of flow-through operation. However, for the 1-hour experiments the LSR was also smaller than the one applied in chapter three (6-7 vs. 10), which should still indicate some potential for this configuration. Moreover, the flow-through system applied is not a real flow-through system, but more of a series of batch cycles with intermediate fast discharge pulses, which does not benefit from the full advantages of real flow-through operation. Furthermore, part of the solubilized lignin could be directly recovered from pretreatment liquor by spontaneous precipitation upon cooling, with the cost advantages already mentioned in the previous chapter.

Results from enzymatic hydrolysis of flow-through -pretreated solids (Figure 41) revealed superior digestibility of ER when compared to WS, for the same conditions during PG-based organosolv pretreatment, as already observed for batch experiments in chapter three. This result is in agreement

with the more extensive xylan and lignin solubilization during ER pretreatment (see above), as determined by quantitative acid hydrolysis. The inversion of this tendency at the highest tested severity condition is justified by the fact xylan and lignin solubilization became similar for both feedstocks at these conditions (Figure 39), together with the less recalcitrant character of WS, as demonstrated in chapter three.

Interestingly, digestibility of ER submitted to flow-through autohydrolysis were always inferior (15 to 20% less glucose yield) than the ones of flow-through PG organosolv (for the same severity conditions), in spite of superior xylan removal and even for the conditions (log Ro below 3.8) in which autohydrolysis resulted in lower solid yields. This highlights the relevance of delignification impact on enzymatic digestibility, which was very limited for autohydrolysis when compared to the organosolv process.

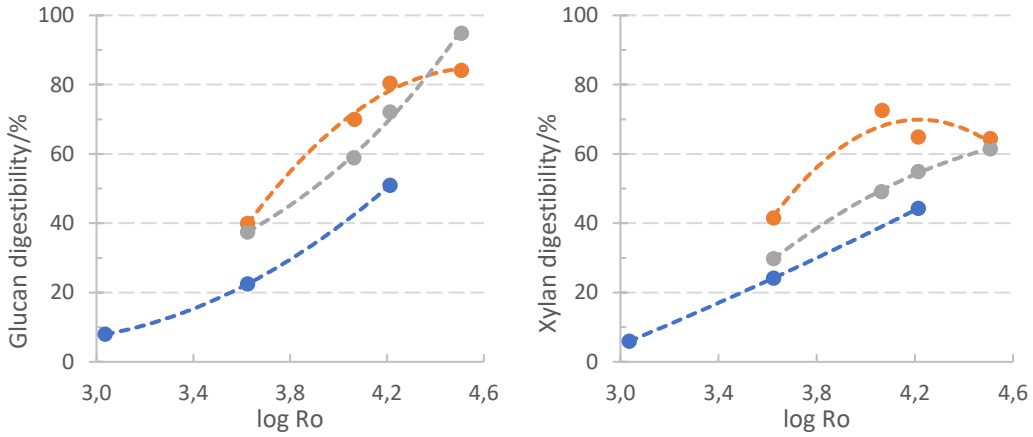

Figure 41. Glucan (left) and xylan (right) digestibility (72h) of solids remaining after flow-through autohydrolysis of ER (●) and flow-through PG-based organosolv with 50%PG of ER (●) and of WS (●)

Cellulose digestibility fell always below 51% (corresponding to 180ºC, 1h) for autohydrolysis of ER at all the tested conditions. Autohydrolysis at 140ºC had practically no impact in ER digestibility. For flow-through organosolv, however, interesting values were obtained, particularly for the harsher severity conditions, i.e. 1 hour at 190ºC, for which glucose yields reached 84.1% for ER and 94.8% for WS, representing very promising values. The obtained digestibility for wheat straw was even much higher than the 78.8% reported for flow-through ethanol organosolv pretreatment of corn stover, at similar conditions (Park, Kim, and Kim 2018).

As for the batch experiments, quadratic correlations were obtained between cellulose digestibility and pretreatment solid yield for both feedstocks (Figure 42). Such dependence implies that a certain level of biomass solubilization must take place in order to obtain significant levels of digestibility, i.e. solid yields need to be below 60% (for ER) and 65% (for WS) to reach digestibility values above 70%, which are still considered low for commercial application. Again, the more recalcitrant nature of ER is highlighted, as for the same level of biomass deconstruction, WS presented higher digestibility values.

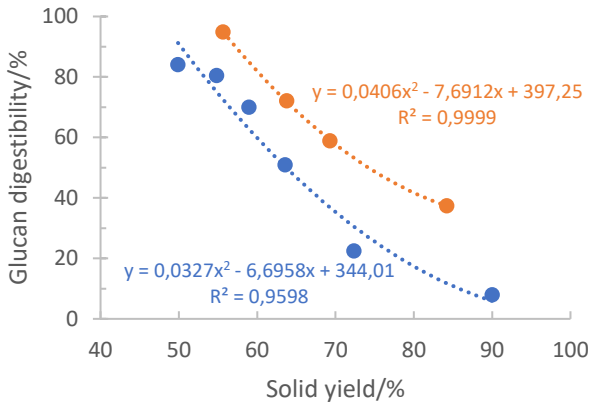

Figure 42. Cellulose digestibility of ER (●) and WS (●) *versus* solid yield resultant after flow-through pretreatment

Comparison of digestibility yields of flow-through operation with the ones presented in chapter three for batch pretreatment is presented in Table XII. As can be observed, flow-through operation had a minimal increasing impact on ER digestibility, while relatively enhancing WS susceptibility to enzymatic hydrolysis. The superior impact on WS might be associated with the greater improvement of xylan solubilization induced by flow-through pretreatment for this feedstock (as presented above). As of right now, increases of solid digestibility due to continuous operation are not that significant, but can potential benefit, in the future, from the application of proper flow-through reactors, instead of the simplistic system applied in this work.

Table XII. Comparison of cellulose digestibility of ER and WS pretreated by autohydrolysis and PG50% organosolv in batch and flow-through configuration

Pretreatment				Glucan digestibility (%)		Difference (%)
Biomass	Solvent	T (ºC)	Time (h)	Batch	FT	
ER	H_2O	140	1	4.3	7.9	3.6
ER	PG 50%	160	1	38.5	40.0	1.5
ER	PG 50%	160	3	61.1	70.0	8.9
WS	PG 50%	160	1	23.9	37.4	13.5
WS	PG 50%	160	3	42.4	58.9	16.5

6.4. Conclusions

Flow-through PG organosolv pretreatment at 190ºC for 1 hour was able to solubilize about 80% of both hemicelluloses and lignin from ER and WS, while preserving cellulose (~85%). Very promising cellulose digestibilities were obtained of 84.1 and 94.8% for ER and WS, respectively. Flow-through operation enabled easy spontaneous recovery of 60-80% of total lignin in feedstocks, simplifying process design with potential gains in CAPEX and OPEX costs. Furthermore, flow-through regime allowed the removal of additional 16% of total lignin from WS and ER and of more 11% and 30% of total xylan from ER and WS, respectively, when compared to batch operation, at similar conditions. This was accompanied by a respective increase in cellulose digestibility of 8.9 and 16.5 percentage points.

7. General conclusions and perspectives

In this work, the development of a selective fractionation pretreatment for the separation of polymeric components from lignocellulosic biomass feedstocks was pursued. Focus was given to the organosolv process as it presents the advantage of the simultaneous solubilization of hemicelluloses and lignin form LM, while yielding a cellulose enriched solid that can undergo enzymatic saccharification to release glucose in concentrations that can then be converted to several products including bioethanol, typically, via fermentation. Propylene glycol was proposed as a suitable solvent for organosolv pretreatment, which revealed similar or superior performance than more conventional solvents, such as methanol, ethanol, or acetone. Additionally, the proposed process solves one of the major challenges of organosolv processes, i.e. the negative environment/safety impact of most organic solvents, by applying a greener solvent, safer for operation, non-flammable, non-toxic, non-volatile, and which can reduce operational costs by allowing operation at near-atmospheric pressure. Moreover, PG benefits include its renewable character as it can be produced by fermentative/chemical pathways from the sugars released during organosolv pretreatment of lignocellulose

The effects of time, temperature and PG content on the PG-based organosolv were studied for two feedstocks – eucalyptus residues, a woody biomass, and wheat straw, an herbaceous biomass. Increasing pretreatment time and temperature were correlated with higher xylan solubilization and delignification, as well as increased enzymatic digestibility of pretreated solids. PG contents of 50% also had a positive impact on those variables because of the synergistic effect of high hydrolysis potential of water fraction together with the superior lignin solubility in organic solvent.

The introduction of a catalyst as a way to enhance the kinetics of deconstruction reactions was also evaluated. Alkali catalyst (sodium hydroxide) did not improve solid yield values nor digestibility level of pretreated solids, but merely resulted in a slight increase of selectivity towards delignification (as opposed to xylan solubilization). Acid catalysis of PG organosolv at 160ºC with 75%PG and 0.5% of sulfuric acid for only 15 minutes allowed for xylan and lignin solubilization values above 80%, versus 58% in the non-catalyzed process. Cellulose recovery of 90% was also obtained leading to a digestibility of 89%, as predicted by the model developed in this work. These values suggest the ability of the proposed process to compete with other organosolv methods, as it originates similar yields using much milder operation conditions, with the corresponding gains in terms of the capital and operational costs.

Flow-through application of the PG-based pretreatment was also developed as an intensification strategy. This process enabled easy spontaneous recovery of 60-80% of total lignin in feedstocks. Therefore, downstream process design for the recovery of the valuable products and recycling of the solvent might be simplified, reducing the operational costs. Furthermore, when compared to batch operation and using similar conditions, flow-through regime allowed the removal of additional 16% of total lignin from WS and ER, as well as more 11% and 30% of total xylan from ER and WS, respectively. Cellulose digestibility also augmented significantly for ER and specially for WS. Hence, these results suggest a higher potential for PG-organosolv when continuous operation is pursued, as it will lead to process intensification, with increased product yields, lower amounts of produced degradation products and simplified lignin recovery steps, with overall potentially reduced CAPEX and OPEX costs.

Based upon the results of the research conducted in this dissertation, the following lines of work are suggested for future research, aiming to further improve the performance of the developed process, as well as to potentiate its future industrialization:

- To test the flexibility of the organosolv process, the evaluation of PG-organosolv process for pretreatment of other biomass types, particularly softwoods and energy crops, along with mixtures of several lignocellulosic materials should be carried out;
- Assessment of other acid catalysts, most likely organic and Lewis acids (e.g. $AlCl_3$), to reproduce the effect of sulfuric acid in enhancing the effectiveness of PG-based organosolv for biomass deconstruction, without the negative environment, safety and corrosion impacts associated with the former;
- Further study of continuous PG-organosolv operation, with application of a proper flow-through reactor, optimizing the main operation conditions for maximum xylan and lignin solubilization, as well as cellulose recovery in the solid fraction, in terms of pretreatment temperature and residence time;
- Assessment of the benefits of application of acid-catalyzed PG-based organosolv in flow-through configuration, which can potentially achieve complete hydrolysis of both hemicellulose and cellulose without the extensive formation of degradation products obtained in batch operation, due to continuous removal of the oligo- and monosaccharides from the reaction mixture;
- Proposal and experimental validation of a solvent recycle scheme, particularly with focus on the separation and purification steps to enable the recovery of commercially valuable fractions from the pretreatment liquor, i.e. hemicelluloses, lignin fragments, phenolic compounds, and degradation products (aliphatic acids, HMF, furfural);
- Study of the performance of all the obtained product fractions, particularly the solubilized fractions (since the potential of the pretreated solids for enzymatic saccharification was already demonstrated), for commercial valorization by integration in already-proven production processes, as well as high-value products;
- Evaluation of the prospect of process scale-up and application of a systematic economical evaluation of the optimized process, including the recycling scheme, to evaluate the potential for commercial deployment.

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Supplementary material

A. Characterization of initial feedstock

The structural composition (in percentage of dry weight) of feedstocks used in this work was determined by quantitative acid hydrolysis (Figure 43). ER had higher content than WS in glucan, lignin and acetyl groups, while WS was richer in hemicelluloses and ash. Miscanthus presented a composition similar to ER, only with less hemicelluloses and more acetyl groups.

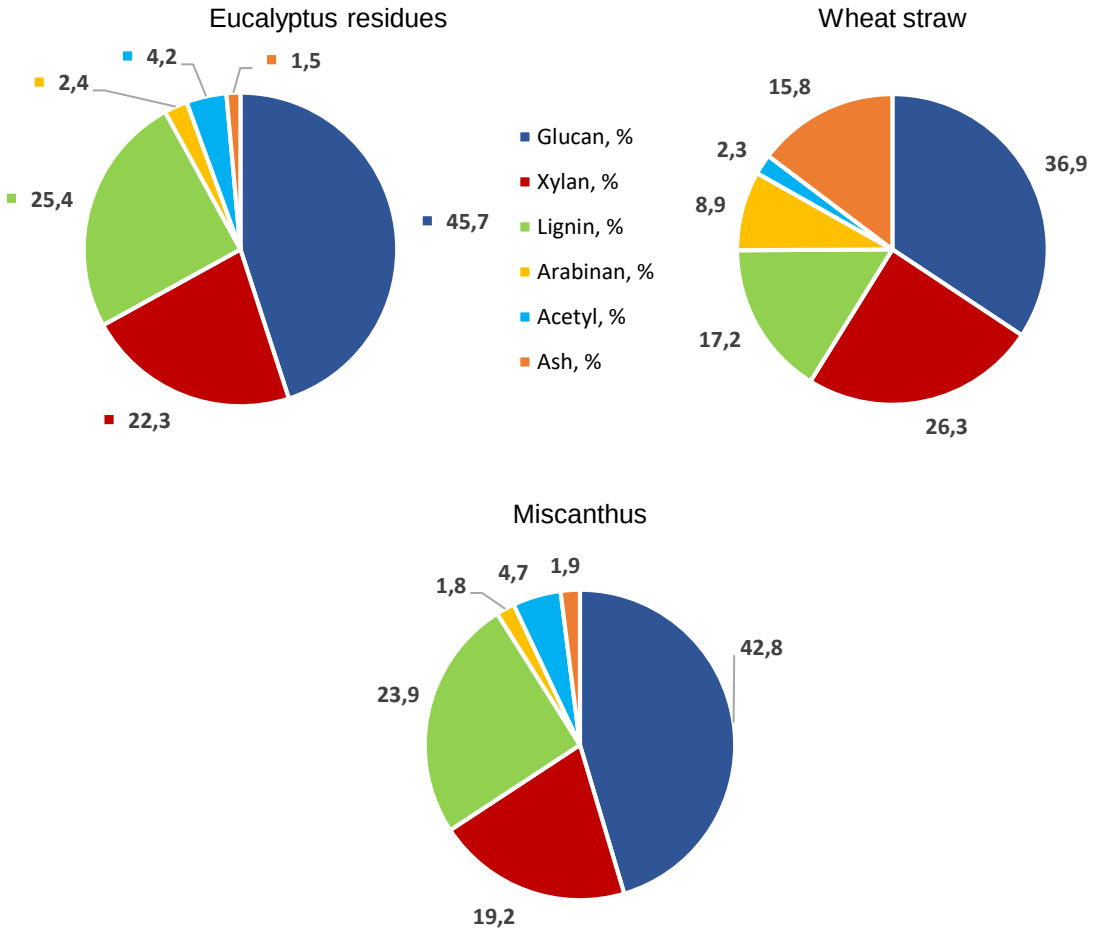

Figure 43. Structural composition of feedstocks (dry weight basis)

B. Mathematical calculations

Analytical methods

The moisture content of the samples was calculated using the following expression (Sluiter et al. 2008):

$$\text{Moisture content (\%)} = \frac{\text{wet sample weight (g)} - \text{oven-dry sample weight (g)}}{\text{wet sample weight (g)}} \times 100$$

The ash content (Ash, %) of the samples was calculated using the following equation (Sluiter et al. 2005):

$$\text{Ash (\%)} = \frac{\text{Ash weight (g)}}{\text{wet sample weight (g)} \times [1 - (\text{Moisture content})/100]} \times 100$$

Concentrations of glucose, xylose, arabinose and acetic acid in the liquors resulting from quantitative acid hydrolysis (Sluiter et al. 2012) of raw materials and pretreated solids were used for the calculation of glucan, xylan, arabinan and acetyl groups content (%), respectively. The acid-insoluble residue, after correction for the ash content, was quantified as Klason lignin. During quantitative acid hydrolysis, a significant percentage of the monosaccharides is degraded, so correction factors (F) are introduced to account for the losses (Browning 1967). The following expressions were used:

$$Gn = F \times \frac{100}{1025} \times \frac{162}{180} \times \frac{Glc \times P_{sol}}{A \times [1 - (\text{Moisture content})/100]}$$

$$Xn = F \times \frac{100}{1025} \times \frac{132}{150} \times \frac{Xyl \times P_{sol}}{A \times [1 - (\text{Moisture content})/100]}$$

$$Arn = F \times \frac{100}{1025} \times \frac{132}{150} \times \frac{Ara \times P_{sol}}{A \times [1 - (\text{Moisture content})/100]}$$

$$Ac = \frac{100}{1025} \times \frac{60}{61} \times \frac{HAc \times P_{sol}}{A \times [1 - (\text{Moisture content})/100]}$$

$$KL = \frac{\text{AIS-Ash}}{A \times [1 - (\text{Moisture content})/100]} \times 100$$

Where,

Gn, Xn, Arn, Ac and KL are the concentrations of glucan, xylan, arabinan, acetyl groups and Klason lignin (g/100 g of dry solid), respectively;

Glc, Xyl, Ara and HAc are the concentrations of glucose, xylose, arabinose, and acetic acid in hydrolysate liquors (g/L), respectively;

The terms $\frac{162}{180}$, $\frac{132}{150}$ are stoichiometric conversion factors of monomers into polysaccharides (loss of a water molecule);

The term $\frac{60}{61}$ is a stoichiometric conversion factors of acetic acid to acetyl groups (loss of a proton);

The term 1025 corresponds to the mass density of the hydrolysate (g/L), a 4% w/w solution of sulfuric acid;

F is the correction factor accounting for sugar degradation (1.04 and 1.09 for hexoses and pentoses, respectively);

W_{sol} and A are the weights of the solution and sample used in the test, respectively (g);

The Moisture content respects to the sample moisture content;

AIS and Ash are the weight of the acid-insoluble residue of the sample and its ash content, respectively (g).

The concentrations of gluco-oligosaccharides (GOS), xylo-oligosaccharides (XOS) and arabino-oligosaccharides (AOS) in organosolv liquors were calculated, after post-hydrolysis (Sluiter et al. 2006), according to the following equations:

$$\text{GOS}=(Glc_{PH} \times \text{F} \times DF - Glc_L) \times \frac{162}{180}$$

$$\text{XOS}=(Xyl_{PH} \times \text{F} \times DF - Xyl_L) \times \frac{132}{150}$$

$$\text{AOS}=(Ara_{PH} \times \text{F} \times DF - Ara_L) \times \frac{132}{150}$$

Where,

Glc_{PH}, Xyl_{PH}, Ara_{PH} are the concentrations of glucose, xylose and arabinose in post-hydrolysis hydrolysates of organosolv liquors, expressed in g/L;

Glc_L, Xyl_L, Ara_L are the percentages of glucose, xylose and arabinose in pretreatment liquors, expressed in g/L;

DF is the dilution factor associated with the addition of sulfuric acid 72% to the organosolv liquor, in mL/mL;

F is the correction factor accounting for sugar degradation (1.04 and 1.09 for hexoses and pentoses, respectively);

The terms $\frac{162}{180}$, $\frac{132}{150}$ are stoichiometric conversion factors of monomers into polysaccharides (loss of a water molecule).

Phenolic content/acid soluble lignin (ASL) of hydrolysates and pretreatment liquors was calculated with the following equation (Sluiter et al. 2006):

$$\text{ASL } (\text{g/L}) = \frac{\text{A}}{\varepsilon \times \text{l}} \times \text{DF}$$

Where,

A is the absorbance of the liquid sample at 320 nm;

l is the length of the measuring cell (1 cm);

ε is the extinction coefficient of the acid soluble lignin, expressed in $Lg^{-1}cm^{-1}$;

DF is the dilution factor to account for the applied liquid sample dilution, in mL/mL.

The glucan (GnDig, %) and xylan (XnDig, %) digestibility of feedstock and pretreated solids after enzymatic hydrolysis (Selig, Weiss, and Ji 2008) was calculated according to the following expressions:

$$\text{GnDig (\%)} = \frac{\text{Glc}}{\text{Glc}_{\max}} \times 100$$

$$\text{XnDig (\%)} = \frac{\text{Xyl}}{\text{Xyl}_{\max}} \times 100$$

Where,

Glc and Xyl are the concentrations of glucose and xylose in the enzymatic hydrolysate corrected with the respective concentrations of glucose and xylose in the sample and enzyme blanks, expressed in g/L;

Glc_{max} and Xyl_{max} are the maximum concentrations possible of glucose and xylose in the enzymatic hydrolysate, assuming total conversion of the polysaccharides initially present in the sample, expressed in g/L, and calculated as follows:

$$Glc_{max} = \frac{S \times [1 - (Moisture\ content)/100] \times Gn \times \frac{162}{180}}{V}$$

$$Xyl_{max} = \frac{S \times [1 - (Moisture\ content)/100] \times Xn \times \frac{162}{180}}{V}$$

Where,

S is the sample mass of the trial experiment;

Moisture content relates to the moisture content of the sample;

Gn and Xn are the glucan and xylan concentrations of the sample;

V is the total volume of the enzymatic hydrolysate;

The terms $\frac{162}{180}$, $\frac{132}{150}$ are stoichiometric conversion factors of monomers into polysaccharides (loss of a water molecule).

Solid yield and solid component recovery in organosolv pretreatment

Pretreatment solid yield (SY, %) was calculated from the following equation:

$$\text{SY (\%)} = \frac{\text{oven-dried mass of pretreated solids}}{\text{mass of initial feedstock} \times [1 - (\text{Moisture content})/100]} \times 100$$

The recovery of glucan, xylan, arabinan, acetyl groups, Klason lignin and ash, expressed as the percentage that remains in the solid residue after organosolv pretreatment, was calculated according to the following equations:

$$\text{Gn}_R = \frac{\text{Gn} \times \text{SY}}{\text{Gn}_F}$$

$$\text{Xn}_R = \frac{\text{Xn} \times \text{SY}}{\text{Xn}_F}$$

$$\text{Arn}_R = \frac{\text{Arn} \times \text{SY}}{\text{Arn}_F}$$

$$Ac_R = \frac{\text{Ac} \times \text{SY}}{\text{Ac}_F}$$

$$\text{KL}_R = \frac{\text{KL} \times \text{SY}}{\text{KL}_F}$$

$$Ash_{\mathrm{R}}=\frac{\mathrm{Ash}\times SY}{\mathrm{Gn_F}}$$

Where,

Gn_R, Xn_R, Arn_R, Ac_R, KL_R and Ash_R are the percentages of glucan, xylan, arabinan, acetyl groups, Klason lignin and ash that remain in the residue after the organosolv pretreatment (g/100g of the respective initial component);

SY is the pretreatment solid yield (g of recovered solid /100 g of raw material).

Gn, Xn, Arn, Ac, KL and Ash are the percentages of glucan, xylan, arabinan, acetyl groups, Klason lignin and ash on the pretreated solids, respectively (g/100 g of raw material), as determined by quantitative acid hydrolysis;

Gn_F, Xn_F, Arn_F, Ac_F, KL_F and Ash_F are the percentages of glucan, xylan, arabinan, acetyl groups, Klason lignin and ash on the feedstock, respectively (g/100 g of raw material).

C. Sugar degradation during biomass pretreatment

The sugars released during acidic pretreatment of lignocellulosic materials, as a result of hydrolysis reactions of cellulose and hemicelluloses, can undergo further reactions with subsequent formation of sugar degradation products (desirable or not). The reactions of formation of these products are slower than the hydrolysis of polysaccharides to monomeric sugars (Dunlop 1948). The rate of those reactions is increased by increasing temperature and/or acid concentration, for which optimization of operation conditions is of extreme importance to tune the selectivity of biomass pretreatment towards a specific product, being oligomeric sugars, monomers, or degradation products (Dunlop 1948).

Glucose (hexose) degrades into HMF during acid pretreatment of LM. With time, HMF further degrades into levulinic and formic acid (1:1), according to Figure 44 (Girisuta, Janssen, and Heeres 2006). HMF can also undergo condensation and polymerization reactions to form humins. Humins are carbonaceous, polymeric by-products formed during acid-catalyzed dehydration of sugars by reactions of HMF, sugars and other reaction intermediates produced during sugar dehydration and subsequent rehydration of HMF with water (Cheng et al. 2018). Figure 45 shows the proposed general structure of a humin fragment by van Zandvoort et al (van Zandvoort et al. 2013). For more discussion of humins formation and characterization see (van Zandvoort et al. 2013; Cheng et al. 2018; van Zandvoort et al. 2015)

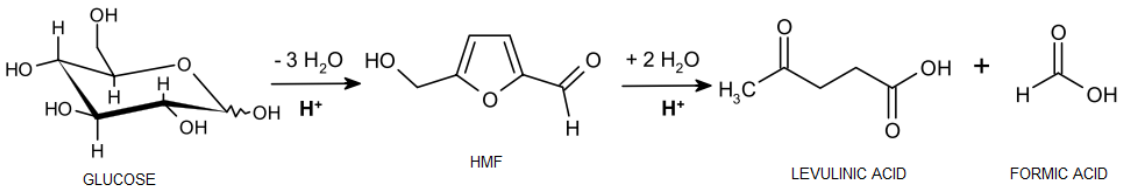

Figure 44. Glucose degradation to HMF and the latter to levulinic and formic acids (Girisuta, Janssen, and Heeres 2006)

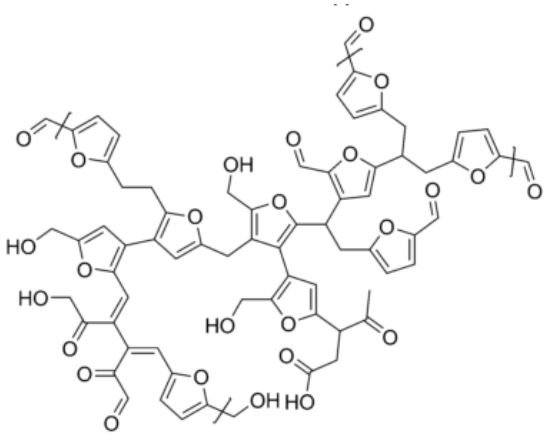

Figure 45. Model structure of a glucose-derived humin fragment as suggested by (van Zandvoort et al. 2013)

Pentoses such as xylose, arabinose and rhamnose are well known to yield furfural (1:1 mol ratio) in acidic media upon heating (Hurd 1932). With extended reaction time in acid environment furfural further degrades into other products following a first order kinetics (Williams and Dunlop 1948). Two

possible pathways are reported for furfural degradation (Figure 46): 1) furfural resignification, in which furfural reacts with itself and 2) furfural condensation, in which furfural reacts with pentose-to-furfural intermediates, typically small aldehydes (Zeitsch 2000; Dunlop 1948; Danon, Marcotullio, and de Jong 2014). The products resulting from these reactions are not completely identified given their complexity. However, formic acid and succindialdehyde are suggested to be formed in the presence of water by hydrolytic fission of the aldehyde group of furfural, with one mol of furfural yielding one mol of formic acid and one mol of succindialdehyde (Dunlop 1948). Succindialdehyde is also proposed to form humins through condensation reactions (Hurd 1932; Dunlop 1948). Condensation and resignification reactions of furfural originate soluble and insoluble polymeric resins and humins, during acid pretreatment. Those insoluble polymers can negatively precipitate into pretreated solids during pretreatment or into precipitated lignin, reducing product purity and the enzymatic digestibility of the former. While increased temperature (as well as time and acid concentration) increases the rate of furfural degradation, very high temperatures will inhibit the formation of insoluble polymeric products (resins) due to entropy effect (Zeitsch 2000).

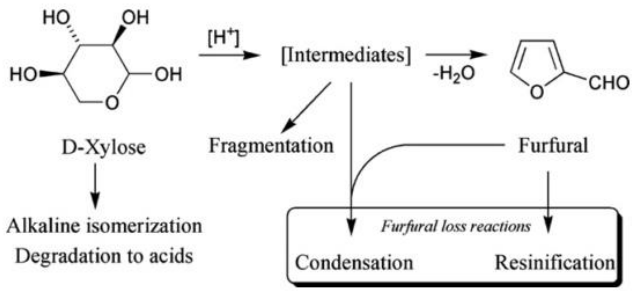

Figure 46. Xylose and furfural degradation pathways (Lima, Pillinger, and Valente 2008)

Additionally, both pentoses and hexoses can directly degrade into various compounds, particularly aldehydes (formaldehyde, pyruvaldehyde, glyceraldehyde) and aliphatic acids (acetic, formic, levulinic) (Lima, Pillinger, and Valente 2008).

The complexity and multiple reaction paths during sugar degradation that can originate formic acid makes its attribution to a specific sugar, in pretreatment mass balances, somewhat dubious. As a simplistic method followed in this work, the quantification of levulinic acid can be used to predict the amount of formic acid prevenient from glucose degradation, as hexose degradation yields HMF which is then degraded into formic and levulinic acid in equimolar proportions. The remaining formic acid should be attributed to degradation reactions of pentoses (xylose, arabinose) by exclusion, even though glucose can also be degraded into formic acid without ever forming HMF. However, as glucose is mostly preserved in the solid fraction during organosolv pretreatment at mild conditions, that approximation should not be too rough of an approach. All conversions of degradation products to sugar monomers should follow a 1:1 molar ratio, as all degradation reactions (except the ones that result in polymers formation, only relevant for harsher severities than the ones of this work), reportedly follow that stoichiometry.

D. Solvent selection

UV-vis spectra were obtained for batch pre-treatment of wheat straw and eucalyptus residues at 140ºC with different solvents (olive oil, frying oil, Baysilone silicone oil, PEG 2000, glycerol and propylene glycol). Additionally, spectra of the solvents treated at the same temperature and time with no biomass were also collected to evaluate the impact on the measured spectra of alterations of the solvents with temperature which could compromise the validity of the analysis if not taken into consideration. Table XIII qualitatively summarizes the results in terms of solvent degradability and effectiveness for delignification of both feedstocks. With respect to thermal stability, Baysilone oil and propylene glycol proved very resistant to thermal treatment, with unchanged UV spectra. All the remaining examined solvents suffered some sort of alteration/degradation with temperature, as implicated by the appearance of new peaks and increase in intensity of already existing ones in their UV spectra after heating. Solvent degradation was particularly evident for olive oil and glycerol.

Delignification effectiveness of tested solvents was assessed by identifying the spectra of treatment liquors of ER and/or WS which suffered a significant increase (in comparison to the pure solvents spectra. considering the degradation effect) of absorption below 400nm, i.e. in the region of lignin absorption (200-240 nm, 280 and 320 nm) (Hyman et al. 2007; Beisl et al. 2018). As such, glycerol and propylene glycol proved to have the most potential for lignin solubilization from lignocellulosic biomass, of all the tested solvent. Baysilone oil also revealed some delignification potential, but was not further considered due to its high cost. PEG 2000 and olive oil had a very limited effect, while frying oil was demonstrated to have no effect in delignification, as its spectrum revealed no apparent alteration after pre-treatment. All these were therefore, not further considered in this work.

Table XIII. Comparison of the delignification performance of selected solvents and their stability under the tested operational conditions

Solvent	ER delignification	WS delignification	Solvent stability
Glycerol	+++	+++	-
PEG 2000	N/A	+	++
Propylene Glycol	+++	++	+++
Baysilone	++	N/A	+++
Olive oil	+	+	-
Frying oil	-	N/A	++

N/A: not tested.

Additionally, it can be added that only for glycerol and propylene glycol treatments were black-liquors obtained (dark brown liquors), a characteristic aspect of lignin solutions. For the remaining treatments, only a slight yellow turbidity was observable. Based on all the above observations, glycerol and propylene glycol were further explored as organosolv solvents in this work.

E. Glycerol Pretreatment

A significant increase in UV-vis absorption in the range of 190-400nm was observed for glycerol pre-treatment of wheat straw at 140ºC after 1, 3, 4, 5 and 6h in comparison to the pure solvent (Figure 47).

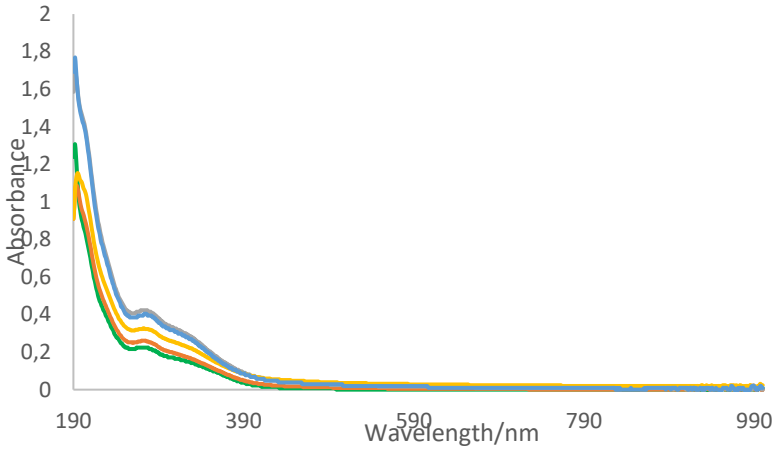

Figure 47. UV-vis spectra of glycerol after batch pre-treatment of wheat straw at 140ºC for 1 (—), 3 (—), 4 (—), 5 (—) and 6 (—) hours.

For all the tested times, two peaks were detected in the UV region: a smaller peak around 280 nm and a more intense peak around 200 nm. These peaks coincide with the characteristic wavelengths of lignin absorption (205, 240, 280 and 320nm) (Hundt et al. 2016; Wang, Xu, and Sun 2010), suggesting that the pre-treatment was effective in delignification of wheat straw. The profile obtained was very identical for all the pre-treatment times varying only the intensity of the absorption, i.e. absorbance increased with pre-treatment time. Therefore, delignification increases with pre-treatment time also, as represented in Figure 48. A linear dependence was obtained until six hours. Pretreatment for 24h yielded a much lower delignification (47.4%) than expected for a linear behavior, suggesting that a saturation is obtained for WS pretreatment at 140ºC. In other words, increasing pretreatment time from a certain value (c.a. 6h) will have a very limited effect on increasing WS delignification, revealing the recalcitrance of the remaining lignin for treatment with glycerol at 140ºC. Harsher temperature conditions would be necessary to enhance the delignification of the feedstock. Exceptionally, the sample corresponding to 4 hours presented the highest absorption values, which resulted in a higher delignification than for 5 hours of pre-treatment. This was correlated with undetermined experimental errors.

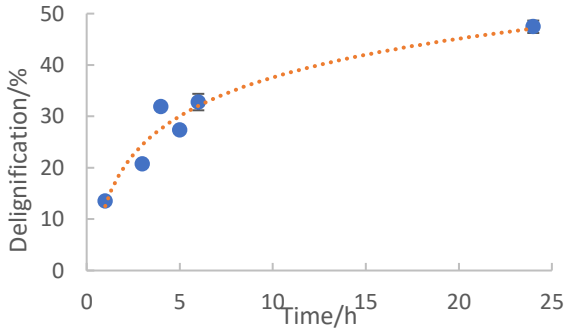

Figure 48. Delignification of wheat straw in glycerol at 140ºC as a function of time

A delignification of 32.8% was obtained for wheat straw pre-treated at 140ºC for 6h. The maximum obtained was 47.4% for 24h. In either case, still very low values if commercialization is to be pursued.

For eucalyptus, the two peaks above mentioned were also observed, for one hour of pre-treatment with glycerol, proving an effective delignification of the feedstock. It was observed that UV-vis spectra of glycerol after 1h pre-treatment at 140ºC presented different profiles according to the type of treated biomass (Figure 49). Particularly, different concavities appeared between 280 and 400nm for the wheat straw and eucalyptus residues. This should be related to the differences in structure and composition of the lignocellulosic material of WS and ER, concretely the differences of structure and composition of lignin and sugar-lignin complexes present in both feedstocks, which should originate distinct lignin fragments during solvolysis treatment. Moreover, these profiles were found independent of the pre-treatment time and the extent of delignification. which varied only the intensity of the radiation absorption.

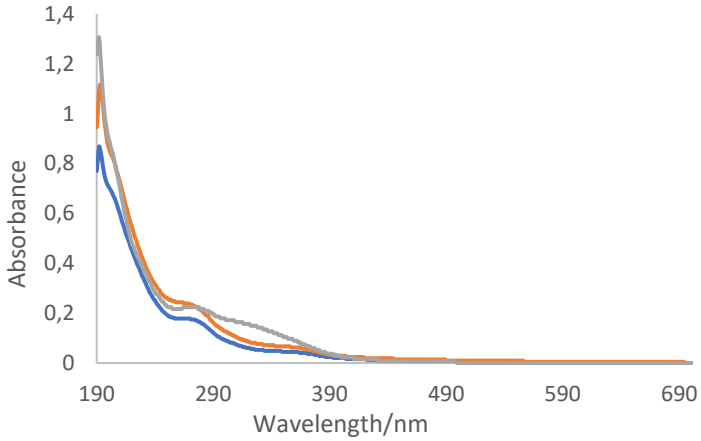

Figure 49. Comparison of UV-vis spectra of pre-treatment liquor of WS (—) and ER (in duplicate) after 1h at 140ºC (—, —)

Delignification of eucalyptus residues with glycerol at 140ºC reached 10.4% and 14.6%, similar to the achieved 13.5% value for wheat straw. As of now, efficiency of glycerol pre-treatment at 140ºC seems to be independent of biomass type.

Delignification of milled WS 0.5mm was 1.4 times higher than that of non-milled WS, on average, as a result of enhanced glycerol contact with biomass, due to increased WS surface area. A similar shape was obtained for the plot of delignification vs. time for milled and non-milled WS, though with a time offset between both, with delignification reaching higher values faster for milled WS (Figure 50). These results agree with the theoretical knowledge of heterogenous reactions, whose reaction rates are decreased by external and internal diffusion limitations to mass transfer (in this case, of solvent molecules into the lignocellulosic structure and of macromolecules from the same structure into the treatment liquor), that are more prevalent for superior particle sizes (Fogler 2001). As better results of delignification are obtained for milled biomass, the following sections will only deal with feedstocks milled to 0.5mm. In practice, for industrial applications, a study needs to be performed to optimize the economical trade-off subjacent to the selection of applicable particle size, i.e. on one side, milling of LM is an energy

and cost intensive operation, but on the other side, it decreases reaction times and increases product recovery, reducing heating costs and increasing gross profit (higher amounts of added-value products per input biomass).

It is important to note that only 1-3 g of the initial 10g of glycerol were recovered after pretreatment of milled WS, much due to the high viscosity of glycerol, which means that on average 84% of total extracted lignin were recovered in the washing liquid. Milled WS 0.5mm absorbed on average twice as much glycerol as non-milled biomass. Hence, future research will require focus on recovery steps.

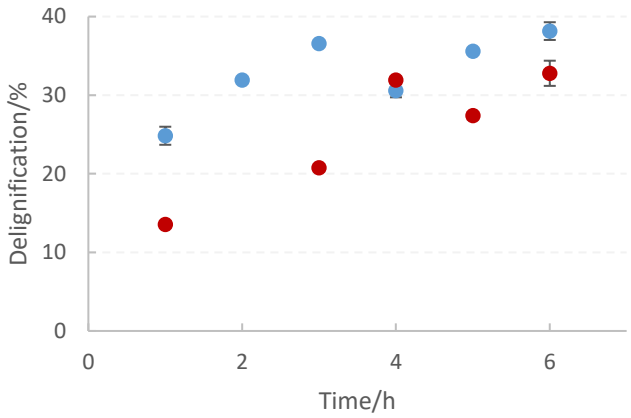

Figure 50. Comparison of delignification yields vs. time for 0.5 mm-milled (●) and non-milled WS (●), pretreated with glycerol at 140ºC

In spite of the promising results obtained with glycerol, further investigation was not carried out, as glycerol's high viscosity was viewed as a limitation to industrial application, particularly for continuous operation, as pumping and high-pressure operation-related costs would be increased. Glycerol also presented a certain thermal degradability, as detected by the appearance of multiple peaks in HPLC analysis of glycerol heated at 140ºC for 3h, putatively associated with the formation of degradation products. This thermal degradation of glycerol was also reported in literature (Demirbaş 2004), though at considerably higher temperatures than the ones applied in this work.

F. Production and identification of propylene glycol-xylosides

Introduction

Zhang et al. found that the xylose and furfural concentrations in the hydrolysate liquid were relatively small, in spite of the extensive xylan removal in acidified polyol and ethylene carbonate-EG pretreatments (Zhang, O'Hara, and Doherty 2013; Zhang et al. 2013). The authors explained this phenomenon not just with the incomplete hydrolysis of solubilized oligomers, but also with the formation of polyol-xylosides (see Figure 51). In fact, when they dissolved pure xylose in ethylene glycol with 10% water and 1.2% sulfuric acid and treated the resulting solution thermally at 130ºC for 30 minutes, only 7% of the initial xylose was detected by HPLC analysis. This yield increases to 39%, if the solution of EG-xylosides is hydrolyzed with water (added to 75%) at the same temperature and for a similar amount of time. Correspondingly, xylose yield in pretreatment liquors also increased after hydrolysis in the same conditions. These results led the authors to conclude that the EG-xylosides could be hydrolyzed back to EG and xylose if water was added to the hydrolysates. Besides, the evidence of changes in xylose yields, a peak in HPLC chromatogram was also detected between xylose and arabinose, which was correlated with EG-xyloside molecules. This peak decreased when hydrolysis was performed, in accordance with the conclusions presented above. The formation of propylene glycol-xylosides similarly to EG-xylosides formation was speculated, although not demonstrated.

Yamada et al. have reported similar observations (Yamada and Ono 1999; Yamada and Ono 2001; Yamada et al. 2007) but with respect to ethylene glycol reaction with glucose. In fact, EG-glucosides accounted for more than 80% of liquefied glucose obtained in the liquefaction of wood with ethylene carbonate and ethylene glycol catalyzed with 3% sulfuric acid at 150ºC, after 30 min. EG-glucosides were identified by ^{13}C NMR and HPLC analysis. In the later, those compounds appeared after the peaks corresponding to sugars and EG. Prolonging the reaction time induced the conversion of the EG-glucosides into levulinates (levulinic acid EG esters) such as 2-hydroxyethyl levulinate, as glucose degraded to levulinic acid (v. mechanism in Figure 52). This reasoning explained why the levulinic structure existed in relatively high concentrations in the liquified product even though there was not much water in the solvent used (as usually levulinic acid forms in aqueous environment from cellulose via HMF).

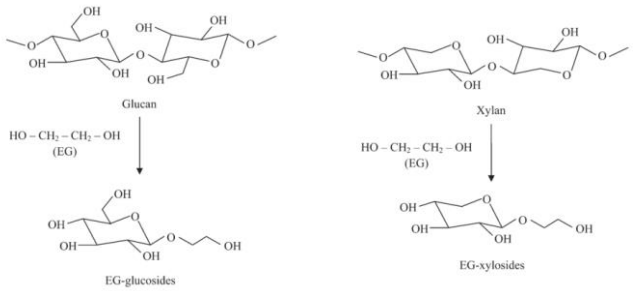

Figure 51. Scheme of formation of ethylene glycol-glycosides (Yamada et al. 2007)

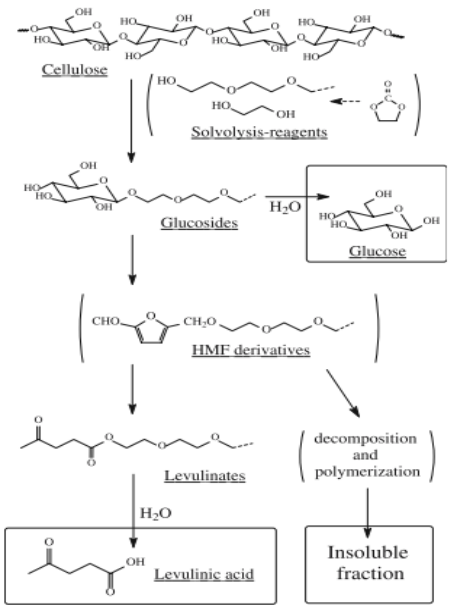

Figure 52. Mechanism of cellulose degradation in glycol liquefaction

It is proposed that the hydrolysate containing polyol-glycosides and lignin may be used in the production of biodegradable polymers, polyesters and resins (Zhang et al. 2013). Yu et al. were able to produce good quality stable biodegradable polyesters directly from the liquid product of the liquefaction of corn stover in acidified ethylene glycol/ethylene carbonate mixture (9:1). The adopted procedure consisted of mixing under heat the liquefied corn stover with crosslinking multifunctional carboxylic acids or anhydrides, after which the resulting mixture was casted in sheet forms and cured in an oven at 140ºC. The properties of the produced polyesters (such as stiffness or flexibility) were adjustable by varying the employed carboxylic donor. Applications for these polymers include use in agriculture, packaging and textile industries (Yu et al. 2006).

Alternatively, polyol-glycosides can be converted into polyurethanes (Mishra and Kumar Sinha 2010; Pan 2011; Galhano dos Santos et al. 2017), a family of polymers characterized by excellent strength and chemical resistance used as foams, coatings, adhesives for glass, wood and plastics. Namely, polyurethane adhesives with good thermal and chemical stability and mechanical resistance superior to the one of commercial brands have been synthesized from liquid enriched with glycosides resultant of acid-catalyzed ethylene glycol liquefaction of waste paper (Mishra and Kumar Sinha 2010). A yield of 93% of glycosides was reported. Hence, the potential for economical valorization of the liquid resultant of alcohol pretreatment (enriched with polyols, including glycosides and xylosides) has been attested, affirming the suitability of this kind of treatment.

Xylosides are also proposed as products with applications in the synthesis of bio-renewable surfactants and wetting agents which can have properties similar or superior to petrochemical derived products (Lancefield et al. 2017). Furthermore, glycoside formation, reversible through hydrolysis, has a positive impact in protecting sugars from degradation (Renders et al. 2017).

The glycosides and lignin fragments produced in polyol pretreatment of biomass can also be separated from the pretreatment solvent and possibly even fractionated amongst themselves in order

to obtain appropriate-sized molecular fragments. Such separation could be fundamental to improve solvent recyclability, a key factor for economic viability of the biorefinery concept. A suggested method for downstream processing is size exclusion chromatography (SEC) (Zhang et al. 2013; Krzan and Zagar 2009). This method was reported to be able to separate ethylene glycol from glycosides, and both from phenolic fragments after microwave assisted glycol liquefaction of wood (Krzan and Zagar 2009).

Incorporation of alcohols into lignin structure during alcoholic pretreatment has also been observed (Lancefield et al. 2017). In one report (Kishimoto and Sano 2002), 1,4-butanediol is introduced into lignin structure to form α and γ ethers, when pretreatment is performed with a 70% aqueous solution of this solvent at 180ºC. In another report (Kubo et al. 2007), the introduction of ethylene glycol molecules in lignin structure during acidified ethylene carbonate-EG pretreatment at 150ºC of Japanese cedar was verified by 13C NMR spectroscopy of recovered lignin. EG, as other alcohols, is nucleophilically substituted for hydroxy groups on α and γ positions of lignin sidechains (Figure 53). This effect is more predominant the longer the time of pretreatment. In the case of EG, a difunctional alcohol, additional molecules of the solvent can be added to the ones substituted in the lignin structure, creating sidechains of EG oligomers. In fact, a degree of polymerization of 3.0 was estimated for 1 hour of pretreatment (Kubo et al. 2007). The introduction of EG linear chains into lignin molecules can improve its plasticity and solubility in various solvents, properties controlled by the amount of added EG chains and their average length, both of which can be tuned by adjusting process conditions. It is proposed that this altered lignin is to be utilized as an amphiphilic polymer or functional gels. The advantage of this pretreatment is based on the fact that extraction and modification of lignin occur simultaneously in a single step, as opposed to other pretreatments where the recovered lignin needs further processing in order to be suitable for commercial application. Moreover, the formation of acetals by reaction of lignin fragments with alcohols stabilizes reactive carbonyl groups, preventing lignin degradation, in line with a “lignin-first” approach (Renders et al. 2017).

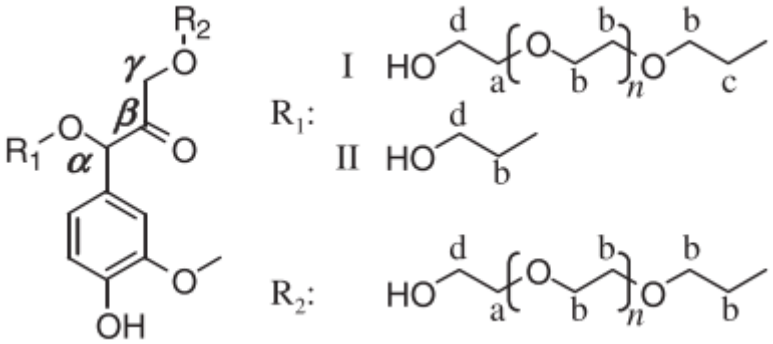

Figure 53. Illustration of ethylene glycol substitution in lignin structure during EG pretreatment (Kubo et al. 2007)

Given the low amounts of xylose recovered in organosolv pretreatment liquors (also including its degradation products), the possibility of side reactions between xylose and the treatment solvent (PG) was investigated.

Methods

In order to evaluate possible reactions of biomass sugars with the pretreatment solvent, four aqueous solutions of xylose were prepared, all with the same concentrations (18 g/L). From those four solutions, one also had propylene glycol (50% weight), another had acetic acid (10% w/w) and another both propylene glycol and acetic acid at the same concentrations. Acetic acid was used to assess the influence of an acid catalyst on the studied reactions, as the proposed pretreatment can include the use of catalysts. The tested concentrations were selected as typical values used in other reported organo-solv treatments. Other two aqueous solutions were additionally prepared without xylose – one with only PG and the other with PG and acetic acid at same concentrations as before –. to evaluate/account for the possibility of solvent degradation. An aliquot of each solution was treated at 140ºC for 3h in a manner analogous to the batch pretreatment, described in chapter three. After the 3 hours, the samples were allowed to cool down, filtered and then analyzed in HPLC system. Samples of the initial unreacted solutions were also analyzed and taken as blanks.

Results

It was observed a decrease in xylose concentration in aqueous solutions heated at 140ºC for 3 hours. While this fact was perfectly accounted for by xylose degradation to furfural and formic acid (assuming reaction stoichiometry of 1:1 mol) when no propylene glycol was present in the solutions, the same was not verified when it was (Table XIV). In fact, 24.5% of initial xylose was not accounted for the aqueous solution with PG, and when acetic acid (10% w/w) was also present this value almost doubled, reaching 42.6%. This observation suggests that propylene glycol reacts with xylose to produce certain products, predicted to be PG-xylosides (PG attached to xylose via a glyosidic bond), as a result of a Fischer glycosidation reaction, and that acetic acid is a catalyst of those reactions. This is also supported by recoveries of PG inferior to 100% (Table XIV), when xylose was present in solution. The possibility of PG degradation was discarded, as PG was fully recovered when the same thermal treatment was applied to aqueous solutions of PG without xylose.

Table XIV. Recoveries of xylose and acetic acid with respect to the untreated xylose standards

Solução	Conditions	Xylose Recovery % (mol)	Acetic Recovery % (mol)	PG Recovery % (mol)
Xylose	no treatment	100.0	-	-
Xylose	140ºC 3h	100.1	-	-
Xylose+PG	140ºC 3h	75.5	-	98.2
Xylose+Acetic	no treatment	100.0	100.0	-
Xylose+Acetic	140ºC 3h	98.6	99.0	-
Xylose+Acetic+PG	140ºC 3h	57.4	76.8	91.4

Additionally, acetic acid was fully recovered from the solutions of xylose and acetic (meaning that acetic acid does not react with xylose, being only a catalyst of its degradation to formic acid and

furfural), meanwhile only 76.8% was recovered when the solutions also contained PG (Table XIV). The latter observation should be a result of the formation of PG-acetates (esters).

Figure 54 illustrates the mass balance (molar basis) with respect to xylose in the solutions kept at 140ºC for 3 hours. The quantities of xylose converted to the various products are referred as percentages (mol) of the initial xylose concentration. The percentage corresponding to PG-xylosides was obtained as the difference between 100% and the sum of the percentages of the other products. It could be observed that acetic acid was a catalyst for not only the production of xylosides (which almost doubled as already mentioned), but also for the degradation of xylose to furfural, which increased from 3.7% to 9.3% for the solutions with no PG. When PG was present these values were significatively lower to 1.5 and 2.1% (without and with acetic acid. respectively). This reveals a protective effect of propylene glycol towards xylose degradation/dehydration to furfural, likely as a result of protection of xylose by formation of PG-xylosides.

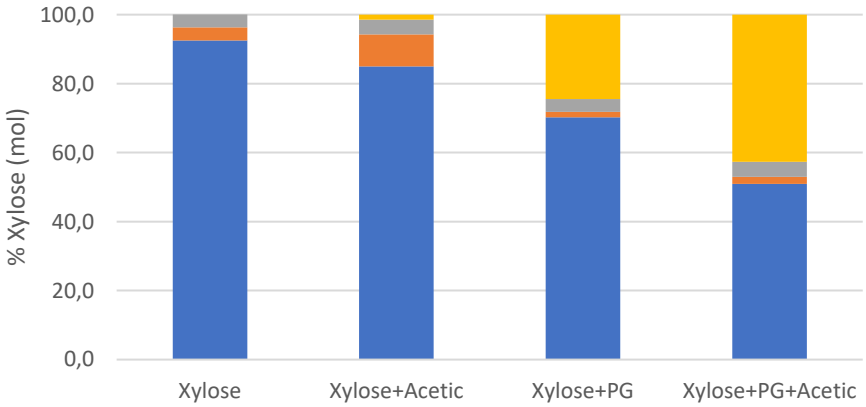

Figure 54. The fate of xylose in aqueous solutions (with/without PG and acetic acid) kept at 140ºC for 3 hours (■ free xylose, ■ xylose to furfural, ■ xylose to formic acid and ■ xylose to xylosides)

Considering the mass balances to xylose, PG and acetic, particularly the amounts that react to form PG-xylosides and PG-esters, the PG to xylose molar ratio in xylosides was calculated. A value of 4.2 was obtained, meaning that, on average, produced xylosides were formed by one xylose molecule and four PG molecules. It is expected that PG-oligomer with an average degree of polymerization (DP) of 4 is substituted to the carbonyl group of each xylose molecule to form the PG-xylosides, as that group is the most reactive of the sugar molecule. Alternatively, the PG-xyloside may also have on average three PG molecules substituted to three of its free hydroxyl groups, instead of a PG oligomer.

The formation of PG-xylosides was also supported by the appearance of two new peak in the HPLC chromatograms.

It is expected that PG also forms similar types of compounds with glucose (glucosides) and other sugars, including sugar oligomers. It is also expected that PG is added to lignin and phenolic compounds (which will be investigated in future work) (Lancefield et al. 2017). These results can explain why the mass balances to xylose, arabinose, acetic acid and phenolics do not close. Additionally, these results will be relevant for the future, since they will determine the process design of solvent recovery steps necessary in the biorefinery concept. Finally, polyol-glycosides are suggested to be useful feedstocks for biodegradable polymers, polyesters, resins, polyurethanes, among others (Mishra and Kumar Sinha 2010; Pan 2011), which may be an interesting valorization path to investigate in the future.

G. Results from sulfuric acid-catalyzed PG organosolv

The complete set of experimental results analyzed in item 4.3.2., which were used to assess the impact of operation conditions on pretreatment liquor composition, is presented in Table XV.

Table XV. Solid yield and hydrolysate concentration of sugars, aliphatic acids, furans and phenolics from sulfuric acid-catalyzed PG organosolv pretreatment of ER

Time (min)	Temperature (ºC)	PG (wt%)	H2SO4 (wt%)	CS	Solid yield (%)	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	GOS (g/L)	XOS (g/L)	Formic acid (g/L)	Acetic acid (g/L)	HMF (g/L)	Furfural (g/L)	Phenolic content (g/L)	Total Sugars (g/L)	Total Degradation Prod. (g/L)	TS-TDP (g/L)
30	120	25	1	1.1	87.5	0.31	2.35	0.83	0.47	3.45	0.32	1.28	0.01	0.01	0.8	7.49	0.34	7.15
60	120	25	0.5	1.2	86.7	0.3	2.5	0.9	0.5	3.8	0.4	1.3	0.0	0.0	1.0	7.9	0.4	7.5
30	120	90	1.5	4.2	63.8	0.72	2.98	1.66	0.55	6.99	0.61	1.90	0.01	0.05	5.2	12.9	0.66	12.28
60	120	90	1.5	4.5	58.7	0.7	3.2	1.7	0.7	6.7	0.8	1.9	0.0	0.1	6.0	13.0	0.9	12.2
30	130	25	1	1.4	73.3	1.10	9.42	1.72	0.24	3.04	0.74	2.95	0.01	0.06	1.4	15.0	0.82	14.21
60	130	25	0.5	1.5	72.3	0.6	6.8	1.2	0.4	3.2	0.3	1.8	0.0	0.0	1.3	12.1	0.4	11.7
30	130	90	1.5	4.5	54.2	1.1	4.0	1.9	0.7	7.0	0.4	1.7	0.0	0.3	6.8	14.4	0.7	13.8
60	130	90	1.5	4.8	49.2	1.6	5.0	2.4	0.7	7.0	1.5	2.6	0.0	0.5	8.1	16.1	2.0	14.1
15	140	25	1	1.4	73.3	0.8	8.6	1.4	0.5	3.8	0.5	2.6	0.01	0.05	1.7	14.9	0.5	14.3
15	140	75	0.5	1.8	70.5	0.7	3.1	1.0	0.2	5.9	0.4	1.6	0.01	0.03	5.0	11.1	0.4	10.7
60	140	25	0.5	2.5	65.4	1.5	13.3	1.8	0.4	1.6	0.7	3.8	0.03	0.3	1.8	18.1	1.0	17.0
60	140	75	1	3.4	44.8	1.7	8.6	2.0	1.3	4.8	1.4	3.0	0.02	1.2	9.8	18.2	2.5	15.7
15	150	25	1.0	1.7	63.5	1.6	12.8	1.9	0.5	3.3	1.1	3.7	0.0	0.2	1.9	19.5	1.3	18.2
15	150	50	1.0	2.1	54.6	1.35	10.9	3.07	0.65	3.95	0.72	3.36	0.02	0.23	5.2	18.5	0.97	17.57
15	150	50	2	2.3	50.3	2.3	12.7	3.4	1.1	3.9	1.1	3.5	0.0	0.9	6.3	21.9	2.0	19.9
15	150	25	2	1.9	61.4	2.0	13.9	1.9	0.7	2.3	0.5	3.7	0.0	0.5	2.0	20.3	1.0	19.4
30	150	50	1	2.4	58.5	1.4	10.6	3.2	0.5	3.6	0.9	3.5	0.0	0.3	5.3	17.7	1.2	16.4
30	150	50	2	2.6	52.7	2.3	11.9	3.7	0.6	3.1	1.4	3.8	0.0	0.8	5.7	19.7	2.2	17.5
30	150	25	1	2.0	59.3	3.1	14.6	2.0	0.5	1.4	1.3	4.0	0.1	1.2	2.4	21.1	2.6	18.5
30	150	25	2	2.2	58.5	4.0	13.2	1.8	0.7	0.9	1.3	4.0	0.1	2.5	2.6	20.0	3.9	16.1
15	160	25	1	2.0	61.1	2.7	14.7	1.7	0.3	1.4	1.1	4.1	0.05	0.9	2.4	20.5	2.0	18.5
15	160	75	0.5	3.1	48.5	1.4	7.8	1.6	0.8	5.5	1.1	2.9	0.02	0.5	9.2	17.3	1.6	15.6
15	160	75	1.5	3.6	46.0	1.8	9.3	2.5	2.2	4.9	2.0	3.3	0.0	1.7	9.9	20.0	3.8	16.3
15	160	75	2	3.6	41.1	3.3	11.2	0.0	2.2	1.8	2.2	3.6	0.0	2.9	11.1	19.9	5.2	14.7
15	160	25	1.5	2.1	58.7	3.04	14.5	1.96	0.48	1.67	1.20	3.93	0.06	1.03	2.6	21.1	2.29	18.8
15	160	25	2	2.2	57.3	3.6	14.3	2.1	0.6	1.6	1.3	4.2	0.1	1.2	2.6	21.4	2.5	18.9
60	160	25	0.5	4.0	57.5	4.1	13.6	1.6	0.5	0.0	1.7	4.2	0.1	3.3	3.0	19.3	5.1	14.2
60	160	75	1	2.4	34.7	2.7	10.8	1.6	5.4	0.0	3.0	3.5	0.06	4.9	13.5	18.5	7.9	10.6
180	160	25	1	3.1	50.0	12.0	4.1	0.5	1.3	0.0	3.4	4.3	0.3	3.4	n.d.	17.9	7.1	10.8
180	160	75	1	4.5	27.2	8.1	7.7	0.6	4.8	0.0	9.3	5.8	0.1	0.3	n.d.	21.1	9.7	11.5