A Study of Organosolv Ethanol-based Processes for the Selective Fractionation of Relevant Agroforest Residues

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Declaration

I declare that this document is an original work of my authorship and that it complies with all the requirements of the Code of Conduct and Good Practices of the University of Lisbon.

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Resumo

Neste trabalho, é apresentado o estudo de processos organosolv usando misturas etanol/água para duas matérias-primas lenhocelulósicas modelo, a palha de trigo (WS), e resíduos de *Eucalyptus globulus* (ER), tendo-se por objetivo o fracionamento integrado da biomassa que remova e despolimerize seletivamente a lenhina e as hemiceluloses, produzindo compostos derivados da lenhina, oligo- e monossacáridos solúveis, bem como um resíduo sólido rico em celulose com digestibilidade elevada.

Numa primeira abordagem, os pré-tratamentos organosolv sem catalisador foram realizados usando misturas etanol/água (50:50) a 190ºC e tempos de reação variando entre 0 e 20 min. Os tratamentos de 120 min provaram ser os mais eficientes para ambos os resíduos, apresentando rendimentos de deslenhificação relativamente altos (59% e 70,9% para WS e ER respetivamente), bem como a formação de xilo-oligossacáridos. Os sólidos, ricos em celulose, sofreram hidrólise enzimática atingindo rendimentos de sacarificação de 68,5% e 71,7% para a WS e ER, respetivamente. Estes tratamentos mostraram-se mais eficientes para ER e por isso, foram otimizados tratamentos organosolv à temperatura de 140ºC e baixas concentrações de catalisador (0-50 mM de ácido sulfúrico), de acordo com uma distribuição estatística de Doehlert. Os rendimentos de deslenhificação e digestibilidade enzimática mais elevados, 86,4% e 84,1%, foram obtidos para concentração de ácido sulfúrico de 25 mM e tempo de reação de 90 min. Foi efetuada uma simulação usando o software Aspen Plus® onde foi realizada uma reconciliação de dados seguida de uma otimização do processo e uma análise *pinch* com vista a prever possíveis reduções de caudais e consumos energéticos.

Palavras-chave: açúcares hemicelulósicos; biorrefinaria; etanol organosolv; lenhina; palha de trigo; resíduos de eucalipto

Abstract

In this work, an organosolv approach based on ethanol/water mixtures was studied for two model lignocellulosic feedstocks, one from agriculture origin (wheat straw, WS) and another from forest origin (*Eucalyptus globulus residues*, ER). The process conditions were studied aiming at an integrated biomass upgrade concept that selectively remove and depolymerise lignin and hemicellulose, producing lignin-derived compounds and soluble oligo- and monosaccharides, as well as an easily digestible polysaccharide (mainly cellulose) containing solid.

In a first approach, non-catalysed organosolv pre-treatments were carried out using ethanol/water mixtures (50:50) at 190ºC and reaction times ranging from 0 to 120 min. The 120 min treatments proved to be the most efficient for both residues with relatively high delignification yields (59% and 70.9% for WS and ER, respectively), as well as xylo-oligosaccharides formation in the hydrolysates. The cellulose-rich solids undergo enzymatic hydrolysis achieving for WS and ER 68.5% and 71.7% of enzymatic digestibility yields, respectively. Non-catalysed organosolv pre-treatments at 190ºC were more efficient for ER than for WS. Thus, ethanol/water organosolv treatments at milder temperature (140ºC) and low catalyst concentration (0-50 mM H_2SO_4) were optimised, using a statistical experimental design, following a Doehlert distribution. The highest delignification yields and enzymatic digestibility, 86.4% and 84.1%, were obtained for 25 mM sulfuric acid concentration and 90 min reaction time. A simulation using Aspen Plus® software was conducted to perform a data reconciliation analysis followed by process optimisation using heat and mass integration. A pinch analysis was made to predict potential energy savings.

Keywords: biorefinery; ethanol organosolv; eucalyptus residues; hemicellulosic sugars; lignin; wheat straw

Table of contents

List of Figures

List of Tables

Notation

AcOS	Oligosaccharides substituted with acetyl groups
AFEX	Ammonia fiber explosion/expansion
AH	Autohydrolysis
ARP	Amonia recyclig percolation
AT	Alkaline Treatment
AXOS	Arabinoxylo-oligossacharides
ER	Eucalyptus Residues
EU	European Union
FAO	Food and Agriculture Organization
GAE	Gallic Acid Equivalents
GDP	Gross Domestic Product
GHG	Greenhouse gases
GlcOS	Gluco-oligosaccharides
HMF	5-hydroxymetylfurfural
HPLC	High Performance Liquid Chromatography
IEA	International Energy Agency
ILs	Ionic liquids
LHW	Liquid Hot Water
LM	Lignocellulosic materials
LNEG	Laboratório Nacional de Energia e Geologia
LSR	Liquid-to-Solid Ratio
NREL	National Renewable Energy Laboratory
OPEC	Organisation of the Petroleum Exporting Countries
OS	Oligosaccharides
PT	Palha de Trigo
R^2	Correlation coefficient
RE	Resíduos de Eucalípto
RP	Reconciliation Problem
Rpm	Rotations per minute
RSM	Response Surface Methodology
SY	Solid Yield

SIADEB	Ibero-American Society for the Development of Biorefinaries
UBB	Bioenergy and Biorefineries Unit (Unidade de Bioenergia)
UN	United Nations
UV	Ultraviolet
Vis	Visible
WS	Wheat Straw
XOS	Xylo-oligosaccharides
Y_{Gn}	Glucan Yield
Y_{Xn}	Xylan Yield

1. Introduction

1.1. Context and motivation

The work presented in this master dissertation was carried out as part of the European project AMBITION, financed by the Horizon 2020 program, which aims to develop new technologies for the production of biofuels that contribute to future energy sustainability, improving the integration of thermal and biological processes for biomass conversion. In this particular work pre-treatment processes for biomass fractionation in order to produce high-value lignin and clean sugars streams using two model relevant feedstocks, one from agriculture origin (wheat straw) and another one from forest origin (eucalyptus residues) were studied.

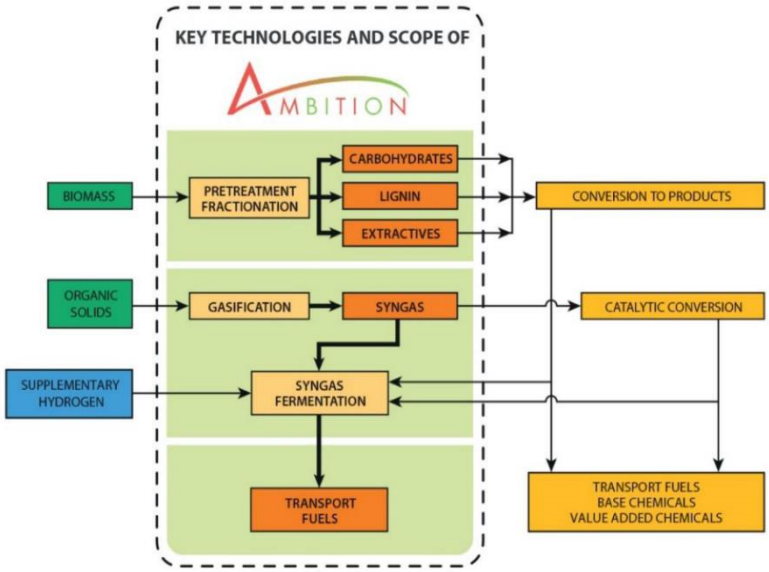

Figure 1. Scheme of AMBITION project, key technologies and objectives

The growing need for alternatives to fossil fuels, the most used resource in chemical industries for decades and the cause of many environmental concerns, has led to the research for greener alternatives. In an attempt to solve these problems, biomass, as a sustainable resource, has been studied. It is in this context that the biorefinery concept takes further importance, as it allows the conversion of biomass into a diversity of (bio)products, which includes energy and added value products, all of them that contribute to the bioeconomy concept.

According to Horizon 2020 *"Bioeconomy is Europe's response to key environmental challenges the world is facing already today. It is meant to reduce the dependence on natural resources, transform manufacturing, promote sustainable production of renewable resources from land, fisheries and aquaculture and their conversion into food, feed, fibre, bio-based products and bio-energy, while growing new jobs and industries."*

In Portugal, the bioeconomy has been growing since 2011, with an added value close to 12 billion euros in 2017 (about 7% of the GDP), corresponding to 13.3% of the total employment and generating

a turnover to 43 billion euros (about 12% of the economy's total turnover). The main contribution to the circular economy comes from the bio-based industry, which represents 62.3% of the total added value generated by the circular economy. [COTEC Portugal, 2020]

Bioproducts are considered highly valuable for the Portuguese economy, as it is estimated that in 2030 a market share of 5% of bioproducts in the Construction, Textile and Plastics markets will correspond to an aggregate increase in revenues of 260-579 million euros per year.

As stated, the basis of a biorefinery is its raw materials which can come from the most diverse origins. The focus of this thesis will be on the agriculture (wheat straw) and forest (Eucalyptus) residues.

The most important world agriculture crops are wheat and corn, mainly cultivated in Asia and Europe being China, India, Russia and France, the most relevant countries in those continents. As of 2018, there were a total of 734 million tons of wheat produced all over the world, with Portugal presenting 67.749 thousand tonnes [FAO, 2018]. Associated with this crop is the production of wheat straw, in general around 50% of the cereal grains [ww.farmprogress.com/grains/what-value-wheat-straw]. Contrarily to wheat, eucalyptus species are widespread in the Iberian Peninsula and well-adapted to the Atlantic climate. In Portugal, according to CELPA, the paper and pulp industry based on eucalyptus represents a Gross Value Added (GVA) of 1.4% of the National GVA while its production corresponds to 2.4% of National Production and 8% of Industry Production. It also produces from forest biomass more than 5% of the total electricity in Portugal, being the largest national green electricity producer. All these numbers points out this sector as an important source of forest residues from the exploitation and processing of eucalyptus.

These and other agroforestry residues are sources of biomass with high availability and abundance in certain contexts and are often treated as waste in the respective process chains, with a low-value valorisation of its applications. However, as lignocellulosic materials, they have high potential to be upgraded. Its cellulose, hemicellulose and lignin fractions can be applied and/or converted to the synthesis of high-value chemicals, thus constituting an excellent alternative to conventional raw materials in the chemical industry. Another advantage, considering the recovery and optimisation of waste, is that its use does not compete with the food or paper industry, falling under the second-generation bioresources category. Furthermore, its renewable nature and low cost, makes its study required in a biorefinery context.

Usually, one of the main issues in the lignocellulosic biomass conversion in a biorefinery, is that it is not simple to break down cell wall polysaccharides, recover high-quality lignin and efficiently achieve high sugar yields, as well as to have low cost and energy inputs allied with environmentally friendly techniques.

To ensure the most desirable conversion of biomass, thermochemical or biochemical processes may be involved. Thermochemical processes occur under severe temperature conditions, for example, where biomass gasification or pyrolysis may take place depending on the temperature and oxygen

availability. On the other hand, biochemical processes occur under less severe conditions. These processes are, in general, focused on the fermentation of sugars extracted from lignocellulosic biomass.

However, the problem arises as high amounts of lignin in cellulosic substrates do not favour bioconversion pathways, creating hindrances. Furthermore, lignin is by itself, a relevant product that can be extracted from lignocellulosic biomass. Thus, it is essential to develop strategies to promote lignin removal, such as different pre-treatment options. Amongst the various types of pre-treatment available, that allow lignin removal from biomass, processes such as Kraft, sulphite, alkaline oxidation and organosolv are usually included. The organosolv pre-treatment potentially allows to recover high-quality, non-degraded lignin and will take special relevance in this work. [Pan et al., 2005]

1.2. Objectives

The research developed in this work was conducted at Laboratório Nacional de Energia e Geologia (LNEG), Unidade de Bioenergia e Biorrefinarias in the framework of the European Project AMBITION (starting June 2019 to December 2020).

This work aims to process wheat and eucalyptus residues using organosolv pre-treatments, carried out in a batch reactor using ethanol/water mixtures under different conditions of temperature, reaction time and without or with addition of catalyst. Carbohydrate- and lignin content and composition in both the liquid and solid streams obtained after pre-treatment will be assessed. The saccharification yields of cellulose-rich pre-treated biomass will also be determined.

1.2.1. Study of Organosolv processes without catalyst adding:

a) Chemical characterisation of biomass: summative chemical composition (determination of ash, extractives in different solvents, cellulose, hemicelluloses and lignin) and monomeric composition of polysaccharides and lignin, using methods gravimetric and HPLC**;**

b) Chemical characterisation of hydrolysates: determination of the monosaccharide content, oligosaccharides, furans, organic acids and phenolic compounds.

1.2.2. Study of Organosolv processes without catalyst adding:

a) Study of operational conditions (time, temperature) for depolymerisation of lignin and hydrolysis of hemicellulose both from wheat straw and eucalyptus residues. Evaluation of the polysaccharide, delignification and hydrolysis yields;

b) Chemical characterisation of the obtained solid residues;

c) Chemical characterisation of liquid fractions (sugars, organic acids, furans and total phenolic compounds);

d) Selective recovery of solubilised lignins: precipitation by dilution.

1.2.3. Study of organosolv processes in the presence of a catalyst (sulfuric acid):

a) For eucalyptus residues, the effect of low catalyst concentrations in depolymerisation of hemicellulose and lignin was studied;

b) The solid residues obtained and the liquid fractions were characterised using the previously described methods.

1.2.4. Enzymatic hydrolysis of pre-treated solids:

For the pre-treated solids obtained will be studied:

a) Characterisation of the percentages of enzymatic saccharification according to protocols established using commercial cellulases;

1.2.5. Scale-up of a non-catalysed eucalyptus organosolv process

a) Use of ASPEN and MatLab tools to scale-up the most promising laboratory assays**.**

1.3. Presentations related to the present work

Carvalheiro, F.; Lukasik, R. M.; Duarte, L.C.; Roseiro, L.B.; Ribeiro, B.; Marques, S.; Bernardo, J.R.; Van-Dúnem, V.; Pires, F.; Costa, D.; Sanfins, L.; Gírio, F.; "Desenvolvimento de Processos de Pré-Tratamento da biomassa para a separação eficiente das correntes de lenhina e de açúcar" - CIES 2020, 701-708, Lisboa, Portugal, November 2020 – Oral/ Proceedings

Pires, F; Van-Dúnem, V.; Sanfins, L.; Duarte, L. C.; Gírio, F.; Carvalheiro, F.; "Optimisation of a mild organosolv ethanol-based process for the selective fraction of *Eucalyptus globulus* residues" 28th European Biomass Conference and Exhibition - EUBCE 2020, Marseille, France (online), July 2020. – Poster/Mini Oral

Carvalheiro, F.; Pires, F.; Van-Dúnem, V.; Sanfins, L.; Duarte, L. C.; Gírio, F.; "Efficient production of sugars and lignin streams using ethanol-based organosolv pre-treatments". 27th European Biomass Conference and Exhibition - EUBCE 2019, Lisboa, Portugal, May 2019. – Poster/Mini Oral

1.4. Structure

This thesis is divided into five chapters.

The first chapter comprises a short introduction to the topic and motivation, followed by the aim of the work and related presentations.

Chapter two includes a bibliographic review over the raw materials, their composition, pre-treatment processes and possible products formed all within the concept of biorefinery.

The third chapter presents a detailed overview of the analytical methods used in experimental work.

Chapter four is where the main results are presented, as well as the a process modelling using ASPEN and MatLab tools

Chapter five includes the conclusions related to the main findings as well as future applications perspectives.

2. State of the Art

2.1. Biorefinery

The world strongly depends on fossil fuels. This dependence comes from the intense consumption and use of petroleum derivatives, which allied with their scarcity, causes political and environmental concerns. Scientific evidence shows that greenhouse gases (GHG) emissions, such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxides (N_2O), that derivates from fossil fuel combustion and land-use change due to human activities, are endangering the planet's climate. The 2018 United Nation Climate Report (IPCC, 2018) clearly states that immediate changes were required to prevent the rise in global temperature to above +1.5ºC, the current manageable scenario.

Over the past 20 years, transport sector has shown the highest rates of growth in terms of GHG emissions since the world's primary source of energy in this sector is oil. In 2019, only CO_2 emissions, due to fossil fuels combustion, reached a value of 35 billion tons with a predicted value of 43 billion tons by 2050 [Energy Information Administration, 2020]. In the same year, the world's oil demand was about 100.1 million barrels a day with prospects to increase up to 110.6 million barrels a day by 2040. However, this latter projected value has been decreasing every year [OPEC, 2019]. Currently, in 2020 due to Covid-19 pandemic situation, which leads to economic and mobility impacts, including widespread shutdowns across the world, this consumption decreased to 91.7 million barrels per day.

Nonetheless, environmentally friendly alternatives to fossil fuels have been studied and implemented, such as the partial replacement of oil by biomass as raw material. This shift has been the driving force for the development of biorefineries. There is not one but several definitions for biorefinery. The definition that is adopted by the International Energy Agency Bioenergy (IEA Bioenergy) Task 42 is as follows: *"Biorefinery is a sustainable processing of biomass into a spectrum of marketable products and energy."*

The Iberian Society for Development of Biorefineries (SIADEB) defines biorefinery as an industrial installation that seeks to extract carbohydrates, oils, lignin, and other materials from biomass, converting them into fuels, high value chemicals and other materials, with a zero waste approach. This concept is illustrated in Figure 2.

Another definition for biorefinery is provided by the American National Renewable Energy Laboratory (NREL) that states that a biorefinery is a facility that integrates biomass conversion process and equipment to produce fuels, power and chemicals from biomass.

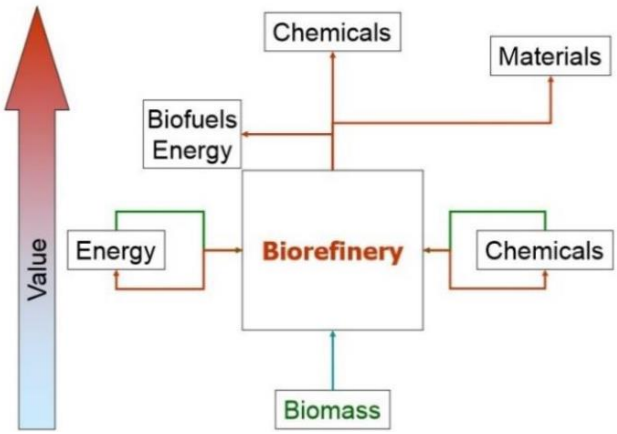

Figure 2. The Biorefinery concept adapted from the SIADEB. [Carvalheiro et al., 2008]

According to all this, a biorefinery can be a facility, a process, a plant or even a cluster of facilities, in which the main driver is its sustainability. In all biorefineries, a life cycle analysis (LCA) should be made to assess the impacts of their entire value chain on the environment, as well as the consequences involving competition for food and biomass resources, the net balance of GHG's and energy efficiency, among others.

All kinds of biomass from forestry, aquaculture, agriculture and residues from households and industries, including wood, forest residues, aquatic biomass, agricultural crops and organic residues, can be used in a biorefinery, making it so that a large spectrum of marketable products and energy are produced. The products can either be intermediates and/or final products, which include feed materials, food and chemicals; while energy includes power, heat and fuels, being the last one the main focus of biorefineries.

Nowadays, biomass-based fuels can be classified as 1st or 2nd generation biofuels depending on the type of raw material used to its formation. First-generation biofuels refer to biofuels produced from sugar, vegetable oil, starch, or animal fats, which are raw materials in competition with feed and food industries. While this type of fuels is produced using conventional technologies, its competition for raw materials originates various ethical, political and environmental concerns [Cherubini, 2010].

On the other hand, 2nd generation fuels derive from lignocellulosic materials such as agriculture, forestry and industrial by-products and residues. This type of biofuels has advantages over the 1st generation ones due to a more efficient land-use and environmental performance according to LCA studies. Even though most technologies and processes for 2nd generation biofuels are still to be implemented in the market, their production is expected to grow in the future as this kind of fuels eliminate the competition problem that arises with the 1st generation ones [Cherubini, 2010] but also to reduce the GGE in order to meet the goal of a net zero carbon footprint "carbon neutrality" and fulfill the Sustainable Development Goals [European Commission and UN]

In Portugal, the *Plano Nacional para a Promoção das Biorrefinarias* (2017) estimated the amount of residual biomass in Portugal and concluded that there is the availability of this biomass to be used as energy (electricity, heat and advanced biofuels), and also in value-added bioproducts in the food,

chemical, pharmaceutical and textile industries, among others. It also stated that the country “has a considerable potential for residual biomass (forest, agricultural, agro-industrial, etc.), as well as biomass of natural origin (shrubs and spontaneous) that can be valorised in the context of biorefineries, with environmental, economic and social benefits”.

2.2. The lignocellulosic materials

Lignocellulosic materials (LM) are very abundant and biodegradable, although they are often viewed as “waste” and can constitute environmental problems due to the large quantities involved. However, the growing interest from biorefineries and chemical industries in agro-industrial residues due to its recovery, availability and low-cost for bioconversion, overcomes these issues [Carvalheiro et al., 2008]. Overall, global lignocellulosic biomass potential is between 10 and 50×10^9 tons/year [Volynets et al., 2016].

Lignocellulosic biomass is composed primarily of three biopolymers: cellulose, hemicellulose, and lignin (Figure 3).

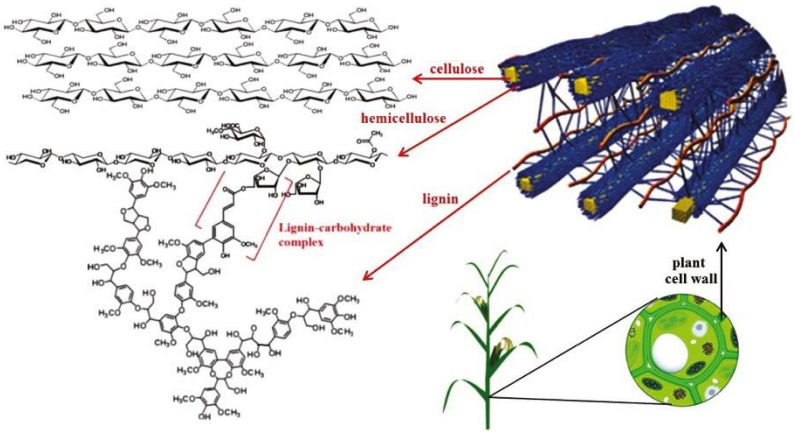

Figure 3. Lignocellulosic structure and its compounds in plant cell wall [Volynets et al., 2016].

Lignocellulosic materials can be classified according to its chemical composition in macromolecular and low molecular weight compounds. Macromolecular compounds that comprise the cell wall are cellulose (35-50%), hemicellulose (20-35%) and lignin (10-25%). Low molecular weight compounds consist of organic compounds (extractives) and inorganic compounds (ash) [Fengel & Wegener, 1984]. It should be noted that LM composition varies according to the specie, origin, genetic and environmental factors [Gírio et al., 2012].

In relation to the structural organisation of the polymers, the cell walls comprise a cellulose microfibril skeleton surrounded by hemicellulose, while lignin occupies the empty spaces left between the hemicellulose molecules. Extractives can be found in the cell lumina, cellular voids or channels [Pereira et al., 2003].

2.2.1. Cellulose

Cellulose is the most abundant biopolymer in the world as well as the major structural fibre in most LM, representing about 40-50% of dry biomass [Finch, 1986]. Structurally it is an unbranched homopolymer of hexoses (glucose monomers) linked by β-1,4-glycosidic bonds with the chemical formula $(C_6H_{10}O_5)_n$, in which "n" represents the degree of polymerisation. As this polymerisation degree increases, various cellulose properties are also changed.

Each glucose molecule is related to the other in a 180º rotation, which means that a molecule of cellobiose is the repetition unit [Fengel & Wegener,1984]. Cellulose chains are grouped into microfibrils of 3-5 nm diameter by and van der Waals and hydrogen bonds, varying predominantly between crystalline and amorphous regions. Those microfibrils are then surrounded by larger structures with diameters of 50-250 nm, in which cellulose is interlocked to hemicellulose through hydrogen bonds, within a complex non-crystalline matrix of hemicellulose and lignin (Figure 4) [Zhang et al., 2016].

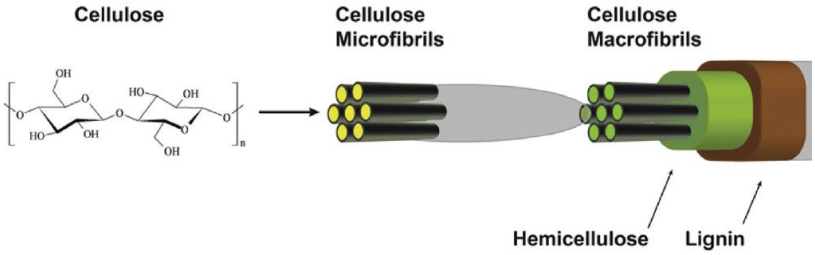

Figure 4. Schematic illustration of cellulose structure within a plant cell wall [adapted from Zhang et al., 2016].

Although cellulose is insoluble in most solvents and presents a high chemical resistance due to its structure, it can suffer hydrolysis by acids and enzymes [Agbor et al., 2011].

Cellulose numerous applications include the production of high value-added chemicals. The glucose formed by cellulose hydrolysis can be fermented to ethanol in high yield. The ethanol gives origin to ethylene and butadiene after being dehydrated. At the same time, the ethylene can be further processed to styrene, polyethylene, ethylene oxide, ethylene glycol or vinyl chloride. Furthermore, glucose fermentation can either be directed to lactic acid formation, from which acrylic acid may be obtained, or to the formation of acetone, butanol, citric acid, butyric acid, glycerine or isopropanol. Glucose can also be converted to chemicals that could become intermediates to other products, such as hydroxymethylfurfural (HMF), ɣ-valerolactone (GVL), levulinic and propionic acids, polyamides, polyesters, polycarbonates and epoxide. Finally, glucose hydrogenation can yield sorbitol and, if higher temperatures are applied, 1,2 propanediol, ethylene glycol, and glycerine [Valentin Popa, 2018; Barbosa et al., 2020].

2.2.2. Hemicellulose

As the second most abundant biopolymer and more complex than cellulose, hemicellulose is a heterogeneous class of polymers that represents 15-35% of plant biomass [Gírio et al., 2012]. It may contain pentoses as β-D-xylose and α-L-arabinose, and hexoses such as β-D-mannose, β-D-glucose, α-D-galactose, while other sugars (α-L-rhamnose and α-L-fucose) and uronic acids (α-D-glucuronic, α-D-4-O- methylgalacturonic and α-D-galacturonic acids) can also be present in small quantities. The hydroxyl groups of these sugars can also be partially replaced by acetyl groups [Pereira et al., 2003]. Xylans and glucomannans are considered the most relevant hemicelluloses, with the firsts being the most abundant [Gírio et al., 2012].

The β-1,4-linkages present in hemicellulose backbone structure, allow the formation of hydrogen bonds with themselves and cellulose conferring to these polysaccharides a support and cohesion role [Zhang et al., 2016]. Depending on their biological origin, hemicelluloses present differences in their structure and composition. Hardwoods, such as Eucalyptus, comprises of mainly xylans and some glucomannans, while softwoods mainly contain galactoglucomannans with some xylans in their hemicellulosic composition. Agriculture residues, such as grass and cereals, have glucuronoarabinoxylans as their main polysaccharide type [Gírio et al., 2012].

Unlike cellulose, the hemicellulose structures vary in such a way that is impossible to identify crystalline microfibers, thus considered amorphous structures. This fact may explain why hemicelluloses present a higher solubility in alkaline solutions and are easily hydrolysed by acids in monomers, conferring it an inferior thermal and chemical stability compared to cellulose [Zhang et al. 2016; Pereira et al., 2003].

To promote hemicellulose fraction valorisation, diluted acid hydrolysis and hydrothermal treatments are ones of the most used, and depending on the severity of such treatments, the resulting products are sugars, i.e., oligosaccharides (OS) and monosaccharides, acetic acid and products derived from monosaccharides degradation (e.g., furfural, HMF, formic acid, levulinic acid). Thus, some high-value chemicals products are produced from hemicellulosic sugars. These include furfural and xylitol, biofuels carriers and biofuels such as ethanol [Gírio et al., 2010; Barbosa et al.; 2020].

2.2.3. Lignin

Lignin, the main constituent of cell walls and in terms of high molecular mass, presents an intricate amorphous heteropolymer (polyphenolic) structure, with high resistance to chemical and biological degradation. This resistance comes from its hydrophobic nature and insolubility in aqueous solution and water, preventing the access to organisms and degrading chemical agents. It also enables the mechanical strength, liquid transport and waterproofing on the LM, representing in average 10 to 30% of the native biomass dry weight, depending on the botanical origin of the specie. In hardwoods, lignin contents can vary from 19 to 28%, while 24 to 33% in softwoods; in cereal straws, lignin contents range

from 15 to 25%. All those lignin contents, however, also depend on the plant age and structure [Gosselink, 2011].

Lignin has a polyphenolic nature in which the monomeric units are linked in a complex three-dimensional network by different types of ether and ester bonds and C-C bonds. The lignin present in plant cell walls is normally related with both cellulose and hemicellulose, although its chemistry is more complicated when compared to the other two. These dispositions make it one of the biopolymers of greatest recalcitrance, an ideal characteristic in cell walls. [Gosselink, 2011] Even though it is difficult to quantify lignin's polymerisation degree as well as a definition to its structure, Figure 5 presents hardwood **(A)** and softwood **(B)** lignin structures as proposed by Chris Lancefield (2015)

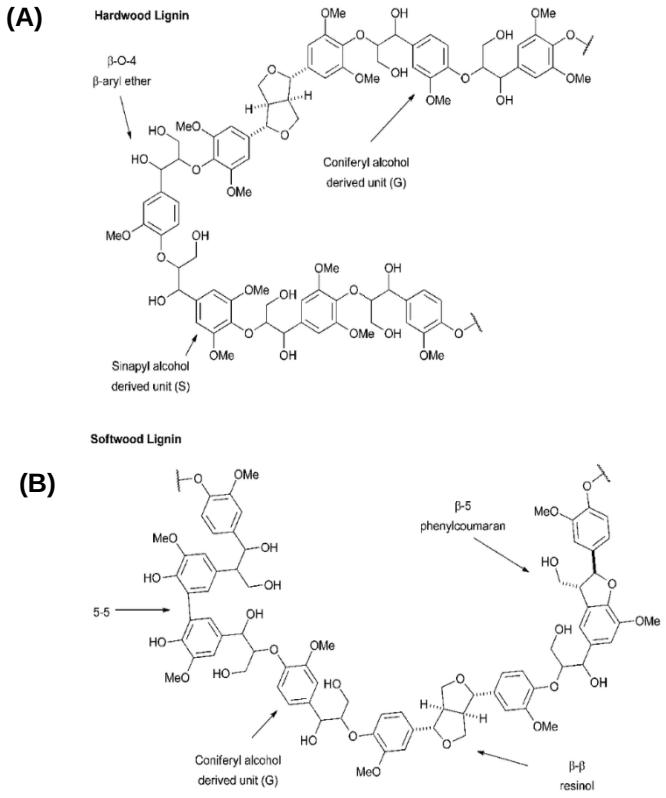

Figure 5. (A) Hardwood lignin structure; (B) Softwood lignin structure [Lancefield, 2015].

Both acid and alkaline depolymerisation of lignin will result in breaking of the ester bonds and some other bonds. However, the released fragments may react, and the result would be a rearrangement of the structure in a way that it would condense even more than it originally was. It leads to the fact that both different conditions and pre-treatments can result in different lignin. Lignin is constituted by three main phenylpropane units, with different substitution in the aromatic ring, syringyl (**S**), guaiacyl (**G**) and hydroxyphenyl (**H**) units (Figure 6).

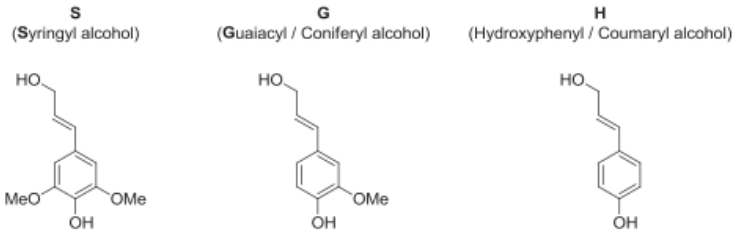

Figure 6. The three lignin strutures. Syringyl (S), Guaiacyl (G) and 4-hydroxyphenol (H) [Gosselink, 2011].

The main functional groups in lignin (see Figure 5) are hydroxyl (aromatic and aliphatic, OH), methoxyl (OMe), carbonyl (C=O), and carboxyl (C(=O)OH), that predominate in lignin according to the extraction method used. In its turn, the proportions of these lignin's functional groups affect its solubility, making it so that most lignins are quite soluble in alkaline solutions because of the ionisation of the hydroxyl and carboxyl groups [Gosselink, 2011].

Lignin is a versatile raw material that can be used in multiple applications which may be classified into three groups, according to its potential uses: power-fuel-syngas, macromolecules and aromatics. In the power-fuel-syngas group, lignin is either used as a source for energy production or converted into energy carries as syngas. The second group uses the macromolecular nature of lignin to convert it into wood adhesives, carbon fibres, and for polymers like polyurethane foams [Gandini and Belgacem 2008; Abe et al., 2010; Gosselink, 2011]. The last group of applications makes use of technologies to split the lignin structure into monomers without breaking the aromatic rings, producing aromatic monomers like benzene, toluene, xylene; phenol and vanillin.

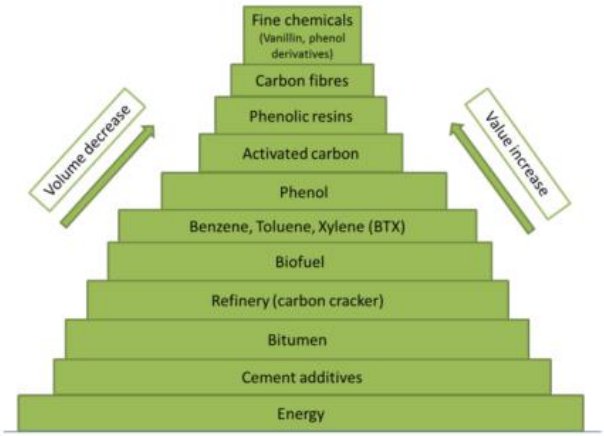

Figure 7. Potential lignin applications in relation to volume and added value [Gosselink, 2011].

Applications from the second and third groups may not be a bulk application, as syngas, however, even with lower volumes, they have the highest added value (Figure 7).

2.2.4. Other compounds

Lignocellulosic biomass contains some other substances though in smaller quantities (< 10%), in addition to macro components, substances this that can have influence in the properties and processing of the biomass. Compounds of low molecular weight can be divided into two groups, the organic (extractives) and the inorganic (ash), whose composition varies according to age, specie, and location as with other lignin compounds [Gírio et al., 2012]. As an example, non-woody materials inorganic compounds content is typically higher than in woods [Fengel & Wegener, 1984].

Extractives can be referred to as compounds that are primarily composed of cyclic hydrocarbons and present solubility in organic solvents or water. They are constituted by a large number of hydrophilic and lipophilic constituents as terpenoids, steroids, waxes, fats, phenolic constituents, amino acids and alkaloids. Most of these compounds play a defensive role against microbiological attacks as well as in cellular development and growth.

In relation to inorganic compounds such as potassium, magnesium and silicon, the quantities found in woods are generally lower than 1% whereas in cereals this value can vary from 10-20%. Such compounds account for ash content as its determination is done by the incineration of the material at 550ºC [Fengel & Wegener, 1984; Moniz et al., 2014; Silva-Fernandes et al., 2015b; Barbosa et al., 2020].

2.3. Agriculture and forestry residues

Even without considering native sources of lignocellulosic biomass, the amount of waste resulting from agricultural and forestry activities is considerable. These residues are generally seen as undesirable by-products, resulting in high costs in their treatment.

Instead of looking towards these large quantities of residues with prejudice, they should be faced as an opportunity, as they are a realistic option for the production of biofuels and other products with great potential and added value. Thus, the transformation of this type of waste, efficiently and in a context of biorefineries can bring several benefits. The bio-refining industries will have the capacity to develop, being able to stimulate the local economy and revitalise rural areas.

In this work, the selected materials were wheat straw and eucalyptus residues, so it is of importance to verify their quantity, availability and distribution in the world. Such data will have great relevance for the strategic planning of a biorefinery oriented towards the processing of more than one type of biomass, preventing this way unpredictable fluctuations in the supply of raw materials, especially from residues of agricultural origin, considering the seasonality of the products involved.

2.3.1. Wheat Straw

Wheat (Triticum spp.) is a cereal with high importance for the humanity over the millennia, being an excellent source of carbohydrates and protein of plant origin, having a protein content of about 13%, which is relatively high compared to other major cereals [CORDIS, 2017]. Worldwide, wheat is the second largest cereal crop, the first being rice and the third corn. Wheat is grown on more land area than any other food crop occupying 220.4 million hectares in 2018. In 2019, world production of wheat was 772 million tonnes, making it the second most-produced cereal after corn.

Global demand for wheat is increasing due to the unique viscoelastic and adhesive properties of gluten proteins that facilitate processed foods production, whose consumption is increasing due to the industrialisation process and modernisation of the diet [Shewry & Hey, 2015].

The top wheat producers in the world comprise China, India, USA, Russia and France, with China providing 45% of the world total wheat production in 2019 (Figure 8) [Food and agriculture organisation, 2019].

Wheat grows best in warmer climates, where temperatures range 21 °C to 24 °C. In addition, the wheat also needs considerable sunshine allied with areas with low humidity. Portugal has a Mediterranean type climate, which is essentially characterised by a very long, hot and dry summer with precipitation concentrated in the autumn and winter. Globally, the regions with this type of climate represent 10-15% of the total production of wheat [Almeida et al., 2016].

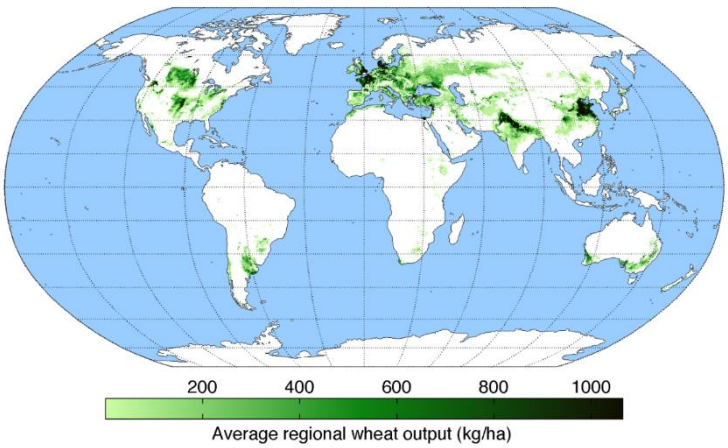

Figure 8. Wheat world distribution [FAO, 2014].

More than two-thirds of the wheat produced in the world are used for human food, generally in the form of flour to produce bread. About 17% of global production is used for livestock feed, although this depends on each country. Portugal still mainly uses wheat residues for that end. In the last decade, by-products from wheat processing, namely wheat straw, have also been used to produce biofuels and bio-based products. By 2018, wheat straw was the dominating feedstock for bioethanol production in Europe [Xu et al., 2019].

Straw is a term used for all harvestable residues after the wheat grain has been collected, including the major parts of the stem, leaves, and spikelets. Other than biofuels production, this residue is currently used in low-value applications, namely for feeding and bedding for livestock, as a vegetation cover to conserve soil moisture and increase its fertility, or in handicrafts [Carvalheiro et al., 2009]. In some regions, there was a process called open field burning in which wheat straw was burned in the field for energy production or most frequently for fast disposal purposes. As this arose, environmental and safety concerns such practices have been increasingly restricted, but still a reality in some countries like India [Xu et al., 2019].

However, wheat straw is a very interesting material to work with as in its composition presents a high amount of polysaccharides that have significative market value (Table 1).

Table 1. Wheat straw composition.

Compounds (%)	*Carvalheiro et al., (2009)*	*Khan et al., (2012)*	*Wildschut et al., (2013)*
Celullose	38.9	33.7-40	35.4
Hemicelullose	23.5	21-26	21.9
Xylan	18.1	-	19.8
Arabinan	3.0	-	2.1
Acetyl groups	2.4	-	-
Lignin	18.0	11-22.9	17.6
Ash	9.7	7-9.9	3.4
Protein	4.5	3.6	-
Others	5.5	4.5-5.5	10.2

2.3.2. Eucalyptus

The eucalyptus tree belongs to the Myrtaceae family and is native to Australia and Tasmania. There are more than 700 species that grow in tropical, subtropical and temperate regions. In its native region, eucalyptus represents 95% of the wooded areas, and it is, globally, one of the most used forest resources in the world [Fernández and Silva-Pando, 2016].

The eucalyptus is widespread in more than 90 countries, extending over more than 22 million ha around the world, of which 13 million are dedicated to industrial production. Among all eucalyptus species available, 37 are of interest to the forestry industry, with only 15 being used for commercial purposes [ENCE, 2009].

It is a species of short rotation, with an average rotation period of 4 to 6 years [Xu et al., 2020]. This short period is due to the high rate of growth and density in wood. In addition, it has high economic benefits associated with its composition mainly due to its high cellulose content and low amount of ash and extracts. (Table 2)

Table 2. Eucalyptus wood and eucalyptus residues composition.

Compounds (%)	*McIntosh et al., (2012[a])*	*Romaní et al., (2016[b])*	*Rigual et al., (2019[b])*	*Penín et al., (2020[c])*
Celullose	46.3	44.70	51.08	51.1-49.6
Hemicelullose	17.1	19.96	22.44	12.2-30.2
Xylan	16.6	16.01	18.14	-
Arabinan	0.5	1.09	0.18	-
Acetyl groups	-	2.96	4.12	-
Lignin	23	27.70	27.21	20.3-30.2
Ash	0.2	0.2	-	-
Extractives	2.4	2.0	-	-

[a]*Eucalyptus dunnii* wood [b]*Eucalyptus globulus* wood; [c] *Eucalyptus nitens* wood

In terms of relative abundance, eucalyptus accounts for 40, 8 and 10% of the forests of plantation in South America, East Asia and South Asia, respectively. It was only employed in plantations in Europe in the XIX century, arriving in Portugal in 1854 [Alves et al., 2007].

The Iberian Peninsula presents climate and soil conditions that are especially favourable for its development and production, mainly along the Portuguese coast (Figure 9).

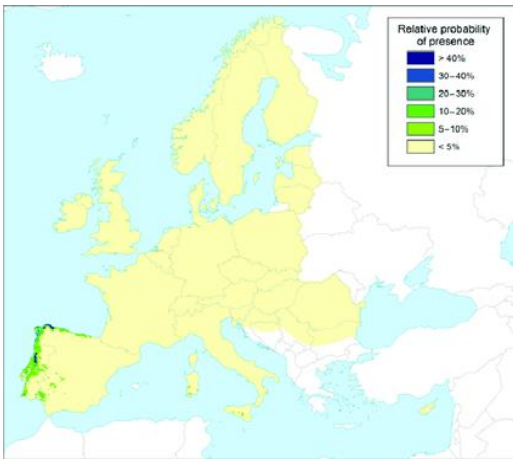

Figure 9. Distribution map of the genus Eucalyptus in the European Union territory from the European Atlas of Forest Tree Species [Jeger et al., 2018].

In Portugal, Eucalyptus occupies the second largest planted area, with 882 thousand hectares, corresponding 17% of this value (approximately 150 thousand hectares) to the exploitation of the pulp and paper industry. [CELPA, 2018]

Eucalyptus (Eucalyptus globulus Labill.) plays an important role in the context of Portuguese economic activity, not only because of the importance of the occupied area in national territory and the high profitability of its culture but also because of the macroeconomic significance of derived products, being the raw material of one of the leading industrial sectors of the country's economy, the pulp and paper industry, with a prominent participation in the external trade balance [Alves et al., 2007]. This industry alone, in Portugal, processed 7.9 million m3 of eucalyptus wood in 2018, with 78% (6.2 million m3) of this value coming from the national market. [CELPA, 2018] Apart from the pulp and paper

industry, eucalyptus wood also has other applications in the construction (boards, pallets, and beams), automobile (car filters) and textile industries.

The exploration and handling of this raw material generate high amounts of by-products. After a tree is cut, the bark, branches and stumps are mechanically removed. These by-products are usually referred to as residual forest biomass and are generally intended for the production of electricity although other applications are also possible. The wood is then cut and crushed into small pieces, producing eucalyptus chips (“estilha”), which is the feedstock for pulp and paper industry and residues (sawdust or “fines”). It is estimated that in an average pulp and paper industry, about 17% of the processed wood results in this type of waste. [CELBI, 2020]

Figure 10. Pile of eucalyptus residues.

2.4. Treatments for fractionation of lignocellulosic materials

In a biorefinery, biomass must first proceed to pre-treatments/fractionation processes which may include a set of physical, physico-chemical, chemical and/or biological processes.

The purpose of these pre-treatments is then to break the rigid structure of the biomass, in order to separate its fractions for later recovery, while minimising the amount of undesirable by-products, such as furfural and hydroxymethylfurfural (HMF), and formic acid and levulinic acid. However, separating a component without degradation the chemical structure of others or by-products formation can create limitations. To overcome it, a careful selection of the method and respective operating conditions is needed.

Several pre-treatments allow biomass deconstruction intending to remove and partially depolymerise hemicellulose, reduce the crystallinity of cellulose and lignin removal.

2.4.1. Delignification Processes

In delignification processes, the main focus is the fractionation and solubilisation of lignin in the LC materials. These processes were initially developed with the objective of enhancing the solid fraction rich in cellulose, with great relevance in the production of cellulose pulp.

These processes are increasingly relevant, especially in a biorefinery context as they promote a decrease in biomass resistance, promoting enzymatic digestibility and thus biofuels production. [Wildschut et al., 2013]. Delignification processes also have influence in lignin quality, so it is very important to adjust the method and its parameters to achieve the desired goals and mainly accomplish a selective fractionation as well as a minordegradation [Gosselink, 2011].

2.4.1.1. Alkaline treatment

Alkaline-based pre-treatment methods target both lignin and hemicellulose fractions, contrary to acid or hydrothermal processes. Depending on the catalyst used, the alkaline pre-treatments can be divided into two main groups, which are, treatments that use sodium, calcium, or potassium hydroxides and those that use ammonia. Besides the removal of hemicellulose and lignin fractions, the increase of cellulose digestibility is the main effect of alkali treatment. These treatments are either used as a primary treatment directly to the biomass or as a subsequent treatment to the acid hydrolysed biomass, in which the aim is to increase cellulose digestibility. Nevertheless, the use of alkali treatment before acid hydrolysis has also been studied.

The Kraft process which is the dominant process in the pulp and paper industry is the most used to separate lignin from the lignocellulosic biomass. In the pulping process via kraft method, aqueous sodium hydroxide along with sulphides are used at a temperature range of 160-180 ºC for 2-6 h to separate lignin from the cellulose. During this process, the lignin is dissolved from the biomass, resulting in a black liquor rich in phenolic compounds. Lignin is recovered from this liquor via acid precipitation techniques. The main advantages of this process lie in the low demands on wood species and wood quality, moderate cooking times and in a well-established processing of the spent liquor. Nevertheless, due to the emission of liquid and gaseous wastes such as sulphur-containing gas emissions, this process becomes a non-environmentally friendly one.

The prominent representatives of ammonia-based processes are the ammonia fibre explosion/expansion (AFEX) treatment and the Ammonia recycling percolation (ARP) processes. Even though both of them can dissolve hemicelluloses, AFEX seems to be efficient when it comes to herbaceous and agricultural residues, working moderately well on hardwoods [Gírio et al., 2010].

2.4.1.2. Sulphite Process

The sulfite process is a very common high yield delignification method, the first patent of which was registered in 1866. It is based on the introduction of sulfonated groups in lignin to make it soluble in water. The conventional process uses aqueous SO2 solutions, together with various sulfite salts, such as sodium, ammonium or magnesium sulfite, under pressure and at temperatures between 140ºC and 170ºC. Under acidic conditions, an extensive dissolution of hemicellulose is obtained, and thus a solid treated with a higher percentage of cellulose than that obtained in the kraft process, but at the expense of longer cooking times. Sulphite process has some advantages over Kraft process, such as, lower

consumption of chemicals, fewer pollution problems, lower installation capital costs, higher flexibility in pulp yields and grades. However, it has disadvantages associated with some limitations of the process, as it is a more suitable process for softwoods, it does not allow the use of biomass with a high resin content [Zinovyev, 2015].

2.4.1.3. Organosolv

Organosolv pre-treatments result in the use of aqueous solutions of organic solvents at temperatures typically in the range of 120-220 ºC. The use of, usually volatile, organic solvents, suggests that relatively high pressures are used in the operation, depending on the solvent volatility.

The solvents used in organosolv processes include aliphatic alcohols, such as ethanol, methanol and butanol; polyols like ethylene glycol and glycerol; ketones such acetone, butanone; organic acids as acetic and formic ones; dioxane; phenol, among others, all this with or without the addition of a catalyst. An advantage in using some of these solvents is that they can be produced from the pre-treated lignocellulosic materials in which they are applied, thus contributing to a more sustainable process. For catalysts, the most usual choices among inorganic and organic acids are hydrochloric and sulfuric acids, while sodium hydroxide, ammonia and lime are used when it comes to bases. The use of water and organic solvents in the reaction medium during organosolv pre-treatment makes possible the simultaneous removal of lignin and hemicelluloses [Zhang et al. 2016; Wildshut et al., 2013].

In an organosolv pre-treatment two fractions are obtained: a solid, enriched in cellulose, which contains some non-solubilised hemicelluloses and lignin; and a liquid fraction containing solubilised sugars, i.e., hemicelluloses in oligomeric and monomeric form as well as acid-soluble lignin, together with some degradation products, organic acids, extractives, and soluble ash. The pre-treated solid fraction is usually subjected to enzymatic hydrolysis and fermentation (that can either be carried out simultaneously or separated) to produce ethanol and other products such as butanol, glycerol, lactic acid, succinic acid or sorbitol. The liquid fraction is usually treated for solvent recovery. Using precipitation methods, by water or acid addition (usually sulphuric), the dissolved lignin can be separated and upgraded, originating high-quality lignin that can be used in several high-value applications.

Therefore, the solvent is separated from the aqueous solution that contains the hemicelluloses, which can be converted into products such as xylitol or furfural (Figure 11).

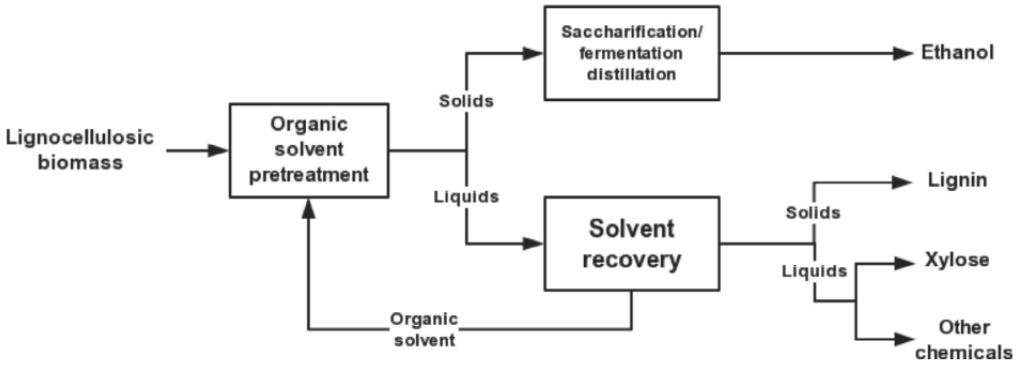

Figure 11. Flowsheet of a typical organosolv process. [Zhang et al., 2016]

The use of organic solvents in these processes improves solvent penetration and biomass dissolution, thus enhancing lignocellulose deconstruction. Product selectivity's, hydrogen transfer and reaction kinetics are also improved. Furthermore, the cost of solvent is relatively low compared to other pre-treatment solvents (e.g., ILs) as the large majority of the organic solvents used are bulk commodity chemicals. However, the overall cost of organosolv pre-treatments and corrosion issues are still factors to take into account when thinking on large-scale applications, as well as the fact that effective recovery and recycling strategies of the solvents have to be implemented. [Zhang et al., 2016]

Table 3 shows some examples of organosolv processes with different solvents, temperatures and residence times.

Table 3. Solvents and operational conditions used in organosolv treatments for different lignocellulosic materials.

Material	Solvent	Temp. (ºC)	Residence time (min)	Reference
Agro-industrial residues				
Wheat	0-50:80-100 acetone:water	160-220	0-120	Huijgen et al., 2010
Straw	60:60 ethanol:water	190	60	Wildschut et al.,
	50:50 ethanol water	190	120	2013
	50:50 ethanol water;15 mMH_2SO_4	190	60	
	65/35:85 acetic/formic acid:water	105	180	Snelders et al.,
				2014
	50:50 ethanol:water; H_2SO_4	140	120	Smit et al., 2017
Corn stover	70:30 methanol:water	160	60	Lee et al., 1987
	70:30 ethanol:water	160	60	
Switch	36.71:25.00:38.9	139.71	20	Cybulska et al.,
grass	Ethyl acetate:ethanol:water			2012
Prairie	36.87:25.49:37.64	140	20	
cordgrass	Ethyl acetate:ethanol:water			
Hardwoods				
Poplar	25:75:75:25 ethanol:water	155-205	26-94	Pan et al., 2006
	50:50 ethanol:water; H_2SO_4	140	120	Smit et al., 2017
Eucalyptus	60:40 ethanol:water	175-200	60-120	Romani et al., 2011
	56:44 glycerol:water	200	69	Romani et al., 2016
	50:80 ethanol:water	170-200	30-90	Romani et al., 2019
	90:10 acetic acid:water	130	180	Vila et al., 2003
Beech	50:50 methanol:water	160	45	Zhao et al., 2009
Softwoods				
Pine	95:5 acetic acid:water	115-130	0-180	Parajó et al., 1995
	60-80:20-40 methanol:water	170	45	Zhao et al., 2009
	50:50 ethanol:water; H_2SO_4	140	120	Smit et al., 2017
Spruce	50:50 ethanol:water; H_2SO_4	140	120	

2.4.1.3.1. Ethanol based Organosolv

Ethanol-based organosolv has been developed since 1931 and in 2001 Lignol Innovations built a pilot installation to produce pulp for paper, using this method, even though the process has been discontinued. It is a process that allows lignin and hemicelluloses removal while the cellulose fraction remains intact. The usual operation for ethanol-based organosolv conditions includes 50-80% ethanol in water solvent with or without catalysts, temperatures that range from 160 ºC to 220 ºC and 15-120 min of reaction times. Acid catalysts are more frequently used with concentrations ranging from 0 to

10%. Organic acids are typically used close to the upper limits whereas mineral acids and salts fall under the lower limits, with limits ranging from 0-2% [Zhang et al. 2016; Zhou et al., 2018].

Ethanol is the most used alcohol solvent for organosolv pre-treatment of lignocellulosic biomass. Many characteristics explain its extensive use, such as low cost, renewability, good lignin solubility, low toxicity, miscibility with water, and easy recovery.

In the organosolv process, ethanol can either be applied alone or with the addition of catalysts. For ethanol pre-treatment of lignocellulose, acid catalysts are preferred to ethanol-alone and base-catalysed ethanol organosolv, once they enhance glucan digestibility while granting lower operating temperatures and reaction times. Zhang et al. (2016) suggest that the improvement in glucan digestibility with the addition of an acid catalyst is not due to the delignification efficiency, but a result of the decrease in the cellulose polymerisation degree, reduction in the average fibre length, and the increasing porosity in the substrate of pre-treated biomass, which leads to increased accessibility of hydrolytic enzymes [Zhang et al. 2016]. Usually, the yields of enzymatic digestibility obtained are above 60% [Zhou et al., 2018].

In comparison with mineral acids (e.g., H_2SO_4 or HCl), the application of organic acids (e.g., formic or acetic acids) and inorganic salts (e.g., $FeCl_3$, $Fe_2(SO_4)_3$, $FeSO_4$, $AlCl_3$, $Al_2(SO_4)_3$, $MgSO_4$) are preferred as they reduce corrosion issues and degradation products formation, even though higher concentrations are needed. Organic acids, as they are products released during pre-treatment of LM, offer the added advantage of not representing an additional contaminant. However, they are applied in high concentrations they also need to be recycled. Furthermore, they can form esters with ethanol.

In ethanol organosolv, ethanol forms ethyl-xylosides and ethyl-glucosides, by reacting with solubilised sugars (xylose and glucose), protecting them from degradation into furfural and HMF, and additionally providing a new source of high-value chemicals. However, ethanol organosolv has a remarkably high demand of energy due to high temperatures and pressures of operation together with the energy costs associated with its recovery by distillation [Zhang et al., 2016; Zhao et al., 2017].

2.4.1.3.2. Organosolv applications: advantages and drawbacks

Applications of organosolv processes at industrial scale do not exist up to date; however, some pilot and laboratory scale ones have been implemented. The most notable are the Alcell and Lignol processes, which are based upon autocatalytic (i.e., without the addition of a catalyst) ethanol delignification of wood. In 2009 in Burnaby (British Columbia, Canada) a pilot plant was implemented that generates 30 ton/year of cellulosic ethanol and high-purity lignin from 1 ton/day of a mixture of hard- and softwood. The working conditions of the plant were: Temperatures of 180-195 ºC with an ethanol concentration of 35%–70% (w/w) with LSR of 4:1 to 10:1 (w/w) for about 30-90 min [Zhou et al., 2018].

Another pilot plant based on organosolv process started to operate in 2012 under the “German Lignocellulose Feedstock Biorefinery Project” at the Fraunhofer CBP at the chemical site of Leuna, Germany. This plant treats about 50 t/h of dry wood chips with an ethanol/water mixture of 1:1 at 180-

200 ºC and 18-36 bar. The LSR used is of 4:1 using sulphuric acid as a catalyst, in concentrations of <1% (based on dry wood) [Laure et al., 2014].

Organosolv processes present a superior performance in comparison with other pre-treatment processes for hemicellulose and lignin solubilisation. One of the main advantages of these processes is that the lignin produced is usually relatively pure, with low sulphur and less condensed in comparison with other pre-treatment technologies, thus allowing it to be used for other added-value products. They also present a significative impact in cellulose accessibility and crystallinity, biomass particle size and porosity while maintaining the cellulose fraction (Figure 12).

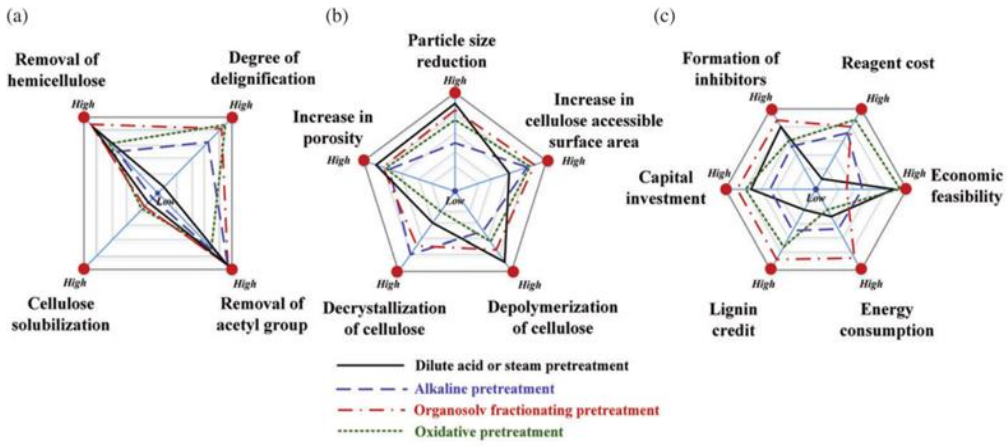

Figure 12. Comparison of organosolv pre-treatment with other pre-treatments processes. (a) removal of lignocellulose constituents; (b) impact on lignocellulosic structure; (c) relevant economical aspects [Zhang et al., 2016].

However, this kind of pre-treatments also faces some drawbacks that prevent them from being applied on a larger scale. In organosolv processes, pre-treated substrates must be directly washed with organic solvents to avoid re-precipitation of dissolved lignin, which at an industrial scale results in cumbersome washing configurations. Another disadvantage lies in the fact that most organic solvents can inhibit processes as the enzymatic hydrolysis and subsequently fermentation, this is mainly due to the high temperatures used in the organosolv process that may led to the considerable formation of inhibitors.

The volatility and flammability of organic solvents constitute hazards to the environment and human health as well as an increase in the capital and operational costs due to the high pressures required. Furthermore, high energy consumption for solvent recovery is needed, as some solvents in addition to being expensive are also toxic to some microorganisms and enzymes [Zhao et al., 2017].

Nonetheless, ever since their initial development in the pulp and paper industry, organosolv pre-treatments have been intensively recognised as very promising pre-treatment technologies.

2.4.2. Hydrothermal Treatments

Non-catalytic processes such as hydrothermal treatments are the simpler and main process options for hemicellulose fractionating which mainly include autohydrolysis and steam explosion. These processes are both economic and environmentally attractive since there is no addition of chemical catalysts.

2.4.2.1. Autohydrolysis

Autohydrolysis, also called Liquid Hot Water (LHW), uses compressed liquid hot water, at temperatures ranging from 150 ºC to 230 ºC, for a reaction time that may vary from seconds up to hours. The liquid-to-solid (LSR) ratio, also known as solids concentration, usually ranges between 2 and 100 (w/w); however, the most common values vary from 7 to 10 (w/w). This ratio depends on the type of reactor used since continuous reactors are associated with higher LSR values, whereas lower LSRs are commonly used in processes employing steam. [Carvalheiro et al. 2004b, Carvalheiro et al. 2009, Gírio et al., 2010]

This process has been successfully used in several LM such as hardwoods (e.g., *Eucalyptus globulus*), energetic cultures (Miscanthus, Arundo donax) and agriculture and agro-industrial materials (e.g., corn residues, wheat straw and rice husk). A high hemicellulose recovery is generally achieved from this process, ranging from 55-84% as well as low levels of inhibitory by-products. Cellulose and lignin are not significantly affected, resulting in cellulose- and lignin-enriched solid phase. [Gírio and al., 2010]

A possible disadvantage of autohydrolysis is that dissolved pentoses mainly appear in the oligomeric form, which requires another post hydrolysis step when the aim is to obtain monomeric sugars. However, this can be advantageous when oligomeric sugars, i.e., xylo-oligosaccharides (XOS) are aimed. These compounds are known as emerging food additives with prebiotic properties and are obtained as a high added-value product from the hemicelluloses.

The gains of these processes are the high selectivity towards hemicelluloses and more economical and environmentally friendly compared with other hydrolytic technologies. However, the biomass delignification efficiency is low in these processes, so a subsequent process is needed to further promote solid phase fractionation. [Moniz et al., 2018]

2.4.2.2. Steam explosion

Steam explosion uses saturated water vapour for heating, enabling an increase of heat transfer rate. This pre-treatment subjects the lignocellulosic material at high pressures (0.69-4-83 MPa) and temperatures (160-240 ºC) for a short reaction time. After completing the requested operation, the material goes through a sudden decompression causing vaporisation of the water entrapped in the fibres, resulting in an explosion. The forces from decompression cause a breakdown of the

lignocellulosic matrix, in which inter and intramolecular bonds are broken. Due to this decompression, ultrastructural changes also occur, which include depolymerisation and considerable degradation of the material fibres. [Carvalheiro et al., 2008]

The effectiveness of steam explosion has already been proved for many LM, being this technique even implemented at pilot and industrial scales. As compared to autohydrolysis, the monosaccharides yield in steam explosion is usually higher and the process is known to produce a significant increase of enzymatic digestibility. The main disadvantage of steam explosion as is that degradation reactions are in general, more relevant. However, the steam explosion is considered to be an environmentally friendly process. [Gírio et al., 2010]

2.4.3. Acid hydrolysis

Acid hydrolysis processes can be divided into two approaches: concentrate-acid/low temperature and dilute-acid/high temperature hydrolysis. The most common acid applied in these processes is sulfuric acid, although others acid such as hydrochloride, nitric and trifluoracetic have also been used in previous works.

Concentrate-acid processes hydrolyse both cellulose and hemicelluloses, using different acid concentration, such as 72% H_2SO_4, 41% HCl or 100% TFA, the last two having the advantage of being easily recovered. This type of hydrolysis has the advantage of operating at moderate to low temperatures, which allows reductions in the operational costs. Temperature is a crucial factor in this process as only slight changes can affect the formation rate of degradation products, even though this formation can be low under appropriate conditions [Gírio et al., 2010]. For the concentrate acid hydrolysis to be economically viable, a process for acid recycling is mandatory. Moreover, the equipment corrosion is an additional drawback.

Dilute-acid hydrolysis processes are more oriented towards hemicelluloses, leaving cellulose un-hydrolysed so that it can go through further enzymatic hydrolysis processes. The most common acid used in this kind of hydrolysis is sulfuric acid in the ranges of 0.5-1.5% (w/w) with temperatures between 121-160 ºC, although HCl, HNO_3 and H_3PO_4 can also be used [Mosier et al., 2005]. Sugar yields recovery from hemicellulose usually are above 70% up to >95% for this of processes. However, a main disadvantage of dilute acid hydrolysis is the degradation of sugars during hydrolysis reactions as well as the formation of undesirable by-products that lower sugar yields and inhibit fermentation processes. Still this disadvantage can be overcome by carry out the dilute acid process in two or more stages. [Taherzadeh et al., 2007]

2.4.4. Ionic liquids

Ionic liquids (ILs) are organic salts formed of cations and anions with a melting point lower than 100ºC. The important parameters affecting this process and defining the interactions between ILs and lignocellulosic materials are cations, anions, temperature, and time. ILs containing chloride, formate,

acetate or alkylphosphonate inions can dissolve cellulose by creating strong hydrogen bonds. [Gírio et al., 2010; Negi et al., 2015]

ILs can be seen as green solvents in comparison to conventional organic solvents due to their excellent recyclability, low volatility, high thermal stability, less toxic formation, and nonflammability. Also, the low vapour pressures make them highly recoverable, reducing exposer risks; making them good substitutes for traditional flammable and volatile solvents. [Negi et al., 2015]

This kind of method has an advantage in LM as it promotes the rapid dissolution at mild temperatures, however not at a complete level. Although ILs as a pre-treatment of biomass are still a fairly new application for this kind of method, requiring further studies. [Gírio et al., 2010; Negi et al., 2015]

2.4.5. Enzymatic hydrolysis

Enzymatic hydrolysis can be described as a process where cellulases are added to the previously treated lignocellulosic biomass releasing fermentable sugars. This process involves several core steps in the following order: transfer of enzymes from the bulk aqueous phase to the surface of the cellulose; adsorption of the enzymes and formation of enzyme-substrate complexes, cellulose hydrolysis; transfer, from the cellulosic particles surface to the bulk aqueous phase, of the hydrolysis products and finally the hydrolysis of cellodextrins and cellobiose to glucose in the aqueous phase [Fan et al., 2014].

The cellulase complex is composed of endo-glucanase, exo-glucanase and β-glucosidase; the first two hydrolyse the internal β-1,4 bonds of cellulose chains, giving rise to a disaccharide, which in turn is hydrolysed to individual glucose units by β-glucosidase [Béguin and Aubert, 1994]. The addition of xylanases during cellulose hydrolysis has the purpose of depolymerizing the hemicellulose in its monomeric pentoses, and thus increase the rate of efficiency of cellulose hydrolysis.

The rate of enzymatic hydrolysis is, however, highly influenced by the structural aspects of the substrate, including the degree and pattern of substitution as well as its polymerisation degree. Furthermore, phenolic compounds, usually present in hydrolysates, can reduce the enzymatic activity as they are particularly toxic and easily form cross-linkage with proteins, inactivating them making this process less competitive and imposing further constraints to the remotion of hemicellulose and lignin [Gírio et al. 2010; Fan et al., 2014].

However, the milder operation conditions of temperature and pH contribute as an advantage to enzymatic hydrolysis processes over acid ones. These milder conditions lead to reaction media free of further sugar degradation compounds, which cause limitations on the microbial performance, providing high saccharification yields, that also depend on the conversion rate and the reactors configuration. Allied to this, there are potential economic advantages associated with energy savings and equipment cost [Gírio et al., 2010].

3. Materials and methods

3.1. Feedstock and storage

Two lignocellulosic materials were used as feedstocks in this work: wheat straw (WS) and wood residues from *Eucalyptus globulus* (ER). The WS residues were purchased in Germany and kindly provided by ECN-TNO (Netherlands). This material was previously milled to particles < 4 mm. The eucalyptus residues (ER), a by-product from the production of wood chips, were kindly provided by The Navigator Company (Cacia, Portugal). This feedstock was not milled, and it was used "as it is".

Both feedstocks were stored in card boxes at room temperature until use.

3.1.1. Particle size characterisation

Upon arrival, a granulometric characterisation of the raw materials was performed using a sieve shaker (Endecotts, England) with seven sieves (Retsch, Germany) of different pore sizes (0.25, 0.355, 0.5,1, 2, 4 and 6 mm). The raw materials (ca. 100 g) were screened in triplicate, for 30 min and the material retained on each sieve was weighed for the determination of the respective mass fraction.

3.2. Investigation strategy

The research strategy followed in this work is shown in Figure 13.

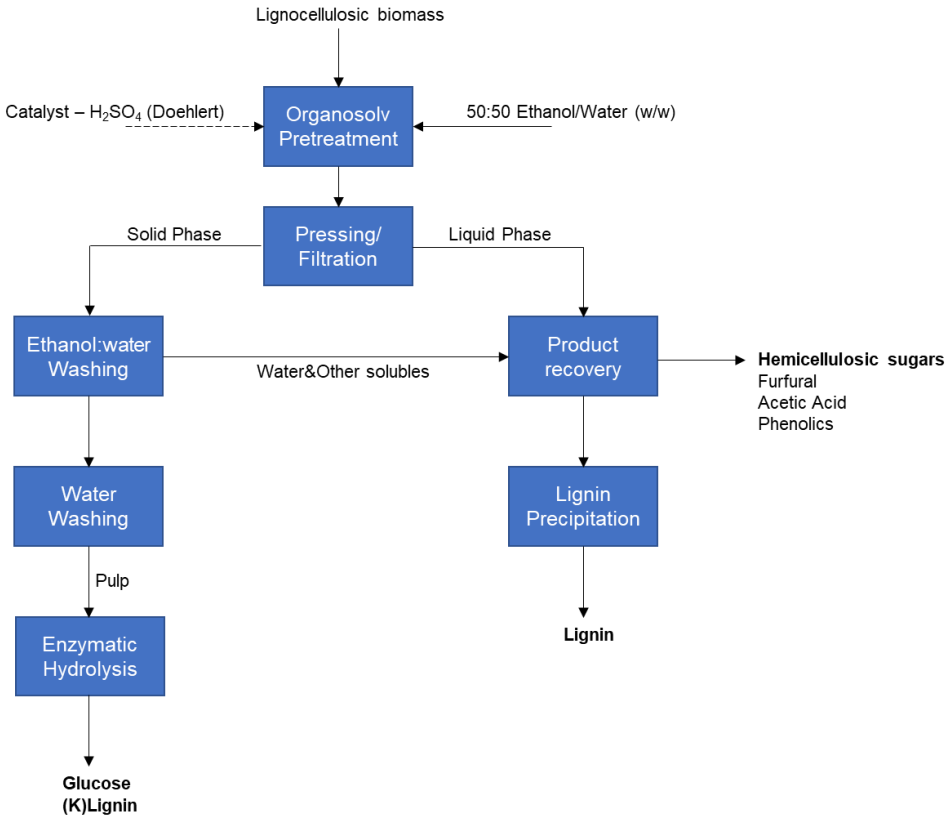

Figure 13. Investigation strategy developed in the present work.

3.3. Organosolv fractionation

3.3.1. Wheat straw and eucalyptus residues fractionation under non-catalysed conditions

Organosolv experiments were carried out at laboratory scale (600 mL) stirred Parr reactor (Parr, USA) using ethanol/water mixtures of 50% (w/w). The amount of biomass used in each treatment (30 g, dry weight) was that needed to reach a liquid-to-solid ratio (LSR) of 10:1. For the first trials (no-catalyst added) and for both feedstocks, the temperature was set at 190ºC and the reaction time ranged from 0-120 min.

After reaction time was over, the reactor is rapidly cooled down by water circulating through a serpentine coil, and the solid and liquid phases were separated by pressing using a manual press (Sotel, Portugal). The resulting liquid was weighted, filtrated under vacuum (Whatman filter paper no. 1), weighted again and stored at 4 ºC for future analysis.

The solid phase was washed twice with the amount of the ethanol/water solution used in the organosolv process, followed by the double amount of water at 70 ºC. After, the solid residue was dried at 45 ºC for at least 48 h before chemical characterisation.

3.3.2. Organosolv fractionation of eucalyptus residues under catalysed conditions - Doehlert statistical experimental design

For ER, ten assays, including four replicates at the centre of the experimental domain, were performed according to a Doehlert statistical distribution for two factors (Doehlert, 1970).

The temperature was set at 140ºC and sulfuric acid concentration (X_1) and time (X_2) were the two factors studied in an experimental domain that ranged from 0-50 mM and 0-180 min, respectively. Table 4 shows the number and conditions in which the assays were carried out.

Table 4. Factors' values in coded units and reals units for the statistical planning assays.

Experiment	Coded units		Real Units	
	H_2SO_4 (X_1)	Time (X_2)	H_2SO_4 (mM) (U_1)	Time (min) (U_2)
1	0	0	25	90
2	1	0	50	90
3	-1	0	0	90
4	0.5	0.866	37.5	167.94
5	-0.5	-0.866	12.5	12.06
6	0.5	-0.866	37.5	12.06
7	-0.5	0.866	12.5	167.94
8	0	0	25	90
9	0	0	25	90
10	0	0	25	90

The numbers +1 and -1 each represent the upper and lower bounds, respectively, while 0 relates to the central of the experimental domain. The distance between each bound and 0 is known as the planning unit.

The values of both factors were calculated according to equation 1.

$$U_i = X_i \,.\, \Delta U_i + U_i^o \qquad (1)$$

In which,

U_i – factor's real units.

X_i – factor's coded units.

ΔU_i – planning unit, in real units.

U_i^o - centre of the experimental domain, in real units.

The organosolv process occurred as described in Section 3.3.1. The model used to express the responses was a second order polynomial equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \qquad (2)$$

β_0 is the regression coefficient at centre point; β_1 and β_2 are the linear coefficients of the variables 1 and 2, respectively; β_{12} is the second-order interaction coefficient between variables 1 and 2; and β_{11} and β_{22} are the quadratic coefficients for variables 1 and 2.

The linear multiple regression to equation 2 and its analysis of variance (ANOVA) were carried out using Microsoft Excel® 2018 Regression tool pack, using all replicates. The best hydrolysis

conditions were determined by using the Microsoft Excel® 2018 Solver tool based on the best-fit equations using a constrained model.

3.4. Analytical methods

3.4.1. Determination of moisture content

The moisture content was determined by oven-drying at 105 ºC, using nickel capsules (dried at 105 ºC for 16 h). The samples were weighted on an analytical balance (Mettler Toledo AG204), in duplicate, and placed in the oven at 105 ºC for 16 h. Afterwards, they were cooled in a desiccator and then weighted again.

Whenever required, moisture content can also be determined by a faster method using an infrared moisture analyser (AMB-50, Germany). Calculations were made according to annexe A.

3.4.2. Determination of ash content

The ash content was determined by incineration at 550 ºC according to NREL/TP-510-42622 protocol (Sluiter et al., 2008b). The samples were weighed, in duplicate, in porcelain crucibles. The crucibles had been previously tared in a muffle furnace at 550 ºC for at least 6 h and then cooled in a desiccator.

The samples were then oven-dried at 105 ºC for 16 h (if the moisture content is also determined) and then burned in a heating plate[1] before being transferred to the muffle furnace, where they were kept overnight at 550 ºC. Finally, the samples were again cooled in a desiccator and weighted. The difference between the final weight of the crucible and its tare gives the value of ash content as it is described in annexe A.

3.4.3. Determination of liquors dry weight

For dry weight determination, 5 g of liquor sample was used in a similar procedure of that described in 3.4.1. for moisture content determination. This determination was carried out in duplicate.

3.4.4. Determination of extractives content

To determine the extractive content, the samples were subsequently extracted with 190 mL of dichloromethane, ethanol and water based on the methods T 12 os-75 method (Gominho et al., 2009) and NREL TP-510-42619 protocol (Sluiter et al., 2008). The samples, 2 g of biomass, were placed on previously weighted (dried at 100 ºC for at least 18 h) extraction thimbles and weighed on an analytical balance. Afterwards, the samples were subsequently extracted with 190 mL of dichloromethane (6 h), ethanol (18 h) and water (18 h), with refluxes of four to five cycles per hour. Finally, after the extractions,

[1] This was made to ensure no ashes would fly and contaminated other samples while being inside the muffle.

the solvent was evaporated in a rotatory evaporator, and then the flasks were placed in an oven at 100 ºC until a constant weight was achieved. Calculations can be found in annexe A.

3.4.5. Determination of protein content

Protein content was estimated by the Kjeldahl method (AOAC, 1975) using the N x 6.25 conversion factor. For protein determination, 0.5 g of biomass were placed in digestion tubes, in which 20 mL concentrated Sulfuric acid of and 10 g of the catalyst mixture (composition w/w: 93% potassium sulphate, 3% copper sulphate, 3% titanium oxide and 1% stearic acid) were added. Afterwards, the tubes were placed in a Tecator (Höganäs, Sweden) Digestion System 6 Model 1007 unit, equipped with a fumes extraction system. The temperature was raised to 420 ºC after 30 min to be after kept for 1.5 h. After a previous cooling 100 ml of water were added and the tubes were placed in a Tecator (Höganäs, Sweden) Kjeltec System Model 1026 distillation unit, that automatically added 75 mL of 50% (w/v) of NaOH. Steam distillation was used to distil the released ammonia, which was collected using 4% (w/v) boric acid (50 mL). Quantification of ammonia was determined by acid-base titration using, as titrant, 0.1 N hydrochloric acid and Tashiro's indicator, with an end point at pH 4.8. For the preparation of this indicator, 2 g of methyl red and 1 g of methylene blue were mixed with ethanol to achieve a final volume of 100 mL. Together with the samples, blank assays were also performed.

3.4.6. Quantitative Acid Hydrolysis

The feedstocks and pre-treated solids obtained from organosolv treatments were subjected to a quantitative acid hydrolysis according to a method based on NREL/TP-510-42618 protocol (Sluiter et al., 2011) in order to determine polysaccharides content (glucan, xylan, arabinan), acetyl groups and Klason lignin.

This method consists in the addition 5 ml of sulphuric acid (72% w/w) to 0.5 g of a solid sample. Afterwards, the test tubes were immersed in a Memmert (Schwabach, Germany) W350 water bath for 1 h at 30 ºC, with occasional manual stirring. Next, the content of the tubes was transferred to 250 ml Schott flasks and water was added in order to reach an H_2SO_4 concentration of 4% (w/w). The samples were then autoclaved at 121 ºC for 1 h. After the reaction time was over, the flasks were cooled down, and the mixture was filtered through) sintered glass crucibles (#3 porosity) previously incinerated in a muffle furnace. Klason lignin corresponds to the solid remaining in the crucibles, which was washed with 100 mL of distilled water and dried in an oven at 100ºC until constant weight and then burned in a muffle furnace for correction for ash content. A sample of the liquid phase obtained was analysed by HPLC as described in 3.4.8 for quantification of sugars, acetyl groups and acid-soluble lignin. The procedure was performed in duplicate for each sample. Calculations can be found in annexe A.

3.4.7. Post-Hydrolysis of liquors

The post-hydrolysis of liquors is an indirect method for oligosaccharides (OS) quantification, which was performed according to NREL/TP-510-42623 protocol (Sluiter et al., 2012). This method consists in the addition of concentrated sulphuric acid (72% (w/w)) to the liquors from the organosolv treatments,

in order to achieve a final H_2SO_4 concentration of 4% (w/w). The mixture was placed in an autoclave for hydrolysis (60 min at 121ºC). After the autoclave cycle was over, the hydrolysates were cooled down to room temperature. Afterwards, a sample was taken and filtered through 0.22 µm membranes (Millipore®) and analysed by HPLC (section 3.4.8). This method allows the quantification of the oligosaccharides (OS) concentration present in the samples, which were calculated considering the increase in sugar monomers in relation to the initial values. All assays were conducted in duplicate. Calculations can be found in annexe A.

3.4.8. HPLC analysis- High Performance Liquid Chromatography

The resulting hydrolysates from the organosolv treatments, ethanol/water washing solutions as well as the hydrolysates obtained from quantitative acid hydrolysis and the enzymatic digests were directed analysed by HPLC (Agilent 1100 Series, Waldbronn, Germany), after being filtered through 0.22 µm syringe filters (Red® analytical). In some cases, the hydrolysates containing high concentrations of lignin were previously filtered through Whatman GD/X, 0.45 µm RC w/GM (Whatman, USA) before filtration through 0.22 µm syringe filters.

For the quantification of sugars (glucose, xylose and arabinose), aliphatic acids (acetic, formic and levulinic acids) and furan derivatives (furfural and 5-hydroxymethylfurfural, HMF); an Aminex HPX-87H column (Bio-Rad, Hercules, USA) was used to perform the analysis. Table 5 shows the operation conditions regarding injection volume, flow rate, temperature, mobile phase and detection for the analysis of solid derived hydrolysates. The column flow rate was 0.4 ml/min while for the liquors, a flow rate of 0.6 ml/min was used. The aliphatic acids and monosaccharides were detected by refractive index while furan derivatives were detected using the UV/vis photodiode detector.

Table 5. Properties and operational conditions of HPLC analysis.

Characteristics	Operational conditions	
	Liquids[1]	**Solids[2]**
Column dimensions	300 x7.8 mm	300 x7.8 mm
Mobile phase	H_2SO_4 5 mM	H_2SO_4 5 mM
Flow	0.6 mL/min	0.4 mL/min
Injection volume	5 µL	20 µL
Column temperature	50ºC	50ºC
IR detector temperature	50ºC	50ºC
UV wavelength	280 nm	-

[1]organosolv hydrolysates and washing solutions; [2]hydrolysates from quantitative acid hydrolysis and enzymatic hydrolysis

The concentrations of the target compounds were calculated considering the calibration curve plotted from the standard solutions. Calculations can be found in annexe A.

3.4.9. Determination of total phenolics content

The Folin-Ciocalteu colorimetric method (Singleton et al., 1999, adapted from Roseiro et al., 2013) was followed for the analysis of total phenolics in eucalyptus and wheat straw organosolv hydrolysates and ethanol/water washing solutions. The diluted extract samples (1:100 dilution at least) and water for blank (all 100 µL) were mixed with 1/10 (v/v) diluted Folin–Ciocalteu reagent (5 mL) and 20% Na_2CO_3 (1250 µL).

Absorbance was measured at 725 nm in a microplate spectrophotometer (Thermo Scientific Multiskan GO UV/Vis), after a 40 min resting in the dark. The results are expressed as Gallic Acid Equivalents (GAE)/mL by comparison to a gallic acid calibration curve. This procedure was performed in triplicate.

3.4.10. Lignin precipitation

Soluble lignin contained in organosolv hydrolysates was recovered by precipitation with water or dilute sulfuric acid. In order to decide about the precipitation method, a first study was carried out, where sulfuric acid (96%) was added as a precipitation agent. As an alternative, a preliminary study of dilution of organosolv hydrolysates with different water volumes (2:1, 3:1, 4:1, 5:1) was also carried out, where water attained better results compared with sulfuric acid.

Water was added to each falcon tube, so that was achieved 6.67 g of liquor to 26.6 g of water (dilution 1:4). This procedure was performed in triplicate, and the tubes were incubated for about 1 or 2 h at 30 ºC and 180 rpm. After incubation time was over, the samples were centrifuged (Digicen 21R ortoalresa) for 20 min at 6000 rpm. After separation of the supernatant, the precipitate was oven-dried at 45 ºC for at least 48 h weighted in order to calculate the amount of lignin.

3.4.11. Enzymatic hydrolysis

The enzymatic hydrolysis method was carried out in duplicate and performed in both pre-treated solids and raw materials. To initiate the procedure, 0.5 g of biomass were weighted (in 40 ml capped plastic tubes). Water was added followed by a solution of 5 mL of a sodium citrate buffer solution (pH 4.8) and 100 µL solution of sodium azide to adjust the volume to 10 mL, considering that the biomass density was 1. Samples were autoclaved at 120 ºC for 10 min and after cooling, 10% of a commercial enzyme complex, Cellic® CTec2 (g enzyme/100 g cellulose) (activity 199.9 FPU/ml), kindly provided by Novozymes (Denmark) was added.

Two blank assays were also prepared in each series of tests. In one blank, to assess the presence of glucose oligomers, no enzyme was added to the substrate, while on the other blank the enzyme was added without a substrate to determine the amount of glucose present in the enzyme.

Samples were incubated (180 rpm) at 50 ºC for 72 h. Once the incubation period was over, the enzyme was deactivated by incubation in a water bath at 100 ºC for 10 min. Finally, the samples were filtered under vacuum conditions through Millipore® membranes (0.45 µm) followed by 0.22 µm syringe filters before HPLC analysis. Saccharification yield was calculated as described in annexe A.

3.5. Process simulation and analysis

Organosolv pre-treatment was modelled and simulated in Aspen Plus®. Here, it is analysed the pre-treatment process of Eucalyptus residues with non-catalytic organosolv at 190 ºC and 120 min (isothermal period). The flowsheet topology here adopted is similar to other reports in the literature (Kautto et al., 2013) and is briefly described in Section 4.5. It follows the modelling.

3.5.1. Process overview

Organosolv pulping process can be generally summarized as follows: biomass cooking; pulp washing; solvent recovery; lignin recovery and finishing; and hemicelluloses conditioning. The process configuration is mostly dependent on the solvent used, as shown in Figure 14.

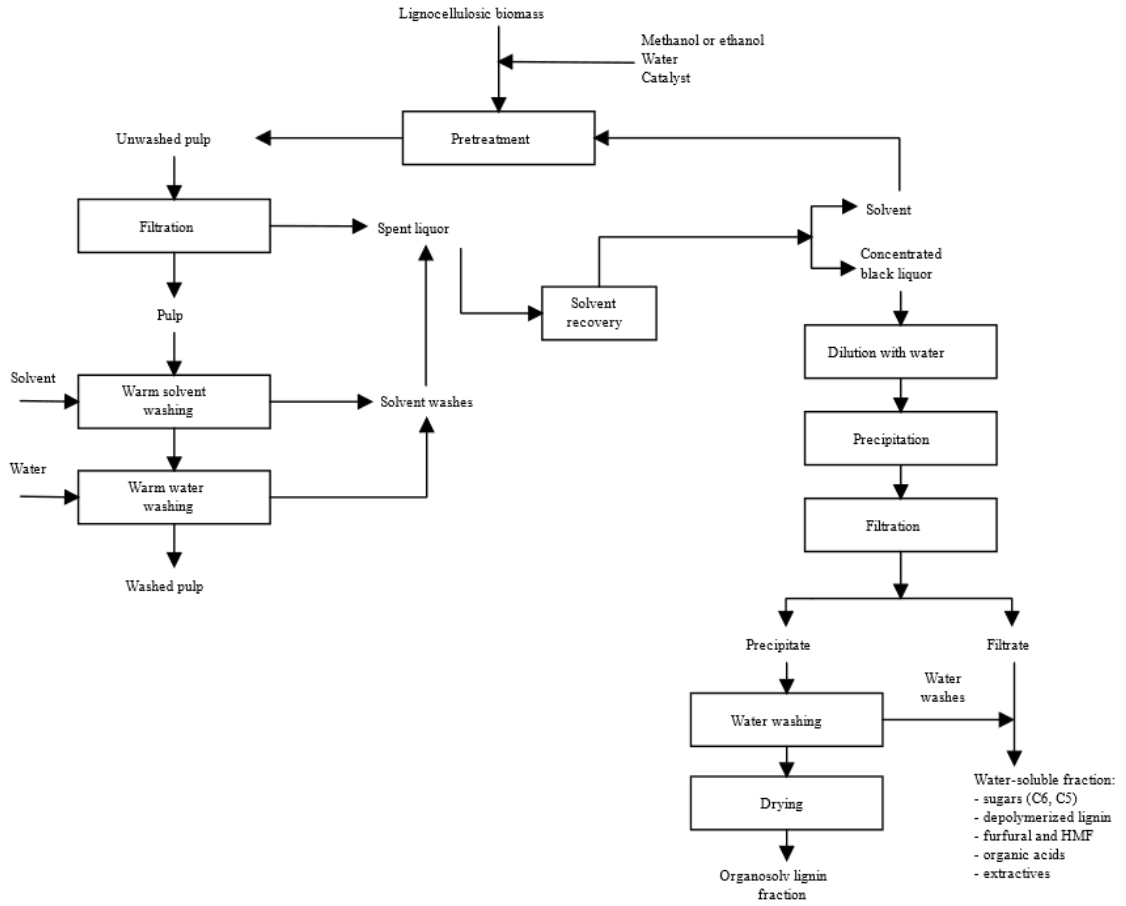

Figure 14. Block diagram of organosolv pretreatment with a low boiling point solvent (methanol/ethanol) Adapted from Zhao et al. (2009).

The unwashed pulp is subjected to a two- up to four-stage washing sequence, where the concentration of organic solvent and temperature are controlled to minimize the lignin reprecipitation. In ethanol organosolv process, temperatures above 60 ºC and concentrations above 50% (v/v) are used to improve lignin solubility in the liquor and increase diffusion rate, thus avoiding a significant reprecipitation of lignin onto the cellulose fibres [Ni et al., 1996; Xu et al., 2007]. The presence of lignin in pulp affects the saccharification negatively, since cellulases bind non-productively to lignin, reducing the area of active sites. As mitigation measures and to enhance enzymatic hydrolysis, non-saccharifying proteins can be added to bind to lignin instead of cellulases [Zhang et al., 2016], or a warm and dilute alkaline aqueous solution can be added to reduce further the lignin content [Kleinert, 1971]. Therefore, organosolv pulp washing arrangement typically includes a first stage with fresh cooking liquor to wash the pulp, followed by a second stage where warm water is used to displace the solvent. Afterwards, pulp consistency is adjusted before saccharification [Kautto et al., 2013].

Cellulose and xylans (partially) are subjected to enzymatic hydrolysis in saccharification, producing the corresponding glucose and xyloses which at large scale are conducted in several parallel bioreactors. Cellulases may also be prepared on-site using enzyme-producing fungus grown aerobically in fed-batch bioreactors [Humbird et al., 2011]. Following the saccharification, cellulases are recovered using filtration or centrifugation and the aqueous solution is concentrated to form syrups.

When a low boiling point solvent is used in organosolv pulping, a multi-effect flashing or distillation of the black liquor is carried out to recycle the cooking liquor condensates for re-use in pulping [Klenart, 2018]. Optionally, a post-hydrolysis step may be added to extend the hydrolysis of sugar oligomers, where an aqueous acid solution (e.g., sulfuric acid) can be added and the reaction runs at temperatures lower than the pulping [Kautto et al., 2013]. However, this step depends on the final products desired since higher amounts of acetic acid and furfural will be obtained. General methods for lignin recovery appear described in the literature include dilution of black liquor with aqueous streams with or without acid addition and the direct evaporation of ethanol from the black liquor. A process setup usually includes a combination of these methods [Kleinert, 1971; Kautto et al., 2013]. Besides, some processes involve membrane separation techniques [Alriols et al., 2010; Wenwurn et al., 2016], liquid-liquid extraction process [Lê et al., 2018; Bozell et al., 2011], and continuous lignin precipitation and agglomeration system consisting of a forced-circulation reactor connected to a falling film evaporator and a rectification column [Schulze et al., 2019]. Lignin isolation is achieved either with filtration or membrane ultrafiltration [Alriols et al., 2010] apparatus followed by lignin drying to tune the final humidity. The hemicelluloses stream is concentrated up to the required specifications, either recurring to a distillation system [Kautto et al., 2013; Alriols et al., 2010] or a membrane-assisted vapour stripping configuration [Vane et al., 2008].

3.5.2. Process modelling and assumptions

The lignocellulosic biomass composition assumed is the one presented in section 4.2. The fractions of lignin resting in the pulp and dissolved in the liquor were defined respectively as solid and liquid

species with chemical structures similar to vanillin. Water-soluble hexoses (e.g., glucose, galactose, and mannose) were modelled as glucose, while pentoses (e.g., xylose, arabinose) were represented by the xylose molecule. Six-carbon and five-carbon monomeric units of cellulose and hemicelluloses (e.g., glucan, xylan, arabinan) and respective oligomers were modelled with solid compounds with chemical formulas $C_6H_{10}O_5$ and $C_6H_8O_4$, respectively. In contrast, other compounds required in the simulation such as acetic acid, water, ethanol, were already present in Aspen Plus® databanks. Pure thermophysical properties and binary interaction parameters were taken from NREL databank for mixtures, while built-in databanks were employed for the remaining compounds [Wooley et al., 1996]. The non-random two-liquid model (NRTL) and Hayden-O'Connell equations of state are used for the thermodynamic modelling framework to predict the non-ideality of liquid solutions, and to take into account effects including chemical association into the gas non-ideal behaviour, respectively.

The organosolv process analysis considered a plant having 7977 hours of annual continuous operation (91% of stream factor) and a biomass processing capacity of 100 ktonne/y (ca. 12,500 kg/h). The feed consists of previously prepared woodchips, which, to improve delignification and hydrolysis, are impregnated with high-pressure live steam during the pulping. The temperature of the impregnated woodchips is raised to the pulping temperature through the amount of injected live steam. The biomass is then pumped into the digester with a discharge pressure similar to the digester operating pressure.

The digester is modelled as a sequence of a stoichiometric reactor unit followed by a pseudo counter-current washing column with the solvent. Table 6 presents the reactions considered and their stoichiometry, as introduced in Aspen Plus®.

Table 6. Occurring reactions in organosolv pulping, respective limiting reagent and stoichiometry[†].

Unit operation	Reaction ID	Limiting reagent	Stoichiometry
Digester	1	$Cellulose_s$	$Cellulose_s \rightarrow Glucoseolig_{sol}$
	2	$Cellulose_s$	$H_2O + 2\ Cellulose_s \rightarrow Cellobiose_{sol}$
	3	$Cellulose_s$	$H_2O + Cellulose_s \rightarrow Glucose_{sol}$
	4	$Cellulose_s$	$Cellulose_s \rightarrow 5\text{-}HMF_{sol} + 2H_2O$
	5	$Xylan_s$	$Xylan_s \rightarrow Xylanolig_{sol}$
	6	$Xylan_s$	$Xylan_s + H2O \rightarrow Xylose_{sol}$
	7	$Xylan_s$	$Xylan_s \rightarrow Furfural_{sol} + H2O$
	19	$Acetate_s$	$Acetate_s \rightarrow Acetateolig_{sol}$
	20	$Acetate_s$	$Acetate_s \rightarrow Acetic\ acid_{sol}$
	21	$Furfural_{sol}$	$Furfural_{sol} + H_2O \rightarrow Tar_s$
	22	HMF_{sol}	$HMF_{sol} + 3H_2O \rightarrow 1.2\ Tar_s$
	23	$Lignin_s$	$Lignin_s \rightarrow Lignin_{sol}$
Lignin precipitation tank		$Lignin_{sol}$	$Lignin_{sol} \rightarrow Lignin_s$
Saccharification reactor	14	$Xylan_s$	$Xylan_s + H_2O \rightarrow Xylose_{sol}$
	15	$Xylanolig_{sol}$	$Xylanolig_{sol} + H_2O \rightarrow Xylose_{sol}$
	3	$Cellulose_s$	$Cellulose_s + H_2O \rightarrow Glucose_{sol}$
	16	$Cellobiose_{sol}$	$Cellobiose_{sol} + H2O \rightarrow Glucose_{sol}$

[†] subscripts *s* and *sol* correspond to the compounds solid and solution states, respectively.

The unwashed pulp stream is subjected to a first washing step where solute-lean cooking liquor is used to displace dissolved substances in pulp and directed to the lignin precipitation area. The second washing stage is performed solely using water to remove the solvent below cellulase inhibition limits. Both washing steps are modelled as single stages in Aspen Plus® using the SWASH model. SWASH simulates a single-stage separation of solid particles from an entrained liquid of a solids stream, not considering the presence of a vapour phase.

In lignin recovery and following the saccharification tank, filter models are used to rate the adiabatic solids separation from liquid, where solids separation efficiency is taken to be 1, so no residual solids are contained in the outlet filtrate stream. The fraction of solids to the solid outlet is 1, while the fraction of liquid to the liquid outlet is set through data reconciliation.

The lignin precipitation tank is modelled with a stoichiometric reactor model working at 30 °C and isobaric where complete precipitation is assumed. Thus, the global yield of lignin recovered is found by adjusting the conversion of soluble lignin and the recovery efficiencies in the first and second washing stages. Other unit models required in the simulation include: HEATER which describes heaters, coolers, valves, pumps, and compressors (whenever work-related results are not needed) by specifying the thermodynamic conditions of the outlet streams or flash calculation; PUMP models which simulate a pump or hydraulic turbine of single liquid phase turbines, wherein power requirement/produced is calculated given an outlet pressure specification or vice-versa; and FLASH2 which performs vapour-liquid or vapour-liquid-liquid equilibrium calculations to model flashes, evaporators, knock-out drums, and other single-stage separators, where when outlet conditions are specified, thermal and phase conditions of a mixture of one or more inlet streams.

3.5.3. Plant model validation

Data retrieved from the experimental work was used to validate the plant model. For this purpose, the following data reconciliation problem (RP) minimizing the weighted least square errors between measured and model-predicted sets of output variables was posed:

$$\begin{aligned} \min_{\boldsymbol{x},\boldsymbol{y}} \varphi \quad = \quad & \sum_{i=1}^{m} \left(\frac{\bar{y}_i - y_i}{\sigma_i} \right)^2 \\ s.t. \quad & \boldsymbol{F}(\boldsymbol{x},\boldsymbol{y}) = \boldsymbol{y} - \boldsymbol{w}(\boldsymbol{x},\boldsymbol{y}) = \boldsymbol{0} \\ & \boldsymbol{c}(\boldsymbol{x},\boldsymbol{y}) = \boldsymbol{0} \\ & \boldsymbol{g}(\boldsymbol{x},\boldsymbol{y}) \leq \boldsymbol{0} \\ & \boldsymbol{x_l} \leq \boldsymbol{x} \leq \boldsymbol{x_u} \\ & \boldsymbol{y_l} \leq \boldsymbol{y} \leq \boldsymbol{y_u}, \end{aligned} \tag{3}$$

where $\varphi \in \mathbb{R}^1$ is the objective function, $\boldsymbol{w}(\boldsymbol{x},\boldsymbol{y}) \in \boldsymbol{Y} \subseteq \mathbb{R}^m$ represents the vector of m outputs from unit models (black-box), $\boldsymbol{c}(\boldsymbol{x},\boldsymbol{y}) \in \boldsymbol{C} \subseteq \mathbb{R}^p$ represents the vector of p extra equality constraints,

$\boldsymbol{g}(\boldsymbol{x},\boldsymbol{y}) \in \boldsymbol{G} \subseteq \mathbb{R}^q$ represents the vector of q extra inequality constraints, $\boldsymbol{x} \in \boldsymbol{X} \subseteq \mathbb{R}^n$ represents the set of n flowsheet decision variables, $\boldsymbol{y} \in \boldsymbol{Y} \subseteq \mathbb{R}^m$ represents the set of m output variables, $\boldsymbol{\sigma} \in \mathbb{R}^m$ represents the vector of the experimental standard error of deviation. Note that residues in $\bar{y}$ are implicitly taken as random, independent, and normally distributed. Consequently, only diagonal terms of the covariance matrix are non-zero. The mathematical programming problem (3) was implemented in Matlab® and solved using direct search solver, which has proven to be effective for smooth or nonsmooth optimization problems [Audet, 2003]. The computation workflow is depicted in Figure 15 and is as follows. A population of samples from the design space ***x*** is generated by the optimizer and the respective values in the Aspen model updated. Once the process simulation is completed successfully, the values of the output variables ***y*** and constraints ***c*** are updated in the Matlab optimizer, the Lagrangian function is computed and the new guess from ***x*** is calculated. Iterations proceed until convergence is attained or the maximum number of iterations is reached. The concrete set of decision variables ***x*** and output variables ***y*** is presented in Section 4.5 while discussing the results of the data model validation.

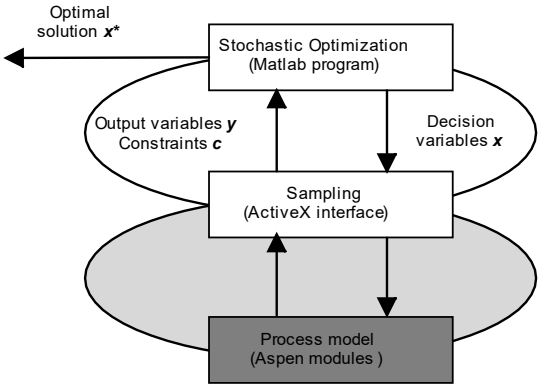

Figure 15. Computational workflow used to solve Problem (RP).

4. Results and Discussion

4.1. Characterisation of raw materials

Chemical and physical characterisations of the raw materials used in this work, wheat straw (WS) and eucalyptus residues (ER), is necessary as to understand its behaviour during pre-treatments. The knowledge gained from the understanding of this characterisations allow us to predict the behaviour of these materials during processing as well as its comparison with other previously studied ones.

4.1.1. Physical Characterisation

Physical characterisation of the feedstocks was assessed through particle size distribution was caried out on WS and ER, that were previously milled to particles smaller than 4 mm and 6 mm, respectively. The distribution results are presented in Figures 16 and 17.

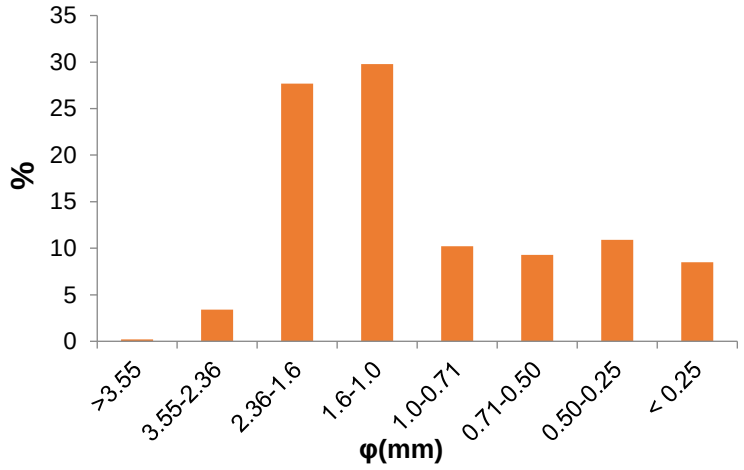

Figure 16. Particle size distribution for WS.

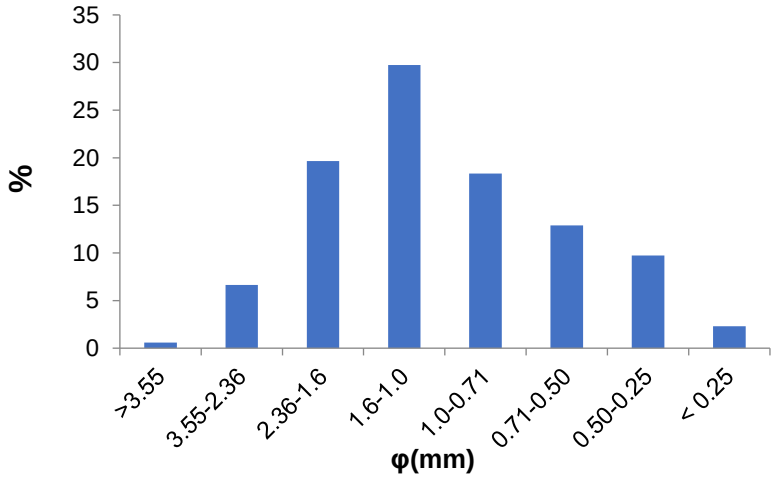

Figure 17. Particle size distribution for ER.

For WS, the most representative fraction, corresponding to 30% of the total mass, is the fraction with particle size ranging between 1.0-1.60 mm, followed by 2.36-1.60 mm fraction with 20%. The fine particles fractions, corresponding to < 0.25 mm, represents 2% of total mass while the largest particles, higher than 3.55 mm correspond only to 1% of the total wheat straw analysed.

Like wheat straw residues, the most representative fraction of ER (30%) corresponds to 1.0-1.6 mm, followed by 2.36-1.60 mm and 0.71-1.0 mm fraction with 20% and 19% of the total mass, respectively. The fines fraction presents only 3% of the total ER analysed whereas 1% comprehends the larger particles, higher than 3.55 mm.

As for both residues, no fraction exceeds 50% of the total and the fine particles percentage is only residual, all fractions were used in the subsequent treatments.

4.1.2. Chemical Characterisation

As previously stated, chemical composition of LM varies with origin, specie and cultivation conditions, with such variations, mainly occurring in the macromolecular components (i.e., cellulose, hemicellulose and lignin), with special focus in hemicellulosic content and composition. To assess the content in these main macromolecular constituents, as well as in ash and other extracts, a chemical characterization of the biomasses was carried out, according to the protocols described in chapter 3. Table 7 presents the results obtained.

Table 7. Average chemical composition (dry basis) of wheat straw and eucalyptus residues.

Compounds (%)	Wheat straw	Eucalyptus residues
Celullose	36.6	44.5
Hemicelullose	26.4	18.5
Xylan	20.8	15.2
Arabinan	2.1	0.5
Acetyl groups	3.5	2.8
Klason lignin	15.7	26.4
Acid soluble lignin	1.72	7.72
Ash	11.4	1.75
Others (by difference)	8.18	1.13

The cellulose content is expressed as “glucan equivalents”, while the hemicellulose content was calculated by the sum of xylan, arabinan and acetyl groups contents.

Both materials present a polysaccharide content of approximately 63%, with cellulose being higher than hemicellulose. It is important to note that ER present lignin quantities, close to 30%. This demonstrates the great potential for valorisation that both materials have.

Cellulose content obtained for WS is slightly higher than the ones reported by Wildschut et al. (2013) but slightly lower than the content found by Carvalheiro et al. (2009). Hemicellulose content is mainly above the values reported in the literature (Carvalheiro et al., 2009 and Wildschut et al., 2013). Contrarily to hemicellulose, lignin content is lower than the values presented by the former authors (Carvalheiro et al., 2009; Wildschut et al., 2013). However, all the previous contents are within the range of values presented by Khan et al. (2012). The ash content is above the values found in the previously presented articles (Carvalheiro et al., 2009; Khan et al., 2012 and Wildschut et al., 2013), but very similar to the values reported by Bruun et al. (2010).

Regarding ER, cellulose content is below most values presented in literature for Eucalyptus wood (McIntosh et al., 2012; Rigual et al., 2019 and Penín et al., 2020), but similar to the ones reported by Romaní et al. (2016). Hemicellulose content is very close to the values presented by McIntosh et al. (2012) and Romaní et al. (2016), being lower than the ones reported by Rigual et al. (2019). The content of lignin is in the range of the values usually reported for this material. The ash content presents higher values when compared to the ones reported in the literature. It is also worth to note that most of the previously mentioned works used eucalyptus wood (*E. globulus*) whereas in this work the residues, i.e., fine particles obtained after grinding to produce chips were utilized.

Comparing both materials there is a higher percentage of cellulose in the ER, as well as close to twice the amount of lignin in relation to WS, as it was already anticipated due to each respective nature. In contrast, the hemicellulose content is higher in WS and the amount of ash is significantly higher in WS than in ER which presented much lower values (< 2%).

4.2. Organosolv pre-treatment

4.2.1. Non-catalysed Organosolv

In order to establish the organosolv kinetics without the addition of an external catalyst, both feedstocks were subjected to organosolv treatments with 50 % (w/w) ethanol/water at 190ºC, for an isothermal reaction time ranging from 0-120 min.

4.2.1.1. Temperature and Pressure profiles

During the multiple pre-treatments carried out for WS and ER, both the temperature and pressure values were collected, and the corresponding profiles were established. This was important not only to check the process reproducibility but also to ensure that the different effects detected after the treatment

are due to the operational conditions used and not to abnormal variation of temperature and pressure, so that a reliable comparison between the results obtained is possible.

Figures 18 and 19 show temperature and pressure profiles for the organosolv treatments. In general temperature profiles are quite similar, with most notable discrepancies in the WS 1 and 2 assays, mainly at an initial stage. Such differences can be explained by the variations in the room temperature each day. However, all the other WS and ER treatments[2] present a significant overlap, as they practically have the same development between the beginning to the end (this is even more evident in the ER). A short plateau can be identified near to 100ºC, which indicates that the temperature of the system only raised when the liquid phase passed to the gaseous phase.

Pressure profiles display an exponential increase, reaching around 300 psi for all treatments. Similar to the temperature profiles, pressure profiles for all the ER and the majority of the WS treatments show an overlapping. The more notorious differences are, once again, for the short reaction times of WS treatments, in which pressure profiles distance themselves more significantly at later stages of the operation. This fact may be explained also by the experimental errors in the pressure measurements.[3]

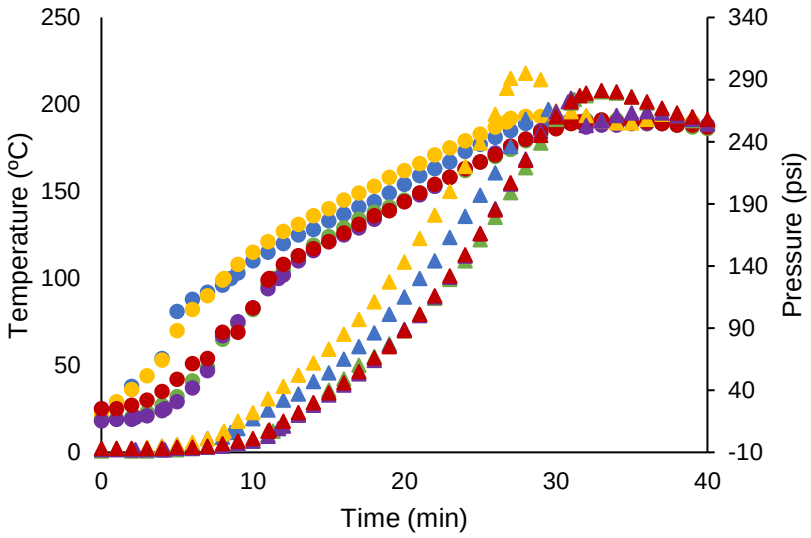

Figure 18. Temperature and Pressure profiles for the heating phase of the organosol process for reaction times 0 min, 15 min, 30 min, 60 min and 120 min for WS. Data for the isothermal period not shown.

[2] The temperature and pressure profiles were not taken to ER 3 and 4 due to technical difficulties at the time.
[3] For both WS 1 and 2 the pressure was measured analogically due to the pressure sensor failure.

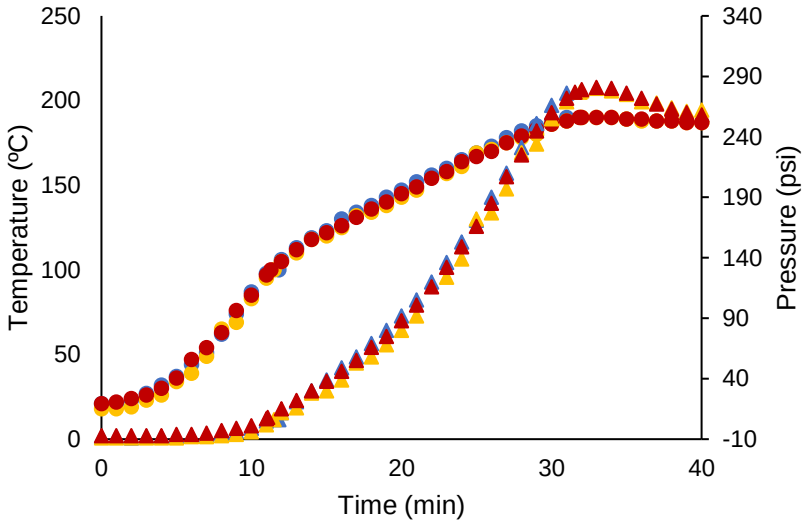

Figure 19. Temperature and Pressure profiles for the heating phase of the organosol process for reaction times 0 min, 15 min, 30 min, 60 min and 120 min for ER. Data for the isothermal period not shown.

4.2.1.2. Organosolv Profile

Figures 20 and 21 show the kinetics of macromolecular components fractionation cellulose, hemicellulose and lignin and the recovery of their derived compounds, in both the solid and liquid phase for WS and ER.

Being organosolv a delignification method, the lignin removal (i.e., delignification yield) as well as the amount of lignin recovered are parameters to consider as well as one of the most interesting points in discussion. For the WS treatment without isothermal phase (0 min) the lignin remaining in the solid is 80.2%, thus the heating phase until 190ºC contribute to a 19.8% delignification of the material (Figure 20a). The treatment with an isothermal phase of 120 min appears to be the most effective of all, with a delignification yield of 59%. The assays in-between followed the tendency in which delignification increased with process severity. A slightly lower delignification yield (48.8%) was obtained by Wildschut et al. (2013) for the treatment of WS using also 50:50 ethanol/water (w/w) at 190ºC for 90 min without the addition of a catalyst.

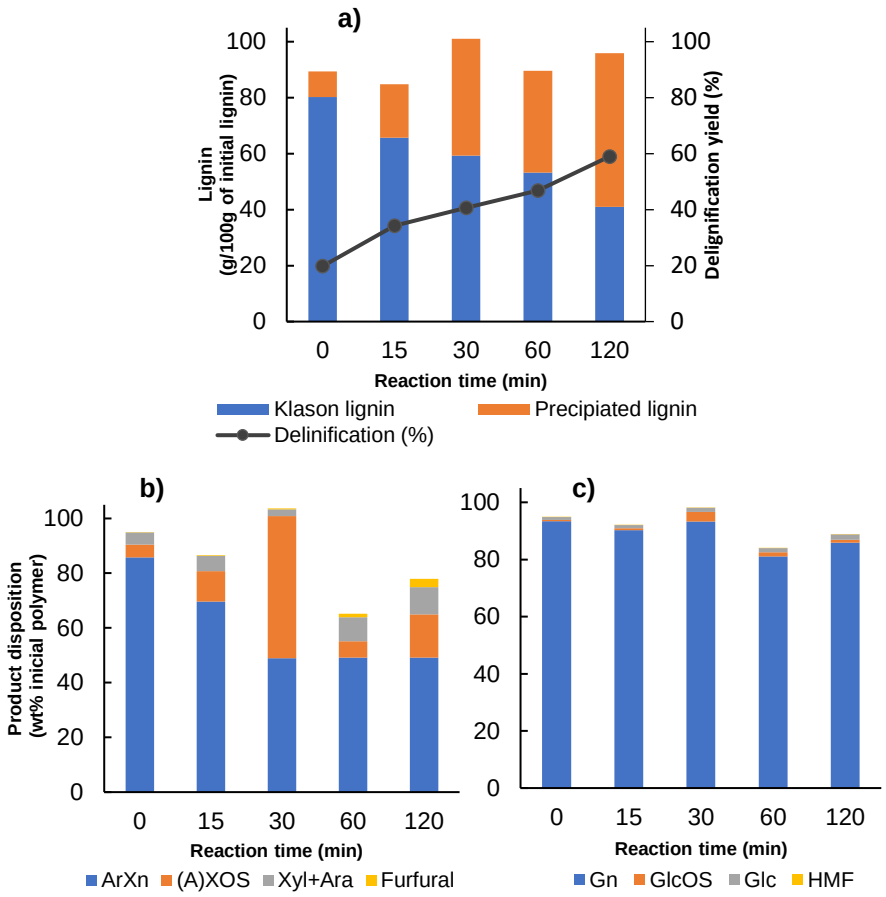

Figure 20. Product distribution obtained for wheat-straw (WS) ethanol-based organosolv fractionation at 190ºC as a function of the isothermal reaction time (no-catalyst added). **a)** Lignin recovery from solid (Klason) and liquid (precipitated) phases and delignification yields; **b)** Recovery of arabinoxylan in solid phase and of (arabino)xylo-oligosaccharides, monomeric pentoses (arabinose+xylose) and furfural in liquid phase; **c)** Recovery of glucan in solid phase and gluco-oligosaccharides, glucose and HMF in liquid phase.

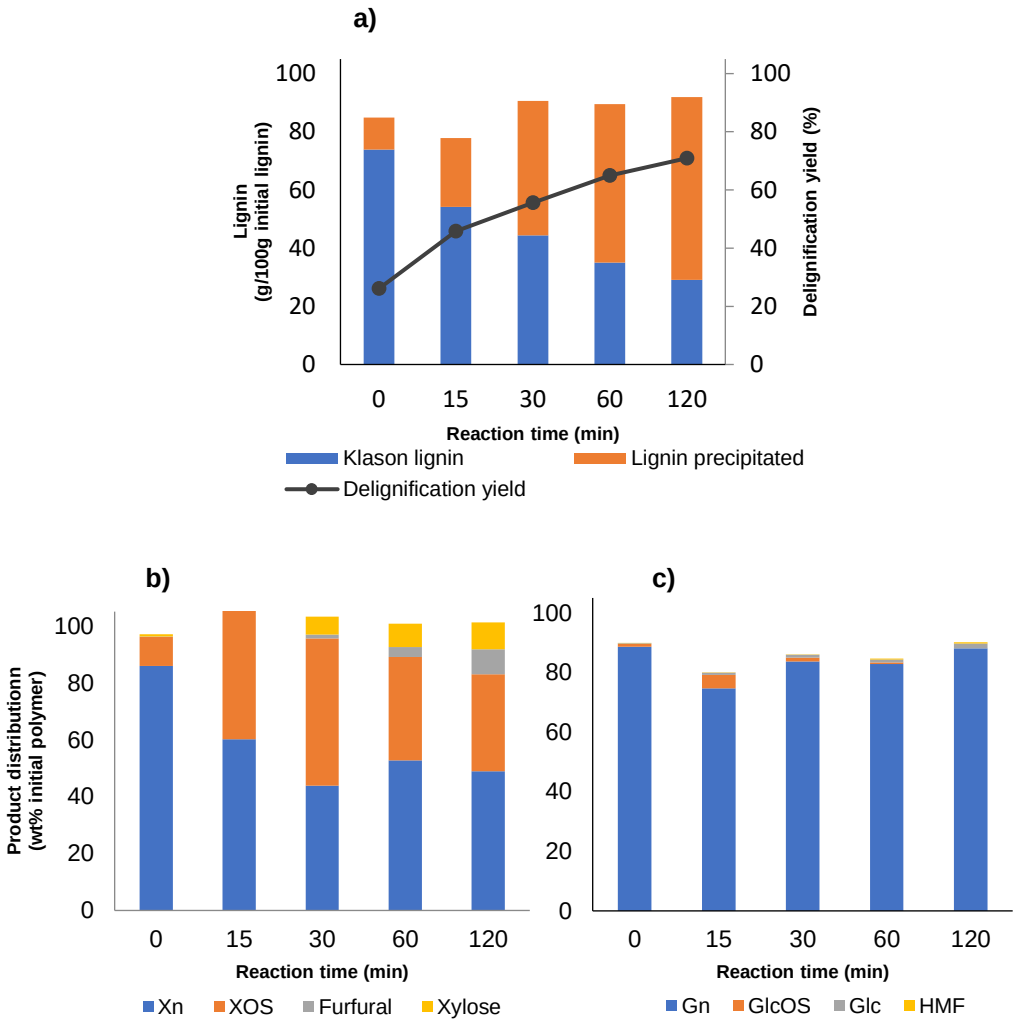

Figure 21. Product distribution obtained for Eucalyptus residues (ER) ethanol-based organosolv fractionation at 190ºC as a function of the isothermal reaction time (no catalyst added). **a)** Lignin recovery from solid (Klason) and liquid (precipitated) phases and delignification yields; **b)** Recovery of xylan in solid phase and of xylo-oligosaccharides, xylose and furfural in liquid phase; **c)** Recovery of glucan in solid phase and gluco-oligosaccharides, glucose and HMF in liquid phase.

For ER (Figure 21a) the highest delignification was also obtained for 120 min treatment. Under these conditions, the Klason lignin percentage remaining in the solid (29.74%), corresponds to a 70.3% delignification yield. The less sever treatment (0 min) presents a delignification yield of 26.1% with 73.9% of lignin still present in the solid. In the case of ER, there was also an increase of delignification with reaction time a similar trend to what happened with WS. Romaní et al. (2016), attained a delignification yield of 65.2%, when eucalyptus wood was treated at 200ºC for 70 min, a similar value to the one achieved in this work for the ER 60 min treatment (64.4%). The solubilised lignin present in the liquid phase was easily recovered by water precipitation (Figure 20a and Figure 21a). Under the best condition (120 min treatments), precipitated lignin can reach 54.8% and 83.3% of Klason lignin, for WS and ER, respectively.

Besides delignification, these organosolv treatments also lead to a partial hydrolysis of hemicellulose and xylan solubilisation, which increases to reach values around 50% for both feedstocks, although slightly higher for ER (Figures 20b and 21b).The formation of xylo-oligosaccharides (XOS) and xylo-oligosaccharides substituted with arabynosil residues (A)XOS, in WS and ER respectively, have

their respective higher values (close to 50% of the recovered hemicellulosic sugars) at 30 min. For the 2 h treatment (A)XOS reach a final composition of 15 and 34.1 g/100g initial xylan for WS and ER, respectively. The monomeric pentoses in WS present its higher concentration value at 2 h with 9.9 g/100g xylan, while its lowest at 30 min with 2.41 g/100g initial component. In ER the best treatment conditions for xylan hydrolysis is 120 min with a xylose concentration of 9.4 g/100g initial xylan. The decrease of xylose and arabinose is mainly due to their degradation, generating an increase of furfural concentration that is also associated with the increase of process severity, reaching its higher values for the 2h treatments for both materials. Even though xylose slightly increases in the ER 2h treatment, there is still a considerably formation of furfural when compared with the previous treatments. Few to any authors speak of XOS recovering, not only because non-catalysed processes are less frequent but they are also not evaluated. However, the recovery of oligomeric sugars is relevant, since this are compounds with bioactive potential i.e. prebiotic properties, which may be relevant to the process economic evaluation.

Cellulose, measured as glucan, was practically not degraded (Figures 20c and 21c) by organosolv treatments, remaining mainly in the solid phase (85.8% of the initial cellulose for WS and 88.1% for ER for the 2h reaction time). Even though as some solubilisation occurs, there is also the formation of glucose oligomers (gluco-oligosaccharides, GlcOS) that presents its maximum values, 3.3 g/100g glucan (30 min assay) and 4.58 g/100g initial glucan for WS and ER, respectively. HMF results from degradation reactions of glucose, that are boosted by the increase of process severity. Thus, this degradation product attains higher values for the 2h treatments, which are considerably more visible in the ER.

Huigen et al. (2010) carried out various organosolv treatments with WS, using acetone:water mixtures under several operational conditions and consistently show cellulose recoveries in the solid phase around 90% of the initial cellulose, which is slightly higher to the 85.8% found in 2h treatment for this material. Romaní et al., (2016) used eucalyptus wood, with 56% glycerol:water, at 200ºC and 70 min, to achieve a cellulose recovery of 99.7% in the solid phase, a value considerably higher to the ones found in this work.

Comparing both residues, eucalyptus exhibited both higher xylan solubilisation and delignification, making ER a more efficient feedstock to be used in a non-catalysed organosolv process. As no acid was added, the behaviour observed can be related to a higher buffer capacity of WS as compared to ER and which can be related to the higher ash content found for WS.

4.2.1.3. Composition of the solid phase

The solids obtained from the treatments, after washing, pressing, and drying were subjected to chemical characterization by means of quantitative acid hydrolysis, in order to assess the changes that

took place during the organosolv treatment. Table 8[4] contains the composition of the pulps obtained for all the performed assays as well as the solid (SY), xylan (Y_{Xn}) and glucan (Y_{Gn}) yields obtained for each case.

For both residues, the solid yield (SY) generally decreases with the severity of the treatment, reaching values of 61.5% and 57.2% for the 2h treatment for WS and ER respectively, even though it tends to stabilise. The solid obtained after 2h treatment mainly contains glucan (51.0% in WS and 68.1% in ER), as glucan fraction tends to increase with process severity (as mentioned before). Wildschut et al., (2013), using WS in an organosolv process (ethanol:water 50:50, at 190ºC, and an isothermal period of 60 min), obtained a less favourable SY percentage of 64.4%, compared to the 65.9% found in this work. Romaní et al. (2016), using eucalyptus wood at 200ºC for 70 min, reported a slightly more favourable SY of 54%.

For both feedstocks hemicellulose fraction solubilisation is lower for the treatments with no isothermal period. Analysing the xylan yields, it should be noted that there is tendency to increase solubilisation with the reaction time, although these values tend to stabilize after 30 min isothermal reaction time, for both materials. At the maximum 48.8% of the initial hemicellulose in WS is solubilised, producing mainly oligomers, monomeric pentoses (xylose and arabinose), acetic acid, and the pentoses degradation product furfural, against 59.3% solubilisation in ER. Arabian in WS decreases with the duration of the treatment, showing total solubilisation for the 60 min treatment. This material also shows a high solubilisation of acetyl groups, mostly for the 60 min treatment. Eucalyptus shows a total solubilisation of arabinose for all treatments. Although the acetyl groups in ER is higher than in WS, it was completely solubilised. For WS it appears that for the conditions with higher isothermal reaction times (30, 60 and 120 min) in average about 46% of the initial hemicellulose is solubilized. This percentage of xylan solubilisation was the highest (56.2%) for the 30 min treatment, followed by 51.5% in the 2h treatment for eucalyptus residues. Wildschut et al. (2013) and Romaní et al. (2016), reported higher solubilisations of hemicellulose, 63.7% and 71.5%, but either with catalyst addition or under more severe operational conditions. However, this solubilization of hemicellulose is much less extensive than that the observed with other processes (i.e., autohydrolysis), where xylan solubilisations can reach up to 96-98% for wheat straw and eucalyptus residues (Carvalheiro et al., 2016). It is important to note that the effect on hemicellulose hydrolysis results mainly from the action of H_3O^+ ions, resulting from the auto-ionization of water and acetyl groups. The fact that ER has a higher content of acetyl groups may also helps to explain the higher reactivity of this material when compared to WS. The amount of ash is quite considerable in the case of WS and high solubilisation could lead to a buffer effect preventing the hydrolysis. However, this is not the case as the residual ash content of pre-treated solids was always high, in contrast to eucalyptus residues where it is almost non-existent as the native biomass itself is practically devoid of ash. The values obtained for the pre-treated biomass of WS show that the ash of this material is kept in the solid itself, not being significantly solubilised by the treatment. The lower

[4] Some of these values have been discussed earlier on 4.2.1.2.

values of eucalyptus ash content may, in part, also justify the greater reactivity of this material, making it again a preferable option for organosolv processes.

To conclude the fractionation of eucalyptus residues using an organosolv treatment for an assay at 190ºC for 120 min, seems to be the most promising process, as it results in a solid phase rich in cellulose, containing 68.6% cellulose, 13.0% xylan and with 11.4% Klason lignin (around 71% delignification yield), making it a suitable substrate for enzymatic hydrolysis and fermentation.

Table 8. Composition of the solid fraction obtained after organosolv treatments at 190ºC and respective solid yield, xylan and glucan yields for both wheat straw and eucalyptus residues.

		Fractionation data (%)								
	Reaction time (min)	**SY**	**Glucan**	**Xylan**	**Arabinan**	**Acetyl groups**	**Klason lignin**	**Ash**	**Y_{Gn}**	**Y_{Xn}**
WS	0	86.4	39.6	21.9	0.8	0.7	14.6	13.4	93.4	90.9
	15	78.6	41.9	19.0	1.2	0.6	13.1	13.1	90.2	72.0
	30	74.9	45.5	14.2	0.7	0.6	12.4	11.9	93.3	51.2
	60	65.9	45.0	17.0	0.0	0.03	12.7	13.8	81.1	54.0
	120	61.5	51.0	18.7	0.0	0.5	10.5	11.2	87.5	55.4
ER	0	87.6	45.0	14.9	0.0	1.8	18.9	0.4	88.7	85.9
	15	68.5	48.5	13.4	0.0	1.2	17.7	0.5	80.0	60.1
	30	68.5	54.3	9.7	0.0	0.8	14.5	0.6	83.7	43.8
	60	59.1	62.3	13.6	0.0	0.8	13.3	0.5	82.8	52.8
	120	57.2	68.6	13.0	0.0	0.7	11.4	1.1	88.1	48.9

4.2.1.4. Composition of the liquid phase

The liquid streams obtained from the organosolv treatments were submitted to chemical characterization as described in chapter 3. The average compositions of the hydrolysate obtained are shown in Table 9.

The data obtained show an interesting solubilisation of the hemicellulose fraction, with its derivatives being the major compounds dissolved in these liquors. The XOS concentration, present very interesting values. In ER it reached a maximum concentration at 30 min with 6.7g/L, while for the highest isothermal period the concentration obtained was 4.1 g/L. Even though it is lower it is still an interesting value. For WS, XOS attained higher values, i.e., 7.2 g/L for the 30 min isothermal period.

Regarding to gluco-oligosaccharides (GlcOS), as the solubilisation of cellulose was very low, the highest values achieved, were 0.6 g/L and 0.7 g/L for WS and ER at 2h, respectively.

The production sugar monomers was also quite low. Glucose concentrations were similar for both residues. For xylose, a profile with an increasing concentration attaining values of 1.0 g/L and 1.3 g/L for the 2h treatments for WS and ER was observed. Arabinose presented a profile with some variations, displaying higher values for WS than for ER which is in agreement with the arabinan content in both substrates. The hydrolysis of acetyl groups from hemicellulose, also produces noticeable concentrations of acetic acid, 2.7g/L and 1.9 g/L at 120 min for WS and ER, mainly in the monomeric form. The acetyl groups linked to the oligosaccharides (AcOS) were not detected for WS and are present in vestigial amounts for the ER, with higher values for the 15 to 60 min treatments (0.6 g/L). When considering other organosolv treatments reported in the literature, the values obtained by Romaní et al. (2016) for organosolv delignification of eucalyptus bark, with glycerol: water (200ºC,69 min), were lower for the concentration of compounds derived from xylan in the respective hydrolysate, with 4.6 g/L compared to 6.0 g /L in the present work, as well as the complete absence of compounds derived from glucan, against the 0.2 g / L to 1.1 g/L in this work at 120 and 15 min respectively for ER.

Monosaccharides may undergo dehydration reactions, leading to the formation of degradation products; monomeric pentoses (i.e. xylose) thus generate furfural, while monomeric hexoses (i.e. glucose) generate hydroxymethylfurfural (HMF). Both were present in the hydrolysates throughout the reaction times, but the HMF concentrations were practically negligible in both feedstocks achieving a maximum of 0.1 g/L in ER. This once again proves low the low reactivity of cellulose. The highlight for the degradation products still goes to furfural, with concentrations around 0.4 and 0.8 g/L in WS and ER hydrolysates, respectively. These values correspond to 3.2% and 5.2% of degradation of the initial hemicellulose, respectively in eucalyptus and wheat straw residues and are within the range found in literature even if a bit lower. Romaní et al. (2016) obtained 6.3% degradation of the initial hemicellulose in furfural for

eucalyptus (glycerol: water, 200ºC, 69 min), while Huijgen et al. (2012) reported values around 5% of the initial hemicellulose for similar conditions of 2h treatment for WS.

During the organosolv treatment, an acidic medium is generated, as can be assessed by the pH of the hydrolysates. The pH values depend on the severity of the treatment. Although the differences are not significant, eucalyptus produces a slightly lower pH. Wildschut et al. (2013) also reported a decrease in pH for longer treatments for ethanol-based organosolv of wheat straw.

The concentration of total phenolics as analysed by the Folin-Ciocalteu method described in 3.4.9. increases with process severity for both feedstocks, with ER presenting considerably higher amounts than WS, reaching for 2 h treatment 9.8 g/L and 7.5 g/L, respectively.

Table 9. Composition of the liquid phase after the organosolv process and the corresponding liquor pH.

			Fractionation data (g/L)										
	Reaction time (min)	**pH**	**GlcOS**	**XOS**	**AOS**	**AcOS**	**Glucose**	**Xylose**	**Arabinose**	**Acetic Acid**	**HMF**	**Furfural**	**Phenolics**
WS	0	5.6	0.1	0.5	0.5	0.0	0.4	0.5	0.5	0.9	0.01	0.01	3.9
	15	5.2	0.2	1.7	0.5	0.0	0.4	0.4	0.9	1.4	0.02	0.02	4.0
	30	5.1	1.0	7.2	3.0	0.0	0.5	0.3	0.2	1.9	0.02	0.1	4.1
	60	4.9	0.4	1.5	0.0	0.0	0.5	0.8	1.1	2.2	0.02	0.2	5.4
	120	4.5	0.3	2.7	0.3	0.0	0.6	1.0	1.1	2.7	0.02	0.4	7.5
ER	0	5.0	0.4	1.4	0.6	0.2	0.1	0.1	0.04	0.3	0.01	0.01	4.6
	15	4.5	1.7	6.2	0.7	0.6	0.3	0.6	0.5	1.0	0.02	0.1	6.8
	30	4.0	0.5	6.7	0.3	0.6	0.4	0.9	0.7	1.1	0.1	0.1	8.1
	60	4.1	0.2	4.6	0.4	0.6	0.4	1.2	0.3	1.6	0.1	0.3	9.1
	120	4.0	0.2	4.3	0.6	0.1	0.7	1.3	0.2	1.9	0.1	0.8	9.8

GlcOs- gluco-oligosaccharides; XOS- Xylo-oligosaccharides; AOS-Arabino-oligosaccharides; HMF- 5-hydroxymethilfurfural

As this method for total phenolics quantification is not selective, these concentrations may represent both phenolic compounds present in the extractives, but also some resulting from the depolymerization of lignin. So, in order to characterize the type of phenolics present in both hydrolysates, an analysis of the hydrolysates by capillary zone electrophoresis (CZE), was also carried out. The data obtained was compared with the standard samples database available at LNEG. As an example, an electropherogram for WS hydrolysate is shown in Figure 22.

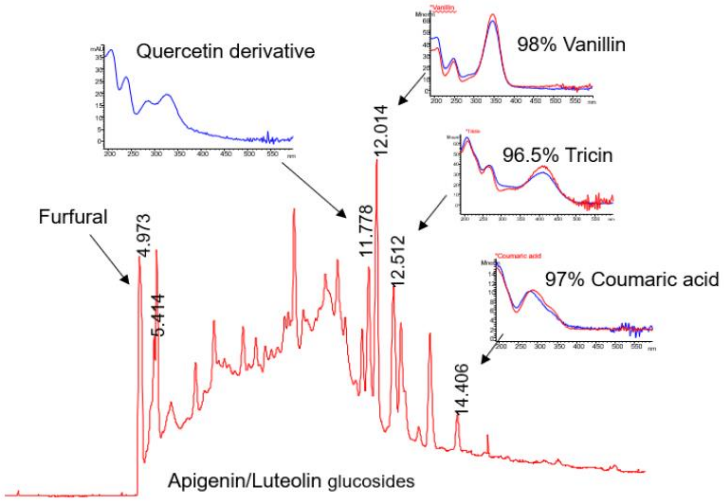

Figure 22. Electropherogram with low molecular weight phenolic compounds for wheat straw ethanol organosolv 120 min hydrolysate.

The furfural peak is clearly observed at 5 min from the start of the run. Between 11 min and 14 min it was possible to find a good matching with the standards available in the database, enabling to identify, in addition to quercetin derivatives, vanillin, tricine and *p*-coumaric acid, with a matching of 98%, 96.5% and 97%, respectively. These were then the main monomeric phenolic compounds found in this hydrolysate, resulting from the hydrolysis of lignin in the organosolv process. The wide variety of phenolic compounds found in the electropherograms meets the characteristics of this herbaceous biomass, whose lignin is rich in all hydroxyphenyl, guaiacyl and syringyl monomers.

In turn, the electropherogram of the hydrolysate of eucalyptus residues (hardwood whose lignin is essentially composed of syringyl and guaiacyl units) also revealed the presence of *p*-coumaric acid, as well as of vanillin and syringaldehyde (data not shown).

In addition to the hydrolysates, the ethanol washing waters obtained from the first washing of the solid fraction were also characterised. These washing waters were presumed to have dragged some hydrolysate that was soaked in the pulp as well as some soluble lignin. It was observed that indeed some sugars were still dragged although in very small, almost in residual quantities with phenolic compounds presenting more significant values, 1.87 and 1.78 g/L for WS and ER respectively. For this reason, this current was also considered as of potential interest for an eventual recovery of phenolic compounds. Figures 23 presents an example pf the electropherogram obtained for the washing water of WS.

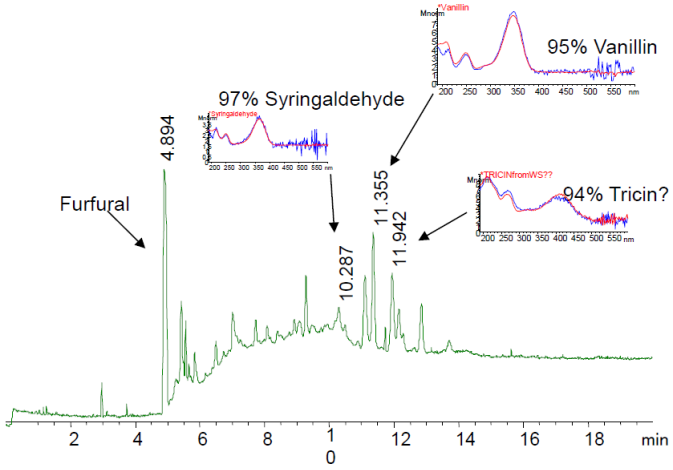

Figure 23. Electropherogram with low molecular weight phenolic compounds for wheat straw ethanol organosolv washing solution.

The furfural peak can also be detected as in hydrolysate. A syringaldehyde peak can be identified at 11 min with 97% matching. Vanillin and tricin can also be identified with 95% and 94% matching, respectively.

To sum up, and likewise of what happened in sections 4.2.1.2. and 4.2.1.3., it is important to highlight that the results obtained for eucalyptus fractionation suggests a possible more interesting feedstock than WS, even though the condition where it was detected a higher formation of XOS (30 min), does not coincide with the highest delignification yield (2 h). However, the amount of phenolic compounds, both in the hydrolysate as in the ethanol washing water associated to the cellulose enriched solids obtained at in the 120 min treatments does contribute to the believe that this treatment is the most efficient one to be carried out in further organosolv treatments.

4.2.1.5. Enzymatic saccharification of pre-treated solids

The efficiency of ethanol-based pre-treatment on WS and ER was also evaluated by enzymatic hydrolysis. The saccharification yields of pre-treated WS and ER were performed as described in 3.4.11. using an enzyme dose of 10% based on pulp glucan. The results obtained are shown in Figure 24.

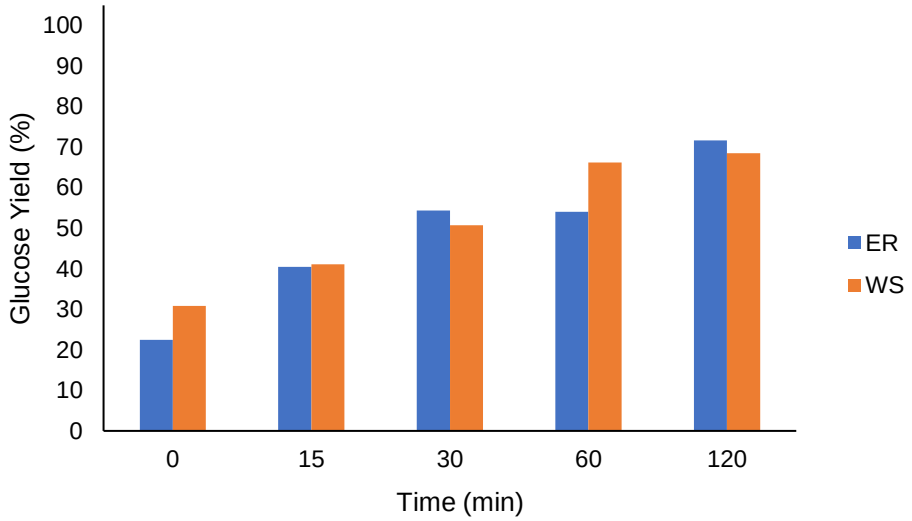

Figure 24. Enzymatic saccharification of ethanol:water organosolv pulp obtained at 190°C (no catalyst added) for wheat straw (WS) and eucalyptus residues (ER).

At 190°C with no catalyst added, enzymatic cellulose conversion to glucose increases with pre-treatment time to reach the highest values for 2 h reaction, respectively 68.5% for WS and 71.7% for ER. These values represent near to 5-fold and 7-fold increase as compared to the original feedstocks, i.e., 10.6% and 14.2% for WS and ER, respectively. According to Wilschut et al. (2013) the enzymatic digestibility achieved was about 42% for wheat straw using 50% etanol:water at 190ºC and 120 min, a much lower value than the one found in this work. Wang et al. (2017) used Eucalyptus wood in an organosolv process with 50% 2-Propanol/water for 2h at 200ºC, and obtained an enzymatic hydrolysis glucose yield of 80.05%, a higher value than the one found in this work, however with a different solvent.

All in all, eucalyptus residues seems to be a slightly efficient biomass out of the two in study in this thesis, with higher delignification and saccharification yields for the 2h treatment, as well as high xylose solubilisation and XOS formation when compared to wheat straw 2h assay.

4.3.1. Organosolv with catalyst addition – Doehlert experimental design

As eucalyptus was the biomass with the most promissing results from the presented autocatalyzed organosolv processes, these residues were submitted to a catalysed organosolv process following a Doehlert experimental design for two factors in oder to maximize both sugars- and delignification yields and establish optimum conditions [Doehlrt,.1970].

In order to fix the reaction temperature several preliminary experiments were carried out at mild temperature (140ºC) with and without addition of catalyst (sulfuric acid).

Therefore, biomass pre-treatments were than optimized at (140ºC), using the same green solvent (ethanol:water 50:50 w/w) and low catalyst (H_2SO_4) concentrations (0-1 M) and different reaction times (0-168 min) .Table 4 of section 3.3.2. already summarized this treatments conditions.

4.3.1.1. Temperature and Pressure Profiles

During the multiple pre-treatments made for both materials, both temperature and pressure profiles were collected, in order to both check the process reproducibility and to ensure that the different effects detected after the treatment are due to the operational conditions used and not to abnormal variation of temperature and pressure, so that comparison between the results obtained can be possible.

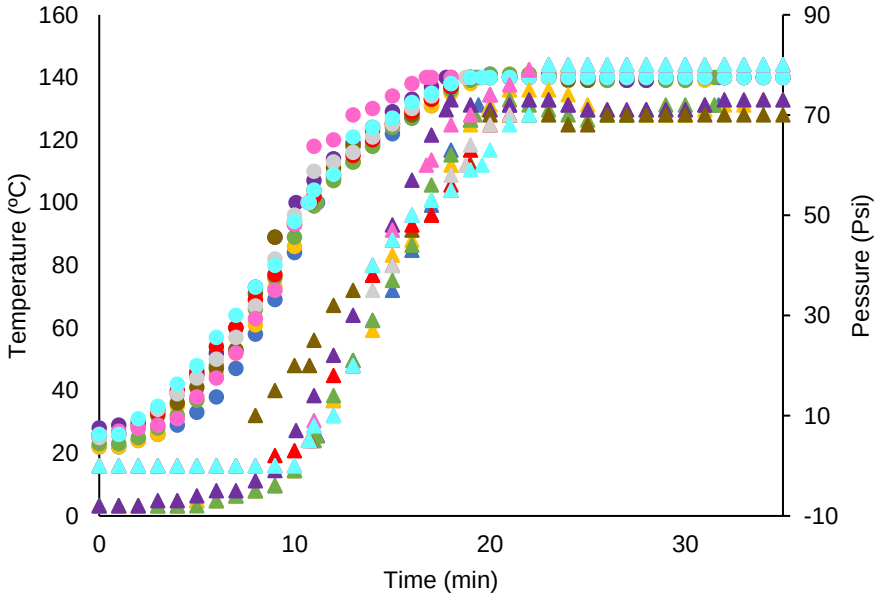

Figure 25. Temperature and Pressure profiles for the heating phase of the 140ºC H_2SO_4 catalysed organosol process for assays 1-10.

Figure 25 show temperature and pressure profiles for the organosolv treatments carried out according to the experimental design. In general temperature profiles are quite similar, almost overlapping each other. The minor lags that can be seen, mainly in the starting region, may be explained due to room temperature differ day by day. When compared with the temperature profiles for eucalyptus at 190ºC (Figure 19), it is noted that their behaviour is quiet the same.

The pressure profiles display an exponential increase, reaching around 80 psi for all treatments. In these profiles there are more notorious differences in comparison to pressure profiles from the non-catalysed organosolv at 190ºC. This differences mainly starting from the 90 min 50 mM H_2SO_4 (exp 1) and continuing to the four middle experiment points (90 min 25 mM H_2SO_4 – exps 7 to 10), are due to the fact that pressures for these assays were taken in an analogical manner due to the equipment pressure sensor failure, which means theses errors falls under experimental errors in measurements.

4.3.1.2. Composition of the solid and liquid phases

The solids obtained from the treatments, after washing, pressing, and drying were subjected to chemical characterization by means of quantitative acid hydrolysis, in order to assess the changes that took place during the organosolv treatment. The liquid streams of the organosolv treatments were also submitted to chemical characterization as described in chapter 3. Table 10 contains the composition of the pulps obtained for all the performed assays as well as the solid (SY), xylan (Y_{Xn}), glucan (Y_{Gn}) and delignification yields obtained for each case. The compositions of the hydrolysates obtained are shown in Table 11.

For the treatment with no acid addition (exp 2) the SY was 91%, the highest value found in all treatments with the lowest being the one with the highest concentration of acid (50 mM H_2SO_4), implying that the increase in acid concentration in a treatment significantly promotes the solibilisation of components to the liquid phase.

Glucan yield in the solid fraction is high for all treatments, even higher than the one found for the un-catalysed organosolv of ER at 190ºC and once again it remains almost unaffected by the process. However, and as expected the xylan yield decreased considerably with catalyst addition when compared with the uncatalyzed process at140ºC (90 min, 0 mM H_2SO_4). On the other hand, the delignification yield attained high values under mild catalysed concentrations and temperature, with a maximum of 86.7% for one of the assays of the centre of the experimental domain (90min, 25mM H_2SO_4).

The acetyl groups remaining in the solid, in general decrease with acid addition, achieving residual values. The ash content remains low and it appears to decrease for the assays with less catalyst addition and reaction times and increase in the ones with both higher temperature and catalyst amounts.

Regarding the liquid stream composition, and compared to the 190ºC uncatalyzed organosolv, the main difference is that the pentoses recovered here are mainly in the monomeric form with a vestigial production of XOS. The AcOS follow the same pattern as XOS with no presence in the various catalysed treatments with the exception in the highest acid concentration one (50 mM H_2SO_4), in which attained values of 1.1 g/L. Xylose concentration attained higher values for the centre of the experimental domain (90 min and 25 mM H_2SO_4) 10.4 g/L with considerable concentration values for the 90 min 50 mM condition too (9.5 g/L).

Table 10. Experimental conditions of the plan and the obtained composition of the solid phase after organosolv treatment of eucalyptus residues at 140ºC ..

			Fractionation data (%)								
Exp.	**Reaction time (min)**	**H2SO4 concentration (mM)**	**SY**	**Glucan**	**Xylan**	**Acetyl groups**	**Klason lignin**	**Ash**	**Y_{Gn}**	**Y_{Xn}**	**Delignification**
1	**90**	**50**	41.9	96.1	4.6	0.01	8.3	1.4	90.4	12.7	84.6
2	**90**	**0**	91.0	47.8	14.5	0.36	22.3	0.6	97.9	86.5	9.4
3	**167.9**	**37.5**	46.9	89.3	9.4	0.01	12.9	1.8	94.2	29.0	73.1
4	**12.1**	**12.5**	67.1	65.3	6.4	0.20	18.3	0.6	98.4	28.1	45.3
5	**12.1**	**37.5**	57.6	74.6	12.5	0.10	10.8	0.5	96.7	47.3	72.2
6	**167.9**	**12.5**	48.5	83.2	4.3	0.04	13.5	0.6	90.8	13.5	70.8
7	**90**	**25**	48.0	87.2	3.9	0.02	6.2	0.8	94.0	12.3	86.7
8	**90**	**25**	45.7	90.2	3.6	0.02	8.0	0.8	92.8	10.9	83.6
9	**90**	**25**	43.5	64.6	2.4	0.01	11.6	0.5	92.5	6.9	77.5
10	**90**	**25**	43.1	93.7	1.6	0.02	9.2	1.2	90.9	4.6	82.3

Table 11. Experimental conditions of the plan and the obtained composition of the liquid phase after organosolv treatment of eucalyptus residues at 140ºC.

				Fractionation data (g/L)									
Exp.	**Reaction time (min)**	**H_2SO_4 concentration (mM)**	**pH**	**GlcOS**	**XOS**	**AcOS**	**Glucose**	**Xylose**	**Arabinose**	**Acetic Acid**	**HMF**	**Furfural**	**Phenolics**
1	**90**	**50**	1.2	0.64	0.00	1.06	2.07	9.49	0.24	0.05	0.02	1.92	2.5
2	**90**	**0**	4.5	0.00	0.03	0.25	0.22	0.12	0.30	0.07	0.05	0.03	2.4
3	**167.9**	**37.5**	1.5	0.21	0.00	0.00	1.31	8.71	1.63	2.69	0.09	0.82	2.7
4	**12.1**	**12.5**	1.7	0.57	0.00	0.00	0.76	4.09	1.06	1.21	0.00	0.00	1.5
5	**12.1**	**37.5**	1.4	0.59	0.00	0.00	1.04	7.56	1.36	2.29	0.07	0.12	2.4
6	**167.9**	**12.5**	2.1	0.00	0.00	0.00	1.84	7.40	2.16	3.12	0.24	2.19	2.6
7	**90**	**25**	1.5	0.00	0.00	0.00	3.94	9.51	2.79	3.50	0.10	1.11	2.4
8	**90**	**25**	1.5	0.00	0.00	0.00	3.46	9.21	2.57	3.65	0.11	1.03	2.1
9	**90**	**25**	1.3	0.00	0.00	0.00	3.90	10.46	4.41	5.95	0.16	2.39	3.1
10	**90**	**25**	1.4	0.00	0.00	0.00	3.65	9.65	2.59	3.96	0.08	0.98	2.3

As no relevant hydrolysis of cellulose occurred. glucose concentration also attained higher values for the 90 min and 25 mM H_2SO_4 conditions with 3.9 g/L followed by the 50 mM H_2SO_4 condition with a concentration of 2.1 g/L. The GlcOS. on the other hand. present very residual values for all treatments, achieving its maximum concentration (0.6 g/L) for the 50 mM H_2SO_4 treatment. The degradation product from glucose. HMF attained generally very low to residual values in all treatments. while furfural obtained higher concentration values for the treatments in the centre of the experimental domain and 50 mM H_2SO_4 although still relatively low in the order of 2 g/L.

The phenolics content on these assays are considerably lower to the ones found in the uncatalyzed processes. For the same reason, no washing waters were analysed for its phenolics content.

Analysing all assays, in special the ones for the central points of the experiment. it is visible. mainly in the liquid phase, a difference in values for the third treatment when compared with the other three. This difference may be mainly due to experimental errors in the filtration operation after the organosolv treatment. Nevertheless. all experiments were taken into account to the elaboration of the response surface method. to a better understanding of these organosolv assays profile.

4.3.1.3. Response Surface Methodology

The traditional 'one-factor at a time' technique that can be used for optimizing a multivariable system is not only time consuming as it also often misses the alternative effects between components. In addition, this method requires to carry out several experiments to determine the optimum levels, which are untrue. These disadvantages associated to the single factor optimization process can be eliminated by optimizing all the affecting parameters collectively by Doehlert experimental design using response surface methodology (RSM).

RSM is a sequential procedure which has an initial objective of leading the experimenter rapidly and efficiently to the general vicinity of the optimum. Since the location of the optimum is unknown prior to running RSM experiments, a design that provides equal precision of estimation in all directions is employed. As previously stated in chapter 3. the two variables of study are reaction time and catalyst concentration. By applying multiple regression analysis on the experimental data, the following second order polynomial equation was found (equation 2 in 3.3.3).

Statistical validation of the polynomial equations was made by analysis of variance (ANOVA) and model analysis by the coefficient of multiple determination (R^2)

The results for the RSM are given in Table 12 and Figure 26 for delignification. Y_{Xyl}. glucan recovery and saccharification. The significance of each coefficient was determined by *p*-values. The smaller the magnitude of the *p*-value, the more significant is the corresponding coefficient.

Table 12. Parameter values for the quadratic polynomial equation.

Yield (%)	β_0	β_1	β_2	β_{12}	β_{11}	β_{22}	R^2
Delignification	**82.6±5.4 (0)**	**29.2±6.3 (0.010)**	7.6±6.3 (0.290)	-16.8±12.5(0.251)	**-35.6±9.4 (0.019)**	-11.1±9.4 (0.303)	**0.91**
Xylose	**63.3±2.9 (0)**	**23.9±3.3 (0.002)**	**8.9±3.3 (0.050)**	-4.2±6.7 (0.559)	**-34.6±5.0 (0.002)**	-13.7±5.0 (0.052)	**0.97**
Glucan Recovery	**92.6±0.6 (0)**	**-3.3±0.7 (0.009)**	**-2.9±0.7 (0.014)**	-0.9±1.4 (0.558)	1.6±1.0 (0.199)	2.8±1.04 (0.060)	**0.93**
Saccharification	**81.7±6.0 (0)**	**29.7±6.9(0.013)**	**19.6±6.9(0.046)**	-24.0±13.8(0.156)	**-36.9±10.3 0.023)**	-22.3±10.3 (0.097)	**0.92**

*Coefficient ± standard error. **Statistically significant (p< 0.05) coefficients are bold**

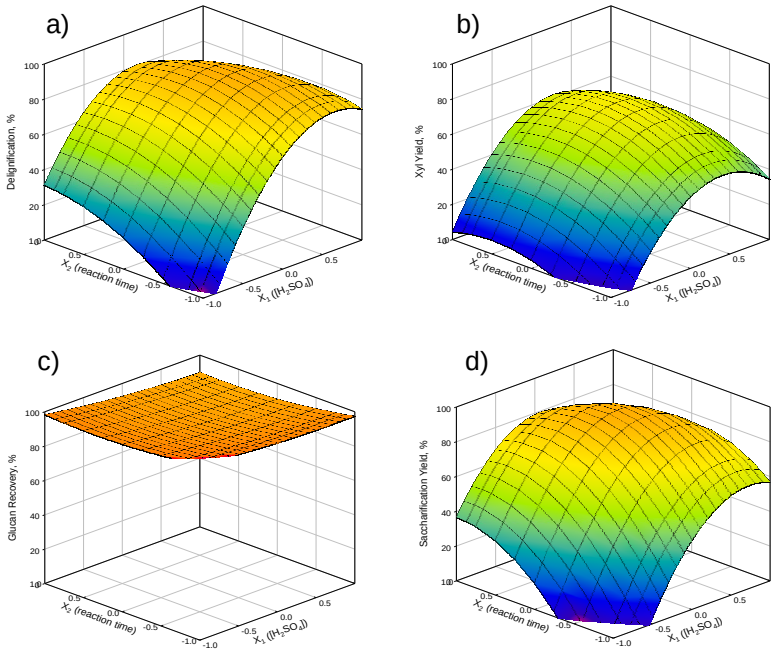

Figure 26. Response surfaces for the most relevant parameters. a) Delignification; b) Xylose yield; c) Glucan Recovery; d) Saccharification

Table 12 show the regression coefficients found for each parameter. By the analysis of both Table 12 and Figure 26 it is possible to see that delignification is influenced by catalyst concentration in a way that low concentrations have a positive effect while higher concentrations induce a detrimental one. As stated, the higher delignification yield achieved for this set of experiments, was 86.7% for conditions close to the centre of the experimental domain (90 min and 25 mM H_2SO_4).

The xylose production yield is both influenced by reaction time and catalyst concentration. It is positively affected by low H_2SO_4 concentrations and to a less extent by reaction time. In addition, a negative effect exists for high catalyst concentration, thus inducing the production of sugar degradation products like furfural.

Glucan once more presents high values in the pulp, regardless of the operational conditions (Figure 26c), thus enabling to obtain pre-treated biomass with an extremely high glucan content, with values above 90% for the optimal conditions. However. as it can be seen and proved by *p*-values glucan recovery is still negatively affected (in a small extent) by both catalyst concentration and reaction time.

This rich glucan pulp obtained was subjected to enzymatic hydrolysis (as described in 3.4.11.) and the saccharification yields obtained are shown (figure 26d). The saccharification yield is positively influenced by both catalyst concentration and reaction time, although higher catalyst concentrations induce a negative effect on the parameter. As for the higher saccharification yields (>84%), those were found for the conditions close to the centre of the experimental domain. with moderate catalyst concentrations.

Analysing the statistical data provided by the analysis of variance and by the coefficient of multiple determination (R^2), the best fit for xylose yield with a R^2 of 0.91, implying that 91% of the sample variation in the xylose production is attributed to the independent variables. All other regression coefficients for the parameters in study were also above 0.90 and the same conclusion can be taken.

Although there are several studies for the fractionation of biomass with ethanol-water mixtures and catalysed with sulfuric acid, in general, they explore higher temperatures and catalyst concentrations, so the available bibliography for comparison of results under similar operational conditions is practically non-existent.

The results obtained in this work show that because eucalyptus is a very reactive material and susceptible to hydrolysis, it can be fractionated, by an organosolv process under very mild conditions of temperature and catalyst concentration, with high efficiency.

4.4. Economical potential

To better understand the application of this study, the mass balances for the main compounds were calculated per tonne of feedstock. These calculations were performed for the optimal conditions of 2h for both ER and WS in the uncatalyzed organosolv process (Figures 27 and 28) and for the 90 min and 25mM H_2SO_4 for ER in the 140ºC catalysed organosolv one (Figure 29).

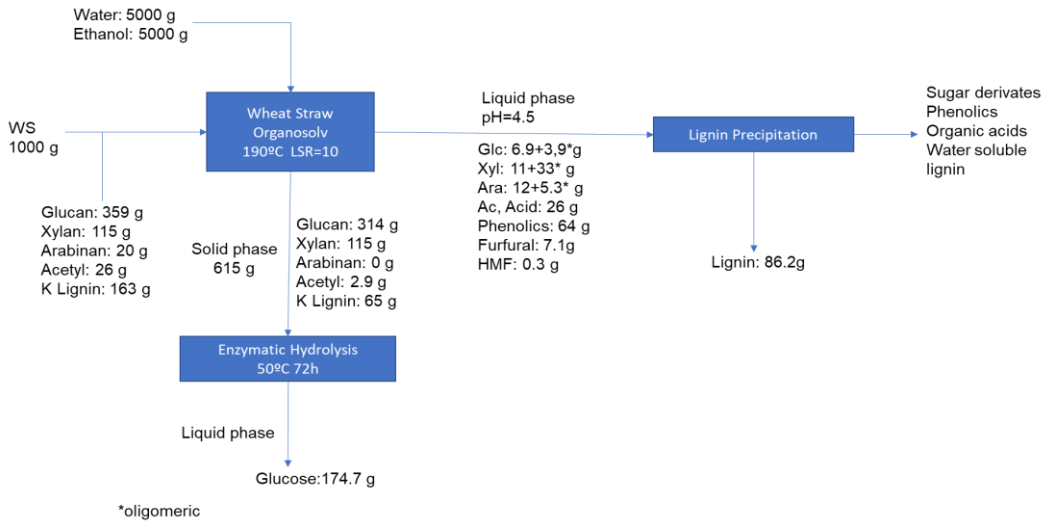

Figure 27. Balance and flow of the main constituent processing Wheat straw by ethanol-organosolv at 190ºC without catalyst and enzymatic hydrolysis.

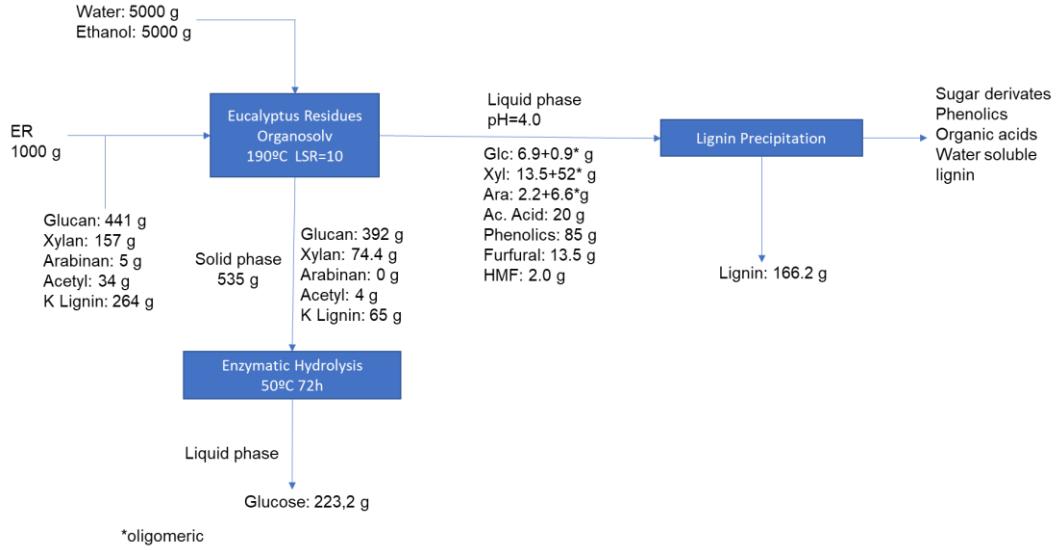

Figure 28. Balance and flow of the main constituent processing Eucalyptus residues by ethanol-organosolv at 190ºC without catalyst and enzymatic hydrolysis.

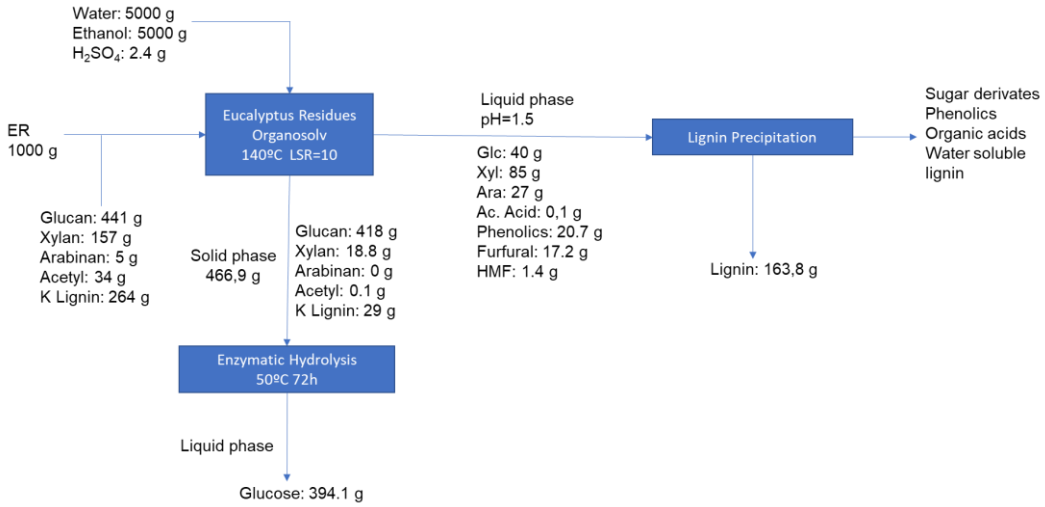

Figure 29. Balance and flow of the main constituent processing Eucalyptus residues by ethanol-organosolv at 140ºC with catalyst (H2SO4) and enzymatic hydrolysis.

Considering the market value for the products[5], a primary economic analysis just for products revenue depending on the feedstock used, was performed. The prices for each product are in table 13. Table 14 contains the values of the initial economic analysis for the processes studied in this work. The feedstock prices were found in Manitoba (2017) and agricultureinformation.com for WS and ER. respectively.

Table 13. Selling prices for the process's products.

Products	Selling Price (€/ton)
XOS	36790
Xyl	4090
Glc	530
Lignin	654

It is noteworthy that lignin and XOS prices heavily depend on its quality, the process used for production, the purity and several other parameters, and so the prices can range to even higher values.

[5] The products market value is not unequivocally available and some change depending on the source, so as much as possible the same source was used (Alibaba).

Table 14. Economic potentials estimated for the organosolv processes in this work.

Material		WS	ER	ER*
Catalyst		No	No	H_2SO_4
Temperature		190	190	140
Time		120	120	90
Revenues (€/dry ton feedstock)	**XOS**	1409.06	1496.99	0.00
	Xyl	94.07	110.43	530.47
	Glc	119.72	152.89	179.61
	Lignin	56.37	119.67	120.85
Total		**1679.22**	**1879.98**	**830.93**
Feedstock cost (€/ton)		37.54	22.20	22.20
Revenue/Feedstock cost		44.73	84.68	37.43

*Catalyst concentration: 25 mM

This economical approach points the uncatalyzed eucalyptus organosolv as the process with potential higher revenues, although no equipment, installations, utilities, or other costs were taken into account. However, looking at feedstock cost alone was already excepted for the ER processes to be more favourable over WS due to the lower price of feedstocks. Also, as stated several times, XOS is a high-value product, making the processes with higher formation of this product more favourable economically.

4.5. Plant model validation

A model for an organosolv pre-treatment was developed and validated to produce xylossacharides and syrups in this work. Process flowsheet (Figure 30) was designed to follow closely the experimental setup used to collect the experimental data for eucalyptus residues at 190ºC without catalyst, as per indication of the preliminary economic analysis.

The cooking process was assumed to be continuously operated. The conditions used in the reactor mirrored the ones of the experimental assay: 190ºC with 120 min of isothermal reaction time, a solvent with 50:50 ethanol/water and LSR of 10:1. In the cooking process, the eucalyptus residues were pre-heated with high-pressure steam in a streaming bin at atmospheric pressure, then fed to the digestor after being heated to the cooking temperature (190 ºC) using a heat exchanger. The solvent was also heated to 190 °C before entering the digester. After the data reconciliation, the adjusted LSR of the discharged pulp was 5.98 and the mixing efficiency of the digester was 0.90.

The pulp was discharged and cooled in a heat exchanger to 50 ºC before the first washing stage. To avoid pulp condensation, the pulp was first washed with ethanol/water solution with a similar composition as the solvent (50:50 ethanol/water) and mass flow rate to remove dissolved lignin. The first washer (WASHER1) has a mixing efficiency of 0.906, and the washed pulp has an LSR of 1 after data reconciliation. The pulp was subsequently washed a second time (in

WASHER2) only with water. The estimated mixing efficiency and pulp LSR were both 1 in WASHER2 after data reconciliation. Afterwards, the washed pulp consistency was adjusted to 10% before saccharification to follow the solid load used in the saccharification experiments. The saccharification tank worked at 48 ºC and 3.9 atm from here the mixture was sent to centrifugation, where the resulting liquid stream goes into a triple effect evaporation system to concentrate the solution and obtain the syrup (glucose and xylose).

The black liquor exiting the digestor is cooled to 50 ºC, mixed with the washing waters and cooled again to 30 ºC via heat exchangers before entering the lignin precipitation tank. After precipitation, the wet lignin is recovered and dried, whereas the liquid fraction proceeds into an evaporation system of triple effect to remove the solvent and concentrate the XOS. For the plant model validation, the process flowsheet does not include recycling streams nor purges (Figure 30), which will only be considered in the following section.

The results obtained by the model were compared to the ones achieved in the experimental assay (Tables 15 to 17). In the pulp results (Table 15) the major deviations are associated with xylose, presenting relative deviations in the order of 20%. However, overall, the pulp itself gave almost a perfect fit (0.02% deviations). Lignin (table 16) presented very good fits in general, while the hydrolysate (Table 17), even though with considerable deviation in the xylose and glucose concentrations as well as in the respective degradation products (~10-20% deviations), the overall hydrolysate presents 0.13% of relative error when compared with the experimental. So, it is safe to conclude that the model is a good fit for the experimental results.

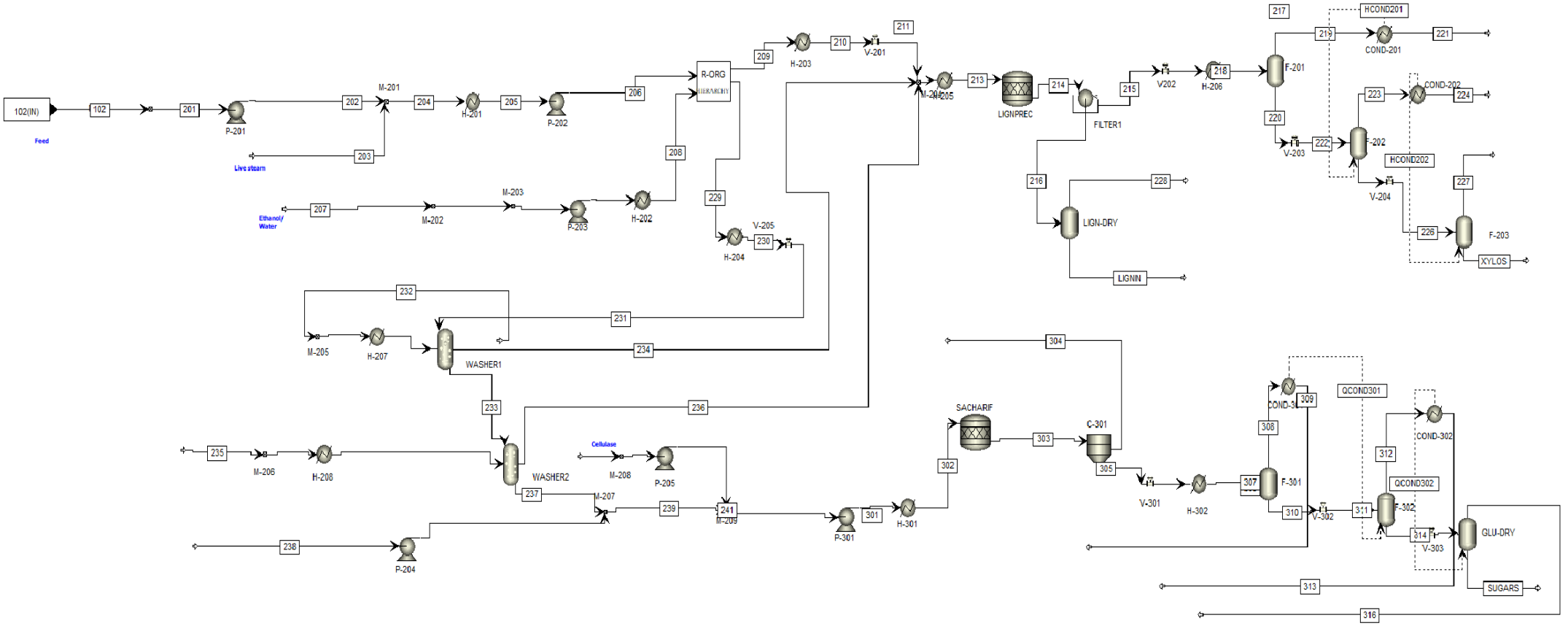

Figure 30. Process flowsheet.

Table 15. Data reconciliation with the experimental results for the pulp.

	g/100g raw material		RD%
	Simulation	**Experimental[6]**	
Pulp (OD)	57.139	57.15	0.02
Klason lignin	5.914	6.51	9.2
Glucan	41.167	38.92	5.8
Xylan	5.885	7.39	20.4
Ash	0.273	0.27	1.1
Acetyl groups	0.652	0.39	67.3
Other	3.247	3.67	11.5

Table 16. Data reconciliation with the experimental results for lignin.

Precipitated lignin (as phenolics)	**g/100g raw material**		**RD%**
	Simulation	**Experimental[6]**	
Lignin	16.46	16.40	0.4
Purity	0.853	0.896	5.1

Table 17. Data reconciliation with the experimental results for the hydrolysate.

Water soluble	**g/100g raw material**		**RD%**
	Simulation	**Experimental[6]**	
Water soluble	15.24	15.22	0.13
Xylose Oligomer	5.60	5.43	3.12
Glucose	2.37	2.15	10.32
Xylose	3.12	2.75	13.64
Furfural	1.08	1.49	27.27
HMF	0.91	1.15	20.75
Acetic acid	2.15	2.25	4.52

[6] These values correspond to a preliminary analysis of the 190ºC 120 min eucalyptus organosolv assay, not the final one given in the mass balances section.

4.6. Process optimisation

Here, the process was optimised by using the plant model previously validated. The process was redesigned, and the operating conditions manipulated to reduce energy consumptions and obtain the products within specifications. Then, pinch analysis was performed to evaluate potential energy savings.

The new process flowsheet shown in Figure 31 now features a double effect distillation system to recover the solvent, an integrated arrangement which has been shown to reduce considerably the energy requirements in diluted aqueous solutions of ethanol. The double effect distillation system was designed as in [Granjo et al., 2020], and the concentrations of ethanol in the distillates were adjusted to match the solvent composition required in the digester and in WASHER1. The operating conditions of the evaporators in the XOS and syrups concentration areas were adjusted to minimize energy consumptions and obtain the products within the specifications. The pulp leaving the saccharification step was partially recycled to increase the overall production of syrups and improve the pulp delignification. In addition, the water streams were redirected to the digester and washing stages to reduce further the need for freshwater and purge streams are included to prevent the accumulation of inhibitors (e.g., HMF, furfural) in the process.

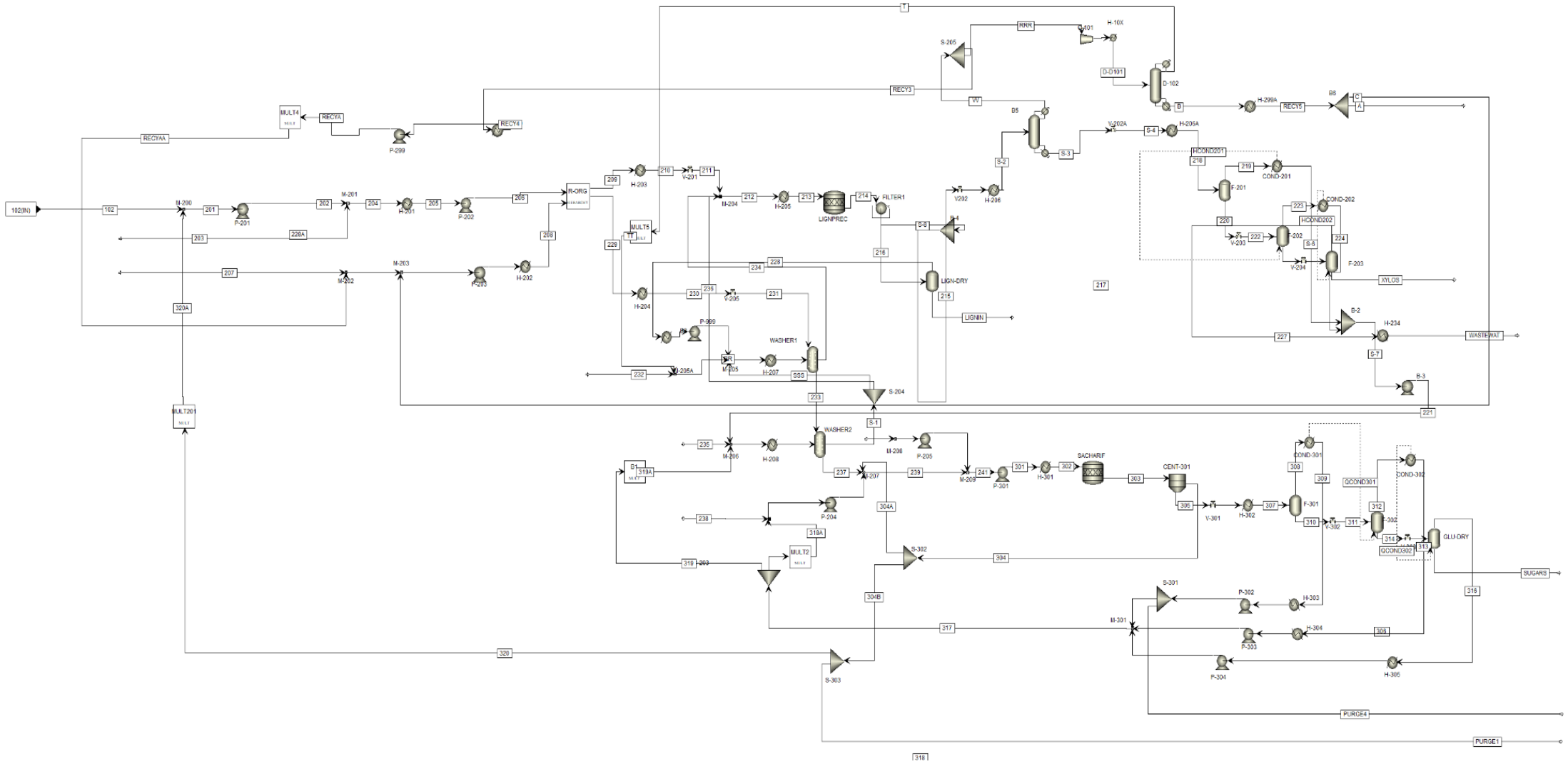

Figure 31. Process optimisation flowsheet.

The results show that the consumption of ethanol and water are greatly reduced with this new process design. A reduction of about 98.4% of ethanol is achieved, from 125328 kg/h to 1964 kg/h. Likewise, freshwater needs are reduced by more than 70%, from 411924 kg/h to 118950 kg/h. By analysing the pinch results, it is clear that energy integration (check Figure 32) can decrease the overall energy consumption, as the total utilities energy values without integration were about 43.7% higher than the minimum achievable. Both hot and cool utilities attained energy saving in the order of 447 GJ/h, while the overall energy saving in utilities were 839.8 GJ/h. In terms of energy demand per kg of product obtained (C5 and C6 sugars, lignins and XOS), heat integration allow us to reduce down to 20.8 MJ/kg-product. This value compares well with the literature, since Kautto et al. (2013) reports energy consumption of 22 MJ/kg-product for an ethanol organosolv process and Humbird et al. (2011) reports 26 MJ/kg-product with the diluted acid process. Overall, the new design here obtained of the organosolv process has an improved performance not only in terms of energy efficiency, but also with lower environmental impact since carbon emissions attained a value of 6430 kg/h against the previous 114300 kg/h through extensive heat and mass integration.

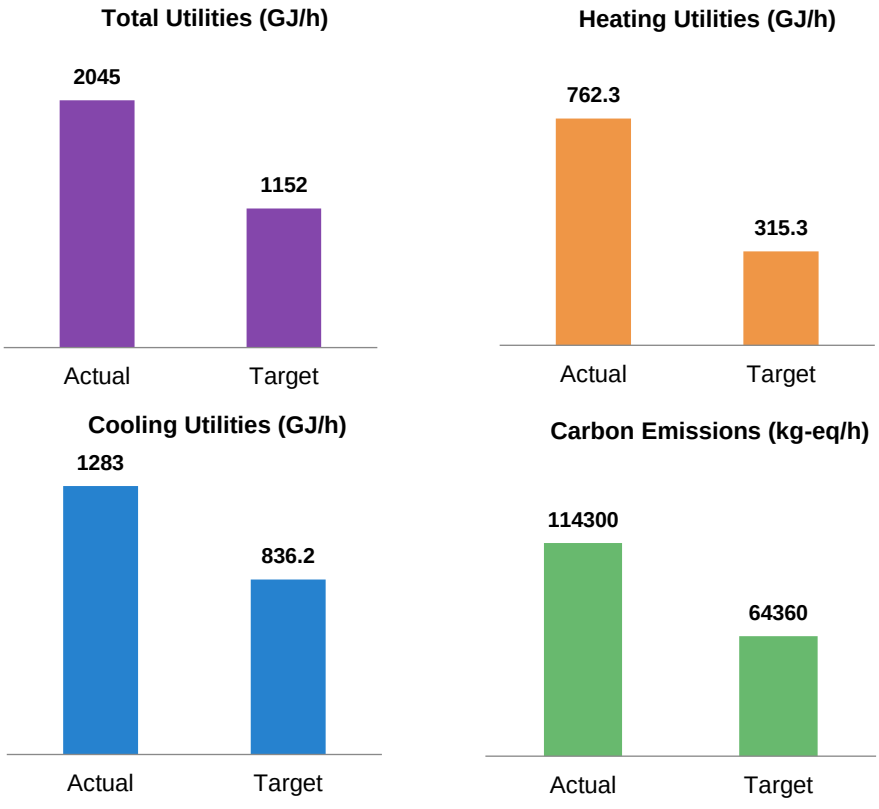

Figure 32. Pinch analysis results.

5. Conclusions and prospects

Despite its abundance and easy access, agroforestry residues are still lignocellulosic materials with few valorisation and applications at industrial level. Alternative and efficient conversion processes to these biomasses are important opportunities for the production of value-added products.

Among the fractionation process options usually considered, the ethanol-based **organosolv process** is one of the most relevant, presenting a potential high industrial applicability, as it enables to take full advantage, of the selective recovery of all biomass fractions namely, hemicellulose, cellulose and lignin. Nevertheless, there are still significant hindrances, as this process requires relatively high temperatures in order to achieve proper efficiency. This is translated into high capital and operational costs that have to be minimized in order that organosolv processes become a real valid industrial option for the biorefineries.

In this work, a series of organosolv fractionation processes were studied, as well as their integration, in order to selectively fractionate lignin, hemicellulose and cellulose from wheat straw and eucalyptus residues. These alternative processes still need improvements as they have no industrial application today. These will include the decrease of operation temperatures and the minimization of catalysts use in order to improve biorefinery economic sustainability, as that could significantly reduce overall operational costs.

In a first approach, an organosolv process was applied to agroforestry residues, eucalyptus and wheat straw using as solvent ethanol in a 50:50 (w/w) solution with water, for different isothermal periods from 0 min to 2h, at 190ºC. With this non-catalysed process, relatively high delignification yields were obtained for the 2h treatments for both feedstocks, 59% and 70.3%, respectively for wheat straw and eucalyptus residues. Simultaneously nearly half of the hemicellulose was recovered in the liquid phase which contain relevant concentrations of XOS (15 and 34.1 g/100g initial xylan for both wheat straw and eucalyptus residues). Cellulose was almost not affected by this process and about 90% of the initial cellulose in both biomasses, is recovered in the solid phase which is enriched in glucan. This solid phase proceeded to enzymatic hydrolysis with commercial cellulases (CellicTec2), achieving saccharification yields of 68.5% and 71.7% for WS and ER respectively. The resulting stream can be further used in applications related to bioconversion processes, namely in the production of bioethanol, organic acids.

Eucalyptus is out of both biomass the one that exhibited the most interesting behaviour in a non-catalysed organosolv process as it exhibited higher xylan solubilisation, higher delignification and higher saccharification. Therefore, in a second stage, those residues were submitted to organosolv pre-treatments at mild temperature (140ºC), low catalyst (sulphuric acid) concentration and different reaction times that were optimized using a Doehlert experimental design. The process conditions were studied aiming at the selectively removal of lignin and hemicellulose, producing soluble lignin and hemicellulosic sugars, as well as an easily digestible

cellulose containing solid. In all conditions tested, a low production of C6 sugars was obtained, with glucan being almost not affected by these treatments. Maximum glucan solubilisation was below 10%. Eucalyptus fractionation proceeds quite fast and for both moderate catalyst concentrations and reaction times almost a complete hydrolysis of hemicellulose occurs, together with a high delignification yield. The process was modelled by a quadratic polynomial equation, that enable the definition of the optimal conditions. The higher delignification yields, >84% were obtained at the centre of the experimental domain, corresponding to 25 mM sulfuric acid and 90 min reaction time. Comparing these data with the non-catalysed process at the same temperature, it is clear that catalyst plays an important role in the delignification process, as similar results can only be achieved without catalyst if the reaction temperature is considerably higher. The cellulose enzymatic digestibility of the processed solids was assessed and modelled based on hemicellulose and lignin removal. The higher yields (>85%) were mainly correlated with high delignification yields treatments and considerably higher to the ones found in the same non-catalysed biomass.

All the organosolv process (uncatalyzed and catalysed) studied gave a cellulose rich solid phase which undergo enzymatic hydrolysis with considerably satisfactory results. The liquid phase of the uncatalyzed organosolv processes were rich in XOS, a product with high market value. Thus, this data obtained for the experimental studies enable the start of a preliminary economic analysis, knowing in advance that the production of the compounds with high added value or high volume from low-cost products as eucalyptus residues increased the viability of the process. Therefore, the most economic viable processes turned out to be the eucalyptus organosolv at 190ºC without the addiction of a catalyst, making this processes data the basis for the construction of an industrial model in ASPEN®.

The ASPEN® model followed the experimental processes steps: organosolv cooking, filtration, enzymatic digestion for the solid phase and lignin and XOS recovery in the liquid phase. In the end this model presented a good fit with the experimental results, with divergences of 20% at maximum in some fractions constituents. Afterwards an optimisation was carried out in order to improved energy recovery and lower water consumption through heat and water integration.

Even though, the overall results presented in this thesis were quite satisfactory, there is still room for suggestions as complement of this study as well as development of new lines of work:

- Study of other organosolv conditions with different LSR, ethanol:water mixtures, catalyst types in order to try to reach an almost complete delignification of the material.
- Study the conditions for an almost complete enzymatic saccharification of cellulose-rich solids to produce high concentration sugar streams for fermentation purposes
- Chemical characterisation and purify evaluation of the lignins obtained.

- Applications and development of strategies to recover and/or purify XOS present in the hydrolysates.
- Applying an economic analysis to the model proposed.
- Application of the studied organosolv processes to other hardwoods residues mixtures

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Annexes

A – Mathematical Formulations

Moisture content

The moisture content (H, %) of the samples was calculated according to the following expression:

$$\mathrm{H}\ (\%) = \frac{\text{wet sample weight (g)} - \text{dry sample weight (g)}}{\text{wet sample weight (g)}} \times 100 \qquad (3)$$

Ash content

The ash content (Ash, %) of the samples was calculated according to the following expression:

$$\text{Ash}\ (\%) = \frac{\text{Ash weight (g)}}{\text{dry sample weight (g)}} \times 100 \qquad (4)$$

Extractives content

The extractives content (Extractives, %) of the samples was calculated according to the following expression:

$$\text{Extractives}\ (\%) = \frac{\mathrm{w} - w_0}{\mathrm{A}(1 - \mathrm{H})} \times 100 \qquad (5)$$

In which:

w = weight of balloon + extractives. after each extraction (g)

w_0 = weight of balloon (g)

A = weight of sample (g)

Protein Determination

The total nitrogen (Total nitrogen, %) of the samples was calculated using the following expression:

$$\text{Total nitrogen}\ (\%) = 0.14 \cdot \left(\frac{\mathrm{V} - \mathrm{V}_0}{\mathrm{A}'}\right) \qquad (6)$$

Where:

V = Volume of 0.1N HCl solution spent during sample titration (mL)

V_0= Volume of 0.1N HCl, spent during titration of blank (mL)

A’ = weight of sample (dry weight) (g)

The conversion factor used in protein calculation was N × 6.25

Quantification of polysaccharides and lignin in solid samples

Glucan, xylan, arabinan and acetyl groups content (%) were calculated from the concentrations of glucose, xylose, arabinose and acetic acid in the raw samples and solid residues after quantitative acid hydrolysis. Kalson lignin was quantified after the correction for ash content of the acid-insoluble residue.

A significant percentage of monosaccharides is degraded during the quantitative acid hydrolysis, providing a need for correction factors to take into account those losses.

$$\mathrm{Gn} = \mathrm{F} \times \frac{100}{1005} \times 0.9 \times \frac{\mathrm{Glc} \times \mathrm{P_{sol}}}{\mathrm{A'}} \qquad (7)$$

$$\mathrm{Xn} = \mathrm{F} \times \frac{100}{1005} \times 0.88 \times \frac{\mathrm{Xyl} \times \mathrm{P_{sol}}}{\mathrm{A'}} \qquad (8)$$

$$\mathrm{Arn} = \mathrm{F} \times \frac{100}{1005} \times 0.88 \times \frac{\mathrm{Ara} \times \mathrm{P_{sol}}}{\mathrm{A'}} \qquad (9)$$

$$\mathrm{Ac} = \frac{100}{1005} \times \frac{60}{61} \times \frac{\mathrm{HAc} \times \mathrm{P_{sol}}}{\mathrm{A'}} \qquad (10)$$

$$\mathrm{LK} = \frac{\mathrm{AIS} - \mathrm{C}}{\mathrm{A'}} \times 100 \qquad (11)$$

Where,

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

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

$\frac{60}{61}$ represents the stoichiometric conversion factors of monomers into polysaccharides for the acetyl groups.

F stands for the correlation factor for sugar degradation. which takes the values of 1.04 for glucose and1.09 for xylose and arabinose.

P_{sol} an A' are the weighs of the solution and the sample dry weigh, respectively.

AIS and C are the weights of acid-insoluble residue and the ashes in the sample, respectively (g).

Polymers Yields

The amount of Kalson lignin and acetyl groups that remain in the solid residues after hydrolysis was calculated by applications of the equations as follows:

$$\mathrm{Gn_R} = \frac{\mathrm{Gn} \times \mathrm{SY}}{\mathrm{Gn_{RM}}} \qquad (12)$$

$$\mathrm{Xn_R} = \frac{\mathrm{Xn} \times \mathrm{SY}}{\mathrm{Xn_{RM}}} \qquad (13)$$

$$\mathrm{Arn_R} = \frac{\mathrm{Arn} \times \mathrm{SY}}{\mathrm{Arn_{RM}}} \qquad (14)$$

$$\mathrm{Ac_R} = \frac{\mathrm{HAc} \times \mathrm{SY}}{\mathrm{Ac_{RM}}} \qquad (15)$$

$$\mathrm{KL} = \frac{\mathrm{LKn} \times \mathrm{SY}}{\mathrm{KL_{RM}}} \qquad (16)$$

Where, Gn_R, Xn_R, Arn_R, Ac_R and KL represent the percentages of glucan, xylan, arabinan, acetyl groups and Klason lignin that remain in the residue after the organosolv processing (g/100g of the respective initial component);

Gn_{RM}, Xn_{RM}, Arn_{RM}, Ac_{RM} and KL_{RM} represent the percentages of glucan, xylan, arabinan, acetyl groups and Klason lignin on the raw materials, respectively (g/100 g of raw material);

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

The percentage of each of the polysaccharides and Ac solubilized into monomers, as well as the percentage of monomers converted into the respective degradation products was attained according to the following equations:

$$\mathrm{Glc_s} = \frac{162}{180} \times \frac{\mathrm{Glc} \times \mathrm{W_H}}{\mathrm{Gn_{RM}} \times \mathrm{A} \times 0.01.\rho_{\mathrm{H}}} \times 100 \qquad (17)$$

$$\mathrm{Xyl_s} = \frac{132}{150} \times \frac{\mathrm{Xyl} \times \mathrm{W_H}}{\mathrm{Xn_{RM}} \times \mathrm{A} \times 0.01.\rho_{\mathrm{H}}} \times 100 \qquad (18)$$

$$\mathrm{Ara_s} = \frac{132}{150} \times \frac{\mathrm{Ara} \times \mathrm{W_H}}{\mathrm{Arn_{RM}} \times \mathrm{A} \times 0.01.\rho_{\mathrm{H}}} \times 100 \qquad (19)$$

$$\mathrm{HAc_s} = \frac{60}{61} \times \frac{\mathrm{HAc} \times \mathrm{W_H}}{\mathrm{Ac_{RM}} \times \mathrm{A} \times 0.01.\rho_{\mathrm{H}}} \times 100 \qquad (20)$$

$$Furf_s = \frac{132}{96} \times \frac{Furf \times W_H}{Xn_{RM} \times A \times 0.01.\rho_H} \times 100 \qquad (21)$$

$$HMF_s = \frac{162}{126.1} \times \frac{HMF \times W_H}{Gn_{RM} \times A \times 0.01.\rho_H} \times 100 \qquad (22)$$

Where,

Glc_s, Xyl_s, Ara_s, HAc_s $Furf_s$, HMF_s, are the percentages of glucose, xylose, arabinose, acetic acid, furfural and 5-hydroxymethylfurfural recovered in the hydrolysate (g/100g of polysaccharides/HAc/Furf/HMF resent in raw material);

Furf and HMF are concentrations of furfural and 5-hydroxymethylfurfural in the liquors (g/L);

WH is the mass of hydrolysate obtained in the assay (g);

ρ_H is the density of hydrolysate (g/L).

Oligosaccharides

The percentage of oligosaccharides with glucose (GOSS), xylose (XOSS) and arabinose (AOSS) from recovery liquors (g/100g (w/w) of glucans, xylans and arabinans, respectively) were calculated according to the following equations:

$$GOS_S = \frac{Gn \cdot P_H}{Gn_{RM} \cdot A'} \cdot 100\text{-}Glc_S \qquad (23)$$

$$XOS_S = \frac{Xn \cdot P_H}{Xn_{RM} \cdot A'} \cdot 100\text{-}Xyl_S \qquad (24)$$

$$AOS_S = \frac{Arn \cdot P_H}{Arn_{RM} \cdot A'} \cdot 100\text{-}Ara_S \qquad (25)$$

Where,

Gn, Xn, Arn are the percentages of glucans, xylans and arabinans, expressed in g/100g (w/w) of solid;

GnRM, XnRM and ArnRM are the percentages of glucans, xylans and arabinans in raw-material, expressed in g/100g of polysaccharides in raw-material;

GlcS, XylS and AraS are the percentages of glucose, xylose and arabinose recovery in liquors, expressed in g/100g of polysaccharides in raw-material;

P_H is weight of hydrolysate obtained in each test realized (g);

A' is the sample dry weight realized in each test

Enzymatic hydrolysis

The percentage of Glucan conversion into Glucose (GC) and Xylan conversion into Xylose (XC) was calculated as follows:

$$\mathrm{GC}\ (\%) = \frac{0.90 \times [[\mathrm{Glucose\ Released}] - \mathrm{blank}\ ]}{[\mathrm{Substrate}] \times \%\ \mathrm{Glucan\ Content}} \times 100 \qquad (26)$$

$$\mathrm{XC}\ (\%) = \frac{0.88 \times [[\mathrm{Xylose\ Released}] - \mathrm{blank}]}{[\mathrm{Substrate}] \times \%\ \mathrm{Xylan\ Content}} \times 100 \qquad (27)$$

For the Concentration of Released Sugars. the assessed concentrations should be corrected with the blank assays as follows as shown. The terms 0.90 and 0.88 correspond to the stoichiometric conversion factors of monomers into polysaccharides for glucose and xylose, respectively.