Universidad de Valladolid

Escuela de Doctorado Universidad de Valladolid

PROGRAMA DE DOCTORADO EN INGENIERÍA QUÍMICA Y AMBIENTAL

TESIS DOCTORAL:

Hemicellulose production using hot pressurized water: from lab to pilot scale.

Presentada por Gianluca Gallina para optar al grado de
Doctor/a por la Universidad de Valladolid

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Universidad de Valladolid

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Siendo los tutores en la Universidad de Valladolid

Memoria para optar al grado de Doctor,

con Mención de Doctorado Internacional

presentada por El Ingeniero Químico

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Valladolid, Septiembre de 2017

Universidad de Valladolid

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Que el Ingeniero Químico GIANLUCA GALLINA ha realizado en el Departamento de Ingeniería Química y Tecnología del Medio Ambiente de la Universidad de Valladolid, bajo nuestra dirección, el trabajo que, para optar al grado de Doctorado Internacional, presenta con el título "*Hemicellulose production using hot pressurized wáter: from lab to pilot scale.*", cuyo título en castellano es "*Producción de hemicelulosa utilizando agua caliente presurizada: desde escala laboratorio hasta escala piloto* ", siendo el Prof. Tapio Salmi su tutor durante la estancia realizada en Åbo Akademi University (Finlandia).

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Reunido el tribunal que ha de juzgar la tesis doctoral titulada "*Producción de hemicelulosa utilizando agua caliente presurizada: desde escala laboratorio hasta escala piloto*" presentada por el ingeniero Gianluca Gallina y en cumplimiento con lo establecido por el Real Decreto 99/2011 de 28 de enero de 2011 acuerda conceder por_____ la calificación de _____.

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The author was financially supported by Spanish Economy and Competitiveness Ministry with the scholarship/predoctoral contract BES-2013-063556, Project FracBioFuel: ENE2012-33613 and Project BioFraHynery: CTQ2015-64892-R.

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ABSTRACT

HEMICELLULOSE PRODUCTION USING HOT PRESSURIZED WATER: FROM LAB TO PILOT SCALE.

The climate change, the decline in fossil resources and the growing demand for energy are some of the main problems of our society. These issues could be cushioned with a greater and responsible use of renewable resources. In addition, it is imperative to optimize the use of resources, creating high benefits and lower amounts of waste. The concept of lignocellulosic biorefinery is based on this idea, with the objective of using agricultural and forest waste to produce energy, fuel and chemicals. An ideal biorefinery, in addition to using renewable raw materials and "green" solvents, would not produce any harmful products for health or the environment.

A promising, clean and cheap way to extract biocompounds and biopolymers consists of treating biomass with liquid pressurized water above 100 ºC.

Depending on the compound to extract, different temperatures are needed. The selective removal of hydrosoluble extractives requires temperatures around 100 °C, the extraction of hemicellulose between 140 and 190 °C, while the extraction of cellulose requires temperatures above 240 ºC. In the liquid phase, the extracted compounds undergo a hydrolysis and degradation process in which, depending on temperature and residence time, oligosaccharides are depolymerized to monosaccharides and then to degradation products. Temperature and residence time are therefore fundamental parameters to increase the selectivity towards certain products than others.

The aim of this thesis is to investigate the possibility of bringing the liquid hot water process, for hemicellulose extraction and transformation into high value compounds, closer to the industrial level.

The route started with investigating the process at a laboratory scale, studying the fractionation of wood chips from *Eucalyptus globulus* with a flow through reactor.

This setup has been chosen due to its many advantages over batch systems and continuous systems. Respect to batch, mass transfer is improved in flow through reactors, thanks to the continuous supply of fresh water to the system; moreover, the rapid removal of products from the system, prevents their degradation. Unlike in continuous reactors, flow-through reactors do not require solid pumping and extreme milling of biomass.

The process was studied at temperatures ranging from 140 to 285 ºC, and liquid flow-rates between 2.5 mL/min and 20 mL/min, identifying the optimal conditions for extracting hemicellulose without obtaining degradation products.

In the second step of our research, we investigated the possibility of degrading selectively the extracted products, to obtain high value chemicals. Flow through reactor was connected in series with a continuous reactor: a flow of hot pressurized water extracted biocompounds from biomass in the first unit, and subsequently it was mixed with a stream of supercritical water in the second unit, where the reaction time was precisely controlled, and extracted compounds were hydrolysed and degraded.

In the flow-through reactor, fractionation of holm oak wood chips was performed in two stages: optimizing the hemicellulose extraction, at 180 ºC, and subsequently extracting cellulose and hemicellulose stronger associated to cellulose, remaining in the biomass structure at 260 ºC. Hydrolysis of extracted compounds was performed in the continuous reactor by a water stream with temperatures between 350 and 400 ºC. The temperature, pressure and reaction time were modified to tune the selectivity of the reaction.

In addition to defining the operating conditions to optimize hydrothermal hydrolysis, it is important to identify which types of biomass are best candidates to obtain high product yields. In this thesis we chose wood as feedstock, due to its high and non-seasonal availability, its low content in inorganic compounds and its ease cultivation with respect

to crops. However, woods from different species have different compositions and different structures, which allows different products to be obtained.

In the third part of this path, we analysed the composition of wood from 10 different species of tree, typical of the Castilla y Leon region. Hydrothermal extraction of hemicelluloses contained in the different woods, was performed at 160 ºC using a batch-wise cascade reactor, located in Åbo Akademi (Finland). The objective was to seek the wood species that allowed to obtain a high concentration, yield and/or molecular weight of hemicelluloses. Finally, by analysing the structure of the raw materials through TGA analysis, we identified a relationship between composition, structure of the raw material and final yield in hemicelluloses.

The experience and knowledge accumulated during the first period of the thesis, brought us closer to the main objective, which was to scale the hydrothermal hydrolysis process and approach it to an industrial level.

The fourth step of this route was therefore to scale-up the laboratory scale flow-through reactor, to design and build a pilot plant. A one pilot reactor system, with a volume 72 times larger than the laboratory scale was built, and subsequently upgraded to become a manifold system composed of 5 flow-through reactors capable of working in series. This novel apparatus permitted a continuous operability, minimizing downtime when replacing the biomass during loading and unloading phases, fundamental characteristics in an industry. To verify the homogeneous working of the plant, a study was performed to determine the evolution of the composition and molecular weight of the extracted solution, by varying the temperature (140, 150, 160 and 170 ºC) and the residence time of the liquid phase and the solid phase within the system.

ABSTRACT

After checking the effectiveness of the pilot plant's operation, we identified the features and advantages that distinguish it from existing installations with the same purpose. The fifth step in our work was the writing of a patent to protect our technology and increase its value at an industrial level.

Finally, as sixth and final step, we explored the potential commercialization of one of the possible products that can be obtained by the processing of the effluent obtained with our technology.

The idea is to produce a sweetener based on xylitol, obtained through the hydrolysis and hydrogenation of the hemicellulose extracted with hot pressurized water, from lignocellulosic biomass. A business plan was made, based on the selling of this sweetener to industries producing confectionery, soft drinks and candies. Mutual benefits are considered: farmers, can sell their waste, otherwise destined to be burned, while customers can get sugar-free products with benefits on health and environment.

INTRODUCTION

HEMICELLULOSE PRODUCTION USING HOT PRESSURIZED WATER: FROM LAB TO PILOT SCALE.

The rapid growth of the human population and the resulting rising demand for food, energy and water are the most serious challenges that the world is facing.

Climatic change is another severe threat to mankind and requires a significant reduction of greenhouse gases to avoid destructive consequences for the environment.

On December 2015, in Paris, COP21, the 21st session of the UN Conference on Climate Change (UNFCCC), found a historic agreement to counter the threat of climate change. The agreement, called the Paris Agreement, will come into force in 2020 when the Kyoto Protocol declines. The agreement took the goal of limiting the rise in global average temperature "below 2 ° C and continuing efforts to limit temperature increase to 1.5 ° C". This implies that by 2050 a fully de-carbonized energy sector has to mature: three quarters of energy should be produced from zero emission sources.

Throughout many discussions driven by the Chemical Industry Community at the World Economic Forum in 2008 and 2009, industrial biorefineries were identified as a possible solution that can help mitigate the threat of climate change and the seemingly unlimited demand for energy, fuels, chemicals and material [1].

The biorefinery concept is analogous to the concept of oil refinery, and assumes the production of fuel, chemicals and energy from different types of biomass. The choice of which type of biomass to treat can be influenced by economic, environmental or geographic factors; there is also a direct dependence between the raw material, the technology used for its conversion into a usable output and the range of products that can be obtained.

First-generation technologies are based on fermenting and distilling the glucose contained in crops as corn or sugarcane to produce ethanol. Criticisms towards the expansion of

biomass use are mainly directed to this type of technology, blamed for providing serious competition for food production [2].

Lignocellulosic biomass is considered the most promising source for the production of bio-fuels and chemicals. Lignocellulosic materials belong to second-generation feedstocks, and can be obtained from various sources, such as wood residues, agricultural or municipal waste, not interfering with direct crops for human consumption. They are composed mainly of lignin, cellulose and hemicellulose, associated in a resistant structure, whose breakup requires a considerable amount of energy; however, thanks to their differentiated composition, allow to obtain multiple products, like high value chemicals and low value but high volume fuels [3].

Figure 1 shows an outline of the concept of a lignocellulosic biorefinery. Raw materials can be pre-treated and fractionated into cellulose, hemicellulose and lignin. Lignin phenolics can be used to produce materials like plastics or adhesives, glucose from cellulose can be converted to methanol or chemicals, while other chemicals, fuels, polymers and materials can be obtained from hemicellulose. Waste cellulose, hemicellulose and lignin, moreover, can be used for cogeneration of power and heat [4].

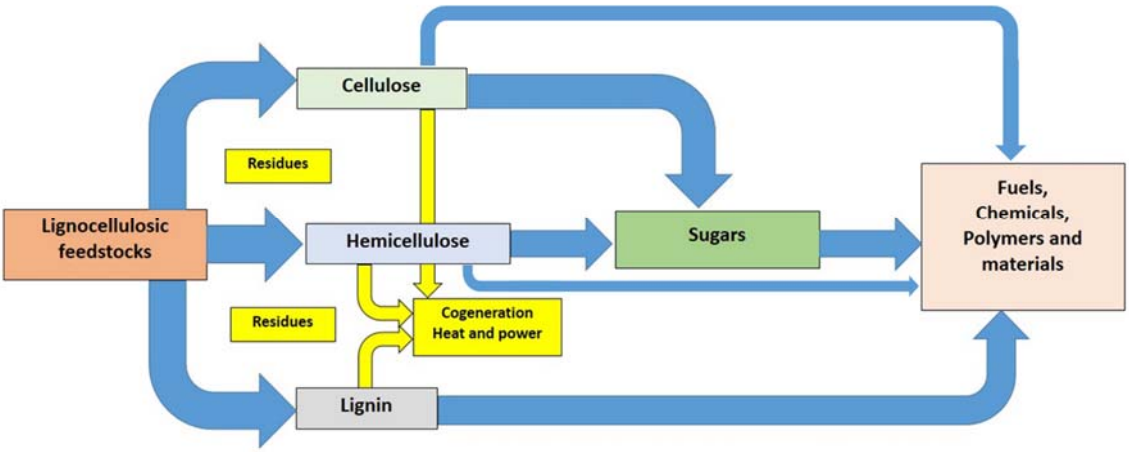

Figure 1. Conceptual scheme of lignocellulosic biorefinery.

Biomass structure and composition

Besides cellulose, hemicelluloses and lignin, most biomasses contain a mixture of other components such as starch, pectins, organic acids, free sugars and other extractives like chitin, proteins, fats and waxes. Laboratory analytical procedures for identifying the composition of biomass were described by the National Renewable Energy Laboratory (NREL), the leading national laboratory of the US Department of Energy, devoted to research and development on renewable energies and energy efficiency [5-7].

It is important to know the structure and composition of these molecules, as the choice and effectiveness of biomass valorization processes depends on them. A brief description of the main constituents of lignocellulosic biomass is given below.

Extractives

Extractives are defined as non-structural components of biomass (non-chemically bound), which are soluble in either water or ethanol during exhaustive extraction. This components include sucrose, nitrate/nitrites, protein, ash, chlorophyll, waxes, terpenes (phenols, hydrocarbons), fatty acids (fats, oil, waxes, resins, resin acids, sterols), colouring matter (phlobaphenes, tannins, stilbenes), inorganic material (calcium, potassium, magnesium, sodium) [8].

Soxhlet method is generally used to perform an exhaustive extraction, which means that most or all of the water and ethanol soluble material has been extracted from the biomass. This process take about 24 hours using water and 24 hours with ethanol.

In the characterization of biomass, it is important to remove the extractives as a first step, because their presence could potentially interfere with the downstream analysis of the biomass sample. Hydrophobic extractives may lead to errors in the determination of

structural sugar, inhibiting the penetration of the sulfuric acid into the sample resulting in incomplete hydrolysis [6].

Pectins

Pectins are heterogeneous polysaccharides present in most primary cell walls and middle lamella, especially in non-woody terrestrial plants. However, up to 10% of pectin can be found in tension wood and is even more abundant in bark, where it plays a part in early stages of cell development.

Pectins main constituent is D-galactouronic acid with residues of D-galactose, L-arabinose, and L-rhamnose [9].

Starch

Starch is a polysaccharide produced as an energy store by most green plants, and consists of a large number of glucose units joined by glycosidic bonds. It consists of two types of molecules: linear amylose and the branched amylopectin. Depending on the plant, starch generally contains 20 to 25% amylose and 75 to 80% amylopectin by weight. They both have very high molecular mass, but can be easily solubilized in hot water (below 100 ºC) [10].

Hemicelluloses

Hemicelluloses are a branched polymer consisting in short chains of about 500 to 3000 units of different monosaccharides [11]. They form covalent bonds with lignin, hydrogen-bonds with cellulose and ester linkages with acetyl groups.

Under acidic conditions, hemicelluloses can be quite easily hydrolysed to different pentoses, exoses and sugar acids like D-xylose and L-arabinose; D-mannose, D-galactose and D-glucose; glucuronic acid, 4-O-methyl-D-glucuronic acid and D-galacturonic acid [12].

From their hydrolysis products, the hemicelluloses take the respective names of xylans, arabino-galactans, galacto-mannans, glucurono-xylan, arabino-xylan, gluco-mannan, xylo-glucan, ecc.

Different species of plant contain different hemicelluloses.

Hemicelluloses from *gramineae* such as cereal straws and grasses are mainly constituted by arabino-glucurono-xylans [13], softwoods mainly contain galacto-gluco-mannans where the hydroxyl groups at C-2 and C-3 in the chain are substituted by O-acetyl groups; hardwoods predominant hemicellulose is glucurono-xylan where the 60% of xylose units carry an acetyl group attached either to the C-2 or C-3 position [14]. Softwoods have a high proportion of mannose units and more galactose units than grasses and hardwoods, while hardwoods have a high proportion of xylose units and more acetyl groups.

Cellulose

Cellulose $(C_6H_{10}O_5)n$ is the most abundant bio polymer on the world, consisting the 40–50% of wood species structure. It presents a crystalline structure, with linear molecules composed by 7000 to 15000 units of glucose which condense through β(1→4)-glycosidic bonds [11]. Hydroxyl groups on the glucose molecules from one chain form hydrogen bonds with oxygen atoms on a neighbor chain, forming microfibrils with crystalline or amorphous regions, depending on the intra and intermolecular hydrogen bonds between cellulose molecules. The crystalline structure have high tensile strength and is difficult to break compared to other polysaccharides.

Lignin

Lignin is a complex three-dimensional bio-polymer with a complex and sophisticated structure. It constitute the cell wall of plants, filling the space between cellulose, hemicellulose, and pectin components, especially in vascular and support tissues

Chemically, lignins are cross-linked phenolic polymers, and are the most abundant source of aromatic compounds in nature, representing the 30% of the organic carbon in the biosphere [15] .

The composition of lignin varies from species to species. Softwood lignins are usually referred to as guaiacyl lignins because more of the 90% of their structural elements are derived principally from coniferyl alcohol; hardwood lignins are referred as guaiacyl–syringyl lignins, and are composed of coniferyl alcohol and sinapyl alcohol-type units in varying ratios; grass lignins are also classified as guaiacyl–syringyl lignins, butunlike hardwoods lignins thei contain p-coumaric, hydroxycinnamic, and ferulic acid residues attached through ester and ether linkages [16].

Wood: structure and composition

Wood is a structural tissue forming the stem and the roots of the so called woody plants (mainly trees, but also shrubs and lianas). It constitutes the xylem, one of the two transport tissue in vascular plants (together with phloem) which function is to transport water and some nutrients from roots to leaves. Among the various lignocellulosic biomasses, wood is an important renewable resource, as it derives from long-living plants with non-seasonal character and with huge availability; no intensive use of fertilizers and pesticides have to be done for the growth of trees, and they generally contain less inorganic substances compared to crops [17].

Despite that, there are many characteristics (economical and anatomical) that have to be taken into account when thinking on which kind of wood to use in a biorefinery process.

1. Chemical composition (cellulose, hemicellulose, lignin and extractives)
2. Wood species

3. Age of the tree and harvesting location.
4. Bark content
5. Moisture content

The composition of wood varies according to species, age, and place where a tree has grown. Depending on whether the aim is to obtain a high quantity of cellulose, hemicellulose, lignin or other compounds it is important to carefully select the wood to be processed. The composition of hemicellulose and lignin also varies according to the species.

A first classification for tree species, which is also used for the deriving wood, distinguishes *hardwoods* (from Angiosperms, which reproduces by flowers and fruits) and *softwoods* (from Gymnosperms, which reproduces through unenclosed seeds). Hardwoods have vessel elements that transport water throughout the wood; under a microscope, these elements appear as pores. This elements are not visible in softwoods. Hardwoods have usually a slower growth rate than softwoods, and have a higher density. Hemicelluloses composition is also very different in softwood and hardwoods, as explained in the previous section.

Location where the trees grow, directly reflects on the structure of the fibers: wet, warm, and sunny locations contribute to accelerate the growth of tree and produce stiff fibers; while cold and dry locations produce trees with fine and dense fibers.

The age of the trees also influence the average length of the fibers, being longer as much as older is the tree.

Tree trunks and branches are protected from external agents by the bark, a layer that comprises about 10-20% of the stem. Chemical composition of bark consists of about 10-30 % of extractives, 15-45 % of polysaccharaides (cellulose and hemicelluloses), 15-40

% of lignin and other compounds like tannins, waxy materials and inorganic compounds. Because of its high content in "impurities", bark is usually removed in pulp processes.

Below the bark, in the trunks of the trees, two regions can be recognized: *sapwood*, the outer part, containing some living cells; and *heartwood*, the inner part of older trees, containing only dead cells [18]. Extractives amount is generally higher in heartwood, but moisture content is higher in sapwood [19].

Moisture is an important parameter, as one pays for the whole weight of wood, not on a dry-basis; for this reason, a low content of moisture is often desired.

Biomass valorization approaches

In order to valorize this heterogeneous raw material, three different approaches can be followed [20]:

1. Convert as much as possible of the components into low molecular weight building blocks, which can be used in the chemical industry. Operations like gasification and pyrolysis follow this straightforward strategy, which implies high costs even if it is the only possible technology for diluted streams.
2. Selectively convert single components into valuable chemicals that can be isolated after the reactions from the complex mixture. Enzymatic conversion of proteins contained in biomass into amino acids and electro-dialysis separation is an example of this procedure.
3. Fractionate the biomass into homogeneous blocks like cellulose, hemicellulose, extractives, or lignin and then degrade each of this platforms into smaller molecules like monosaccharaides, polyphenols and other base chemicals. The

same treatments, such as hot-water extraction, can be used both to separate the main blocks from biomass and to degrade them to smaller molecules.

The choice of the optimum process depends on the type of feedstocks, its economic assessment and environmental impact [20]. The third approach allows obtaining a differentiated range of products, from each of the fundamental constituents of the biomass: the fundamental problem is to achieve high selectivity and efficient product separation. The crucial step in then the separation of the major components of biomass ie hemicellulose, cellulose and lignin [21] .When the main purpose is to produce fuels, through the fermentation of the glucose that constitutes the cellulose in biomass, the separation processes are defined as pretreatments.

Biomass pretreatments

Different typologies of pretreatments can be conducted: physical, physic-chemical, chemical and biological pretreatments, as represented in Figure 2 [21].

Physical pretreatments are used to reduce the dimension of biomass particles. Several methods, such as milling, extrusion, ultrasound radiation can be used for this purpose. The cost of the operation depends on the size of the final particles, and thus on the energy required.

Physic-chemical pretreatments include steam- explosion, ammonia fiber explosion, liquid hot water process and microwave pretreatment.

Steam-explosion process consists in treating biomass with high pressure saturated steam (160-260 ºC and 7-480 bar) for a few minutes and then suddenly depressurizing to atmospheric pressure. After the process, hemicellulose is almost completely degraded into monosaccharaides, but also side-products like furfural, levulinic acid and 5-

hydroxymethylfurfural are generated [22]. Lignin structure is partially destroyed, increasing the potential of cellulose hydrolysis.

Ammonia fiber explosion consists in exposing biomass to liquid ammonia (1-2 Kg/ Kg of dry biomass) at moderate pressure and temperature (7-30 bar and 70-200 ºC) [23] for about 30 minutes of time and then suddenly depressurizing. This method is highly efficient in removing lignin and hemicelluloses, but it is not very efficient when species with high amount of lignin such as wood or nut-shells [24]. The cost of the operation is high due to the necessity to recover ammonia at the end of the process.

Liquid hot water pretreatments utilize water at 160-240 ºC, maintained liquid by the pressure [25], to extract hemicellulose from biomass and making cellulose more accessible for enzyme attach. Depending on the temperature, hemicellulose can be more or less degraded into monomers, and lignin removed by the biomass. Water is the only solvent used, making the process clean and environmentally sustainable.

Microwave pretreatments are found to be more efficient than conventional heating pretreatments [26], as reactions are accelerated and cellulose is also depolymerized.

Chemical pretreatments are usually used in paper industry and have the primary goal to remove hemicellulose and lignin from biomass.

Acid pretreatments with concentrated or diluted acids (commonly sulphuric acid) have been studied to extract completely the hemicellulose and hydrolyze it into monosaccharaides like xylose, which can be degraded to furfural. This kind of treatments require the recovery of the acid, which is toxic and corrosive.

Alkaline pretreatments use bases like sodium or potassium hydroxide to degrade and disrupting the structure of lignin, removing hemicellulose and also partially decrystallizing cellulose. A neutralizing step is required to remove lignin and inhibitors

like salts, phenolic acids and furfural. Solvents has to be recovered at the end of the process.

Ionic liquids are solvents generally consisting of salts which have a very low melting point, being liquids at ambient temperature. They have been confirmed to be very efficient in dissolving cellulose and lignocellulosic materials, even if they are not very efficient for biomasses with high lignin content. The study of this materials is still at the early stages, they are quite expensives and there are not many information about toxicological data and physic-chemical characteristics.

Biological pretreatments involves microorganisms such as bacteria or fungi to modify the structure of lignocellulosic biomass and degrading cellulose or lignin. This technique imply very low energy inputs, even if it is very slow and require careful controls and large spaces.

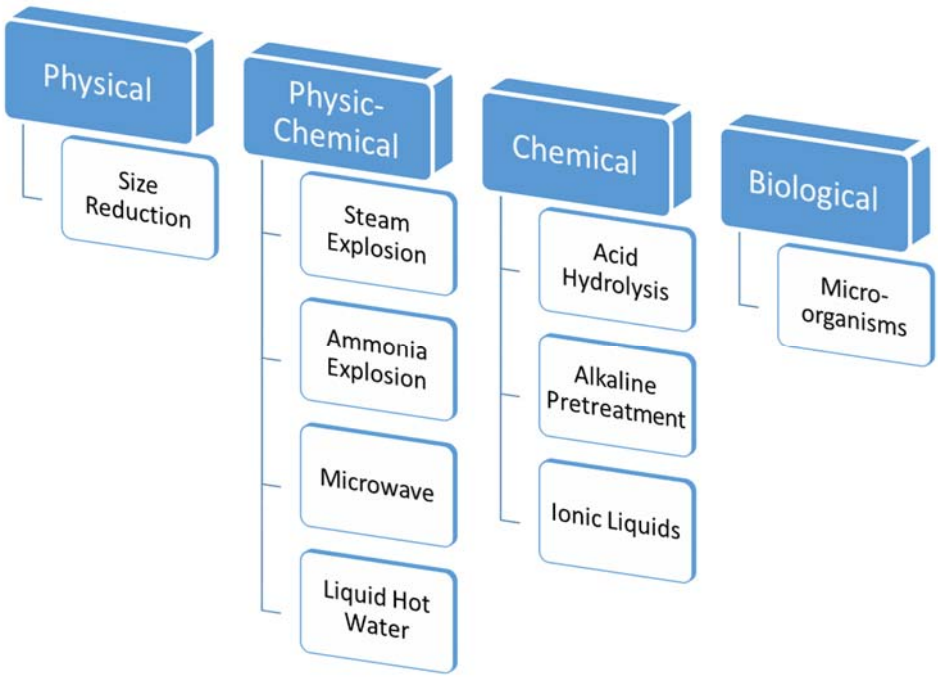

Figure 2. Pretreatments employed for lignocellulosic biomasses.

Liquid hot water extraction

Compared to other methods such as acid or alkaline pretreatments, hydrothermal pretreatments offers several advantages: no toxic or corrosive solvents are used, no special reactors are required, and there are not costs associated to the recovery or disposals of chemicals [27].

In hot water media, the fractionation of lignocellulosic biomass takes place in solid phase, where cellulose and hemicellulose start to break into oligomers, with a decrease of their molecular weight. When a certain molecular weight is reached, they became water-soluble and the hydrolysis proceeds both in liquid and in solid phase.[28, 29]

Depending on the temperature and on the residence time, the oligomers extracted undergo a hydrolysis process: they are depolymerised into monomers, which are subsequently decomposed in a broad range of products.

Due to the differences in structure, whereas temperatures between 140 and 190 ºC are sufficient for the extraction of hemicellulose, the depolymerisation of cellulose requires temperatures above 230 ºC.[30-32]

The main factors influencing the reactions of depolymerization and hydrolysis of lignocellulosic biomass in aqueous media are therefore:

- Temperature
- Residence/reaction time of the compounds inside the reaction medium
- pH at which the reaction takes place (i.e. proton concentration)

The different properties that pressurised water may assume varying these conditions, can be used to control the selectivity of the reactions, in other words, to tune the reaction.

Main reactions of structural carbohydrates in hot pressurized water

After the extraction, where molecules are detached from the solid phase, extracted oligomers are solubilized and start a hydrolysis and degradation process in the liquid phase.

The hydrolysis of hemicellulose in water begins at temperatures around 100 °C, the two principal reactions are represented in Figure 3 for xylans [33]:

- Acetyl groups bonded to oligomers are cleaved and reduced to acetic acid, catalysing the autohydrolysis of the oligomers.
- The oligomer chain is hydrolysed to obtain monomeric sugars, which are dehydrated to furfural at harsher conditions.

At temperatures above 100 °C hydrolysis of hemicellulose begins and can be more or less intense, depending on the composition of the treated biomass. Xylans from hardwood contain greater amounts of acetyl groups respect to softwoods and herbaceous plants [14]; for this reason, auto-hydrolytic capacity in hemicellulose from hardwood is much more intense compared to that of other species. The dehydration of xylose to furfural begins at around 170ºC, and gradually becomes more pronounced as the temperature of the water increases [34].

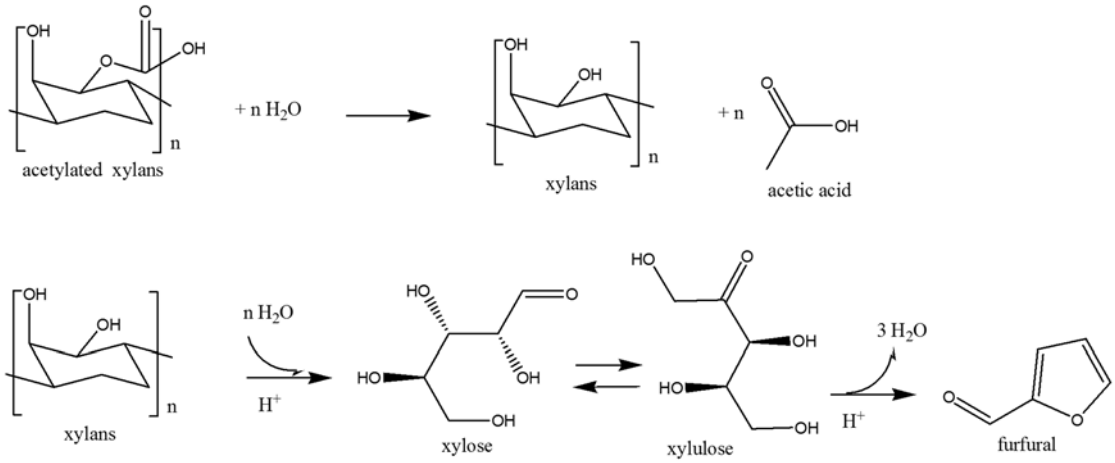

Figure 3. Hydrolysis of xylans in hot pressurized water.

As mentioned previously, cellulose structure presents a crystalline domain where strong intra-chain hydrogen bonds result in a stable and linear configuration of the fibrils [35] that require high temperatures for the depolymerization to glucose units. Once formed, among the glucose molecules, reactions of isomerization and dehydration take place at temperatures above 200 ºC [36].

Glucose undergoes isomerization to fructose, the reverse reaction (fructose to glucose) is almost inhibited. Fructose dehydration leads to the formation of 5-hydroxymethylfurfural and the HMF formation is directly proportional to the temperature rise of the medium until about 350 ºC. 5-HMF can follow principally two pathways: it can be decomposed into furfural and formaldehyde, or can be hydrated to form levulinic acid and formic acid. Reaction pathways are proposed in Figure 4.

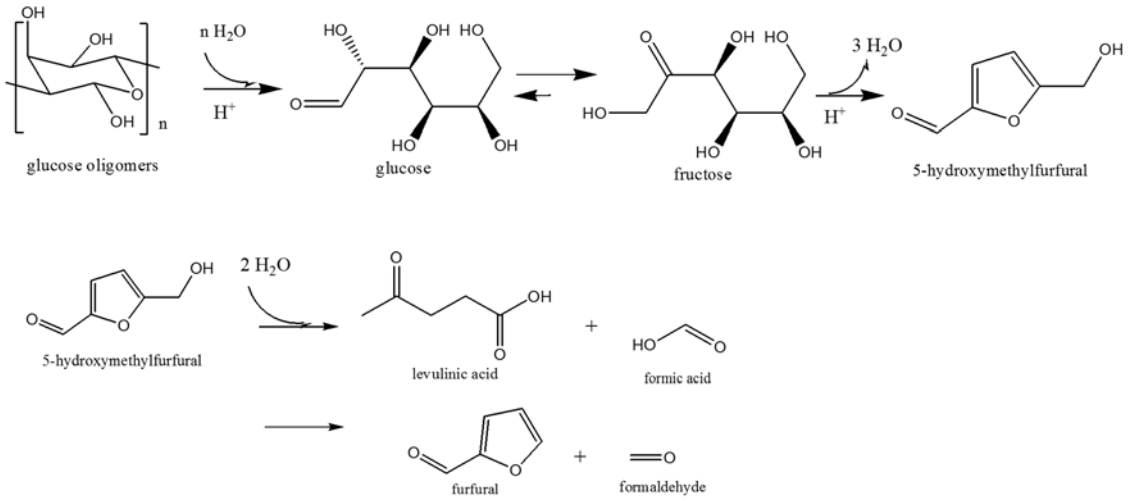

Figure 4. Dehydration of glucose oligomers from cellulose in hot pressurized water

In addition to the reactions listed above, the glucose molecule is also subject to aldol reactions, producing one molecule of two carbons and one molecule of four carbons; fructose produces two molecules of three carbons (like glyceraldehyde) [37].

Glucose, through an aldol reaction, can lead to the production of erythrose and glycolaldehyde. Fructose, through a first aldol reaction can produce glyceraldehyde and its isomer dihydroxyacetone. Another aldol reaction and subsequent dehydration can convert the glyceraldehyde into pyruvaldehyde, which can be further converted into lactic acid, with the loss of another water molecule. Dehydration as well as isomerisation reactions are favoured in acidic media [37], while aldol reactions are promoted in neutral media [38]. Principal aldol reactions involving glucose and fructose monomers are represented in Figure 5.

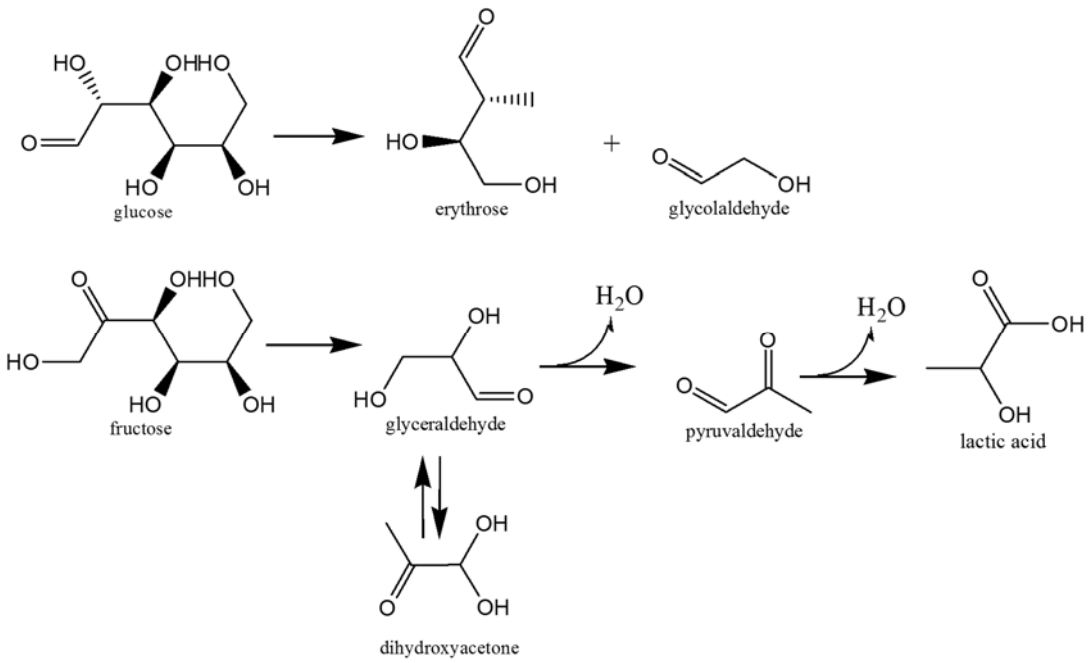

Figure 6. Aldol reactions of glucose and fructose in hot pressurized water.

Also xylose can participate in aldol reactions inside a hydrothermal medium, producing a molecule with three atoms of carbon and a molecule with two atoms of carbon (Figure 7). Glyceraldehyde, consequently, through another aldolic reaction can lead to the production of glycolaldehyde and formaldehyde, or can be isomerised into dihydroxyacetone.

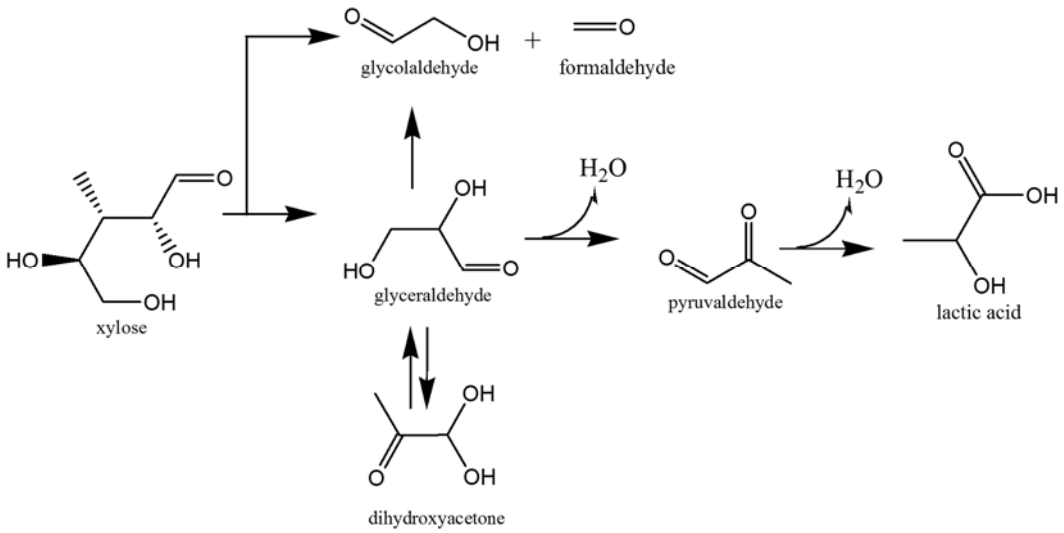

Figure 7. Aldol reactions of xylose in hot pressurized water.

Figure 8 shows schematically a simplified pathway for compounds resulting from the depolymerisation of cellulose and hemicellulose oligomers and decomposition of the monomers (xylose oligomers were chosen as representatives of hemicellulose oligomers) [39-45].

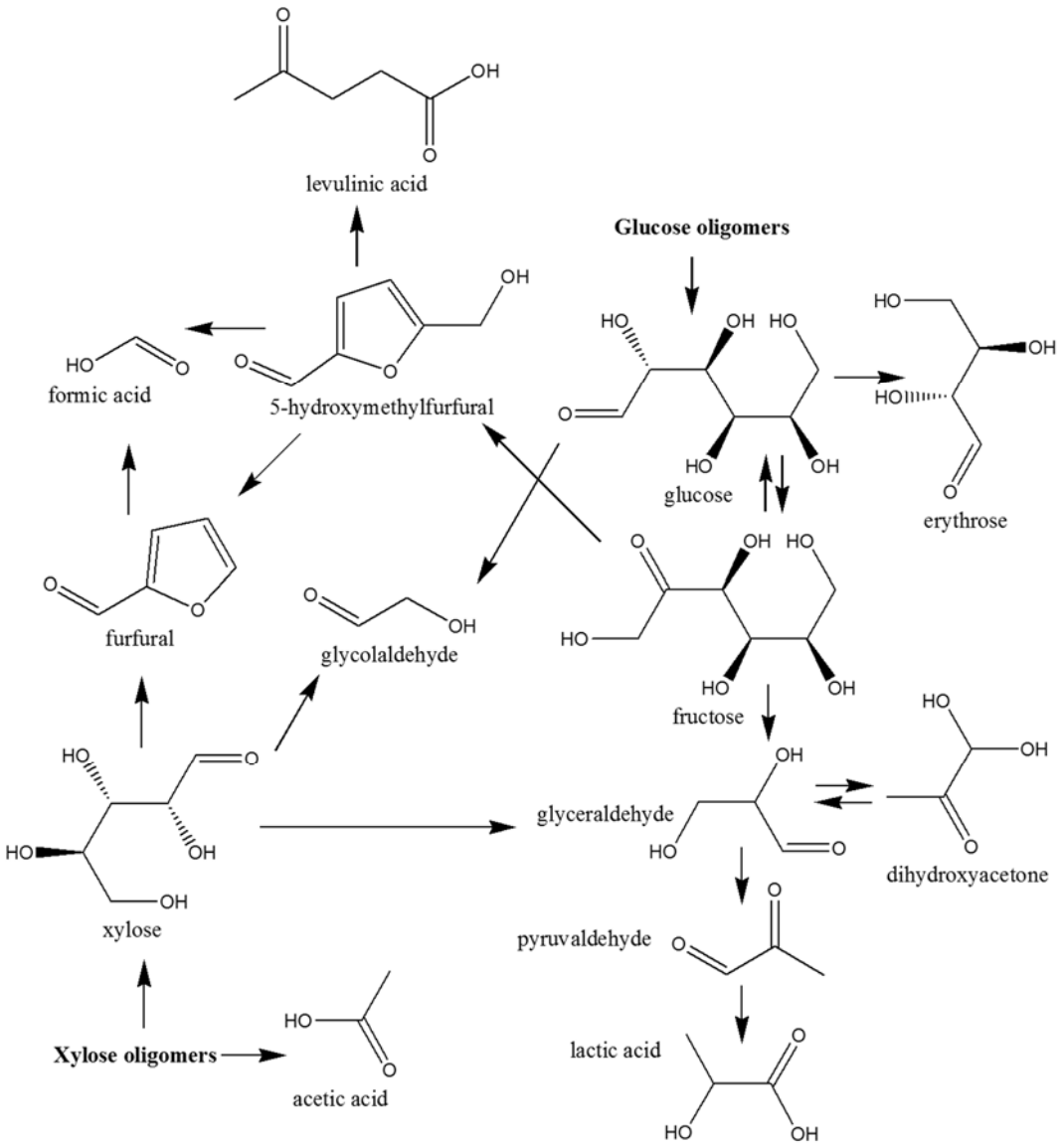

Figure 8. Reaction pathway for hemicellulose and cellulose oligomers in hot pressurized water.

Reactions in supercritical water

Water is under supercritical conditions at temperatures higher than 374ºC and pressures higher than 22.1 MPa. The intermolecular structure of water, at these conditions, varies significantly as hydrogen bonds are significantly reduced in number, giving both gas-like properties (like high diffusivity and low viscosity) and liquid-like properties like high density.

The dielectric constant is subject to significant variations, reaching at supercritical conditions values inside the common range of most organic solvents. At normal conditions of 25 °C and pressure of 1 bar, water has a dielectric constant of about 78 [46]. Figure 9 shows that at a pressure of 25 MPa bar and temperature of 375 °C the dielectric constant of water is around 12, and decrease rapidly at increasing the temperature. A range between 2 and 30 is typical for most organic solvents for dissolving organic macromolecules such as cellulose.

Another property which varies significantly at supercritical conditions is the ionic product of water (K_w). At ambient conditions, the value of K_w, represented as the product of H^+ and OH^- concentrations, is 10^{-13}, at temperatures around 300 ºC it reaches its maximum value (10^{-11}), which creates a medium with high ions concentration, favouring acid/basis catalysed reactions. Under supercritical conditions, K_w decreases drastically to 10^{-25} [47], promoting non-ionic reactions.[46, 48]

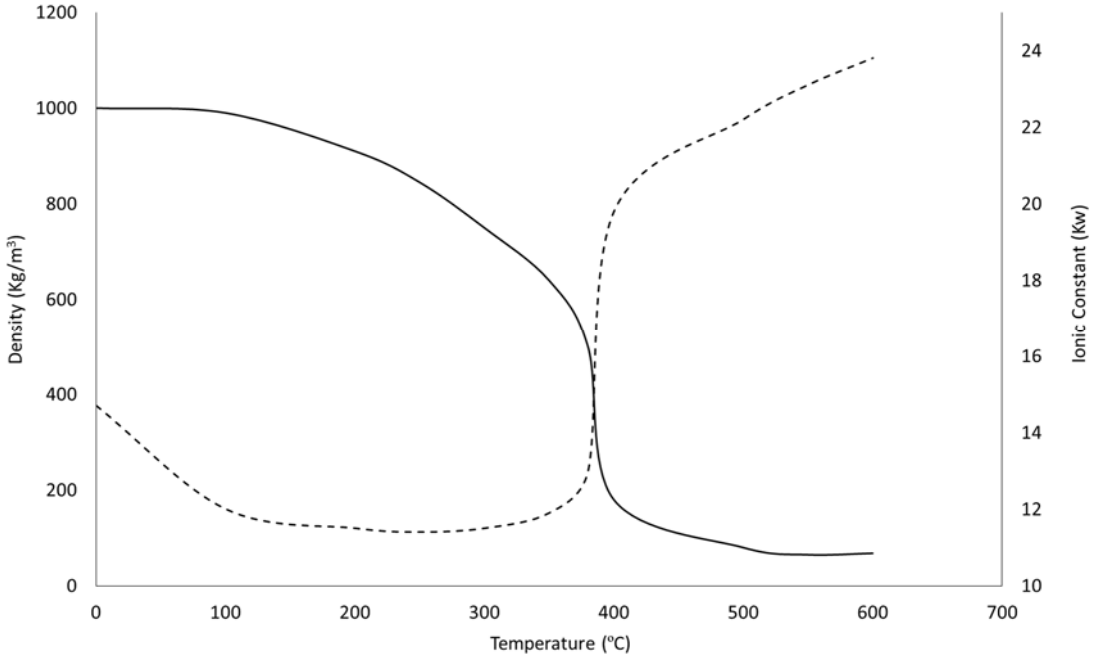

Figure 9. Properties of water at supercritical conditions.

The combination of these properties and the high temperature that allows high reaction rates makes supercritical water an effective solvent and an excellent reaction medium in the hydrolysis processes of lignocellulosic materials. At near critical conditions, therefore, the drastic changes in the ionic product and in the density of water influences the reaction of degradation of monosaccharaides. In the case of xylose, while at subcritical temperatures, the main product of degradation is furfural, with a maximum production between 300 and 350ºC, at supercritical conditions aldolic reactions are favoured, with an increase in the formation of glycolaldehyde and glyceraldehyde [42, 49].

Similarly, with glucose as base monomer, the dehydration reaction leading to the formation of 5-hydroxymethylfurfural is promoted at subcritical conditions with a maximum at around 350 °C. On the other hand, retro-aldol condensations are favoured at higher temperatures, with water under supercritical conditions; glycolaldehyde is the

main product deriving from glucose, and at 450ºC and 35 MPa, yields as high as 70 wt.% can be reached [50].

Hemicellulose application

Unlike cellulose, whose industrial applications have been widely studied and developed since many years in paper, textile, plastic, pharmaceutical, fuel and many other industries [21], hemicellulose investigation is more recent, and valuable characteristics and possible usages of this biopolymer have been recently discovered. Differently from synthetic polymers, hemicelluloses have a high hydrophilic behaviour, due to the free hydroxyl groups along their chains. Moreover, their non-homogeneous and amorphous structure make them different from linear biopolymer like cellulose and starch. However, these features that limit the use of hemicellulose in the industry can be modified through chemical transformations such as oxidation, reduction, partial hydrolysis, esterification or etherification of the hydroxyl groups and cross-linking [12].

Xylo-oligosaccharides, which are oligomers constituted mainly by xylose with substitute groups like acetyl, uronic or phenolic acids, have great prebiotic potential, and have low calorific value and good organoleptic properties [51, 52]. They can be incorporated without further modifications into many food products and work as dietary fibres, promoting moreover the growth of *Bifidobacteria* in the colon [53]. The major use of xylo-oligosaccharides is in beverages; an example is “Bikkle”, a popular Japanese yogurt-based drink produce by the company Suntory. Hydrothermal hydrolysis is the best method for the extraction of oligosaccharides for food, as it does not modify the flavours and ensure the absence of toxic compounds and solvents.

Hydrogels with excellent water absorbency can be produced by using hemicelluloses (xylans or arabino-galactans) as skeletal material, on which acrylic acid or acrylamide can graft so as to form cross-linked hydrogels [54, 55]. These super-absorbent hydrogels can absorb large amounts of water, reaching several hundred times higher than their original weight. Their functional groups can be modify to enhance their selectivity on the capture of ions, making them useful for example in the removal of toxic and polluting heavy metals from water [56], as drug delivers (if they do not contain toxic compounds) [57], in agriculture as seed coatings for relay cropping (a special version of double cropping, where the second crop is planted into the first crop before harvest). In this way, both crops share a portion of the growing season, increasing solar radiation and heat available to each [58].

O-acetyl-galactoglucomannan (AcGGM) hemicellulose modified through benzylation have demonstrated to be a good barrier material in the protection of food from oxygen and water [59] . Hemicellulose-based hybrid films with good gas barrier properties where produced also by adding polyvinyl alcohol (PVA) and chitin nanowhiskers (NCH), into the hemicelluloses matrices [60].

Another chemical with high added value, produced from xylose (the principal monosaccharide in many types of hemicellulose), is xylitol. Xylitol is a sugar alcohol used as a sweetener, obtain by catalytic hydrogenation of xylose. It has the same taste as sugar, with a 40% less calories, it can be used as sugar substitute for diabetics, as it does not require insulin for its metabolism and have more dental health benefits than other polyalcohols [51, 61]. The world xylitol market is valued at 725.9 million dollars in 2016. According to a new research report from Global Market Insights, Inc., the size of the

xylitol market is expected to reach 1 , 12 billion dollars in 2023 (4% more in 7 years) [62].

Other valuable products from hydrothermal processes

Hydrothermal pre-treatments, as well as facilitate the enzymatic attack by reducing the recalcitrance of the biomass [63], result in the production of different compounds, through the extraction and hydrolysis of the lignocellulosic biopolymers.

The monomeric sugars constituting cellulose and hemicellulose, under particular conditions of temperature, acidity or residence time, can originate furfural, 5-hydroxymethylfurfural, glycolaldehyde, acetic acid, pyruvhaldehyde, lactic acid and other products resulting from their dehydration or through aldolic reactions [44, 64].

Although many of them are often considered undesirable by-products, as their presence inhibit a further enzymatic treatment [65], often their commercial value exceeds that of sugars, or alcohols produced from their fermentation. For this reason, in some cases it is more correct to define these compounds as added-value products, rather than by-products.

Among these chemicals, furfural, 5-hydroxymethylfurfural and lactic acid are of great economic interest: furfural can be the starting material for polymers such as nylon 6 and nylon 6,6; 5-HMF has the potential to replace terephthalic acid [66-68]; lactic acid can be used as a food preservative, flavouring agent and is also employed in pharmaceutical technology to produce water-soluble lactates.

A list of the top value added compounds from biomass is widely discussed in two volumes from NRE Laboratories [69, 70], identifying the products deriving from biomass, and the processes that would economically and technically support the production of fuels, power and chemicals in an integrated biorefinery. After a careful screening, 30 foundation

chemicals were selected, which may be competitive with compounds deriving from the petrochemical industry. Some of these products, such as furfural, levulinic acid, formic acid, 5-hydroxymethylfurfural, acetic acid, glycolaldehyde, glyceraldehyde, pyruvaldehyde, lactic acid can be produced via saccharification and subsequent hydrolysis of cellulose and hemicellulose, using only water.

Reaction configuration choice

Thus raise the question: which is the best reaction technology for each case study?

The products obtained with the biomass fractionation processes, highly depend on the residence time, which is set according with the operating temperature. When subcritical water is used (e.g. typically between 160ºC and 250ºC) hemicellulose usually takes from 10 to 30 min to be extracted, while cellulose takes from 40 to 60 min at least. In that case the use of a batch reactor is feasible, although heating-up takes from 10 to 20 min, and cooling down might take from 5 to 10 min; temperature-time profile must be therefore considered in kinetics calculation.

The use of a semi-continuous reactor is also valid; the residence time of the liquid phase can be regulated through the liquid flow-rate and is usually between 1 and 10 min. The residence time of the solid in the reactor is generally much larger, as solid is not removed until the complete extraction of the soluble products, which yield depend on the operating temperature and takes between 30 and 120 min [29, 71, 72].

When supercritical water is used, the temperature goes over 374ºC, but even operating at 300ºC (not supercritical) accelerates the hydrolysis so much that the produced sugars degrade quite easily and sometimes even re-polymerise. In such cases, the use of batch reactors is not recommended. Semi-continuous systems at high flowrate is an option, but

some degradation products might appear. The optimum system will be the continuous reactor; in this case biomass particles need to be milled under 200-300 μm, as a slurry has to be pumped at high pressure through the system. To assure low residence times (less than 1 s) an effective system for heating-up and quenching is required.

Batch and semi-continuous systems do not require extensive milling, biomass can be used as it is, e.g. coffee beans, chips, pellets, etc. Only a filter is required to hold the particle bed during the process.

On the other hand, continuous operation requires very fine particles. At lab scale, equipment (e.g. pipes of 1/8", 1/4", etc.) pumps like HPLC pumps, or membrane pumps can handle some solids (not always) under 100 μm. The bottleneck is normally the check-valve of the pump. At a higher scale, i.e. pilot or demo, there are pumps that can even transfer slurries containing particles of 1 mm.

Pilot plants for hot pressurized water fractionation

When scaling-up a process to pilot scale or industrial scale, it is necessary to minimize and simplify the operations to handle the systems. Safety is important, but also the minimization of downtimes is a fundamental parameter to economize the process and make it viable.

Several authors consider the semi-continuous set-up as the best option for the extraction of hemicelluloses with hot-pressurize water [29, 72, 73]. The main reasons are that extreme milling and solid pumping and are avoided; a continuous supply of fresh water and a rapid removal of the products prevent the degradation and guarantees a high concentration gradient at the solid-liquid interface, enhancing the mass transfer and the extraction yields. The disadvantages of this type of plant are: 1) the difficulty of removing

biomass at the end of the process, which is compacts inside the reactor and 2) the need to stop the production to open the reactor and replace the biomass at the end of each extraction cycle, which results in long dead times. Resolution of these issues decides whether the process can be or not used at an industrial scale.

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AIMS AND CONTENTS

Liquid hot pressurized water is an effective, cheap and clean solvent to extract natural products in general. Especifically, it can extract biopolymers from lignocellulosic biomass, mostly hemicelluloses and less amount of cellulose and lignin at moderate temperatures.

Second generation biomass, such as wood residues, does not compete with food but has two difficulties: first, producing hydrolysates with many side products and second the handling of the swelling solids.

The main hyphothesis of this research work was that:

"A sustainable process for the selective extraction of hemicelluloses from woody biomass using subcritical water autohydrolisis can be scaled-up from lab to pilot plant"

Therefore, the main purpose is to scale-up the subcritical autohydrolysis process, to get an easy to handle pilot plant that could operate with any type of biomass, working continuously and minimising down-times.

In order to achieve the aim of this thesis, the following partial objectives were defined, and described in the 6 chapters constituting the thesis:

1. *Search for optimal operating conditions for hemicellulose extraction using a semi-continuous laboratory scale reactor.*
    - Fractionation and hydrolysis of hemicellulose and cellulose from *Eucalyptus globulus* wood, at different temperature and different liquid flow-rates.
2. *Processing of lignocellulosic biomass derived oligomers and monomers, with subcritical and supercritical water, to obtain high value products.*
    - Development of a hydrothermal process that combined the fractionation of lignocellulosic biomass into a semi-continuous reactor, with the hydrolysis of the extracted components using supercritical water.

- Tuning of operating temperatures and liquid flowrates to enhance the selectivity towards different degradation products.

3. *Investigation on hemicellulose extraction from 10 different species of trees (urban ornamental trees); detection of a correlation between the composition of the biomass, its structure and the yield of hemicellulose extracted.*
    - Process conducted at constant temperature in a batch-cascade reactor located in Åbo Akademi (Finland), which allowed to collect liquid samples, with 5 different residence times, during the same experiment. Some technical features of the reactor were used in the design of the pilot plant, the final objective of this thesis.
4. *Scale-up of semi-continuous reactor from laboratory-scale to pilot-scale for the extraction of hemicelluloses from lignocellulosic biomass with liquid hot pressurized water.*
    - Implementation of technological innovations allowed to design and build a multistage pilot plant with a continuous operability, minimum downtimes and easiness in the operation.
    - Study on the efficiency of scale-up and the ability of the system to operate at different temperatures.
    - Study on the evolution of the characteristics of the liquid extract when changing its residence time within the system.
5. *Detailed description of the pilot plant and its operation.*
    - Claims on the novelty of the invention with respect to existing systems for the extraction of water-soluble compounds from lignocellulosic biomass.
    - Redaction of a national spanish patent.

6. *Design of a business model and evaluation of the viability of a company created around the production and sale of xylitol.*
    - The business model considers the production of xylitol by catalytic hydrogenation of the liquid extract obtained with the pilot plant developed during the thesis. It focuses on the health, social and ecological characteristics of a sweetener produced using water as the only solvent.

CHAPTER 1

OPTIMAL CONDITIONS FOR HEMICELLULOSES EXTRACTION FROM EUCALYPTUS GLOBULUS WOOD: HYDROTHERMAL TREATMENT IN A SEMI-CONTINUOUS REACTOR.

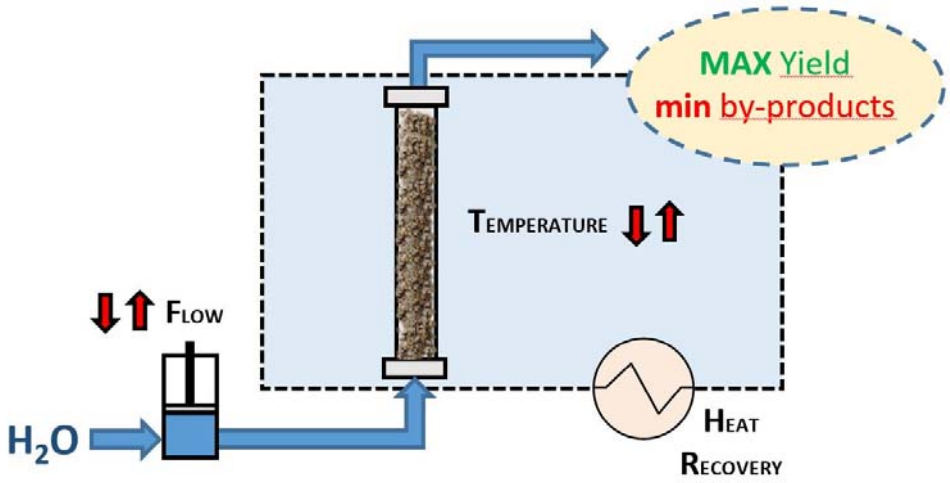
OPTIMAL CONDITIONS FOR HEMICELLULOSES EXTRACTION FROM EUCALYPTUS GLOBULES WOOD: HYDROTHERMAL TREATMENT IN A SEMI-CONTINUOUS REACTOR

Abstract

Optimal conditions for hemicellulose extraction from wooden biomass in a semi-continuous system have been assessed in this work. This study would constitute the first stage for a profitable and green industrial process. *Eucalyptus globulus* was selected as raw material due to its low water consumption, high growth and its efficiency in lignocellulose production. Moreover its cultivation is very popular in southern Europe. Samples of 5.0 g of wood were fractioned using a pressurized hot water semi-continuous system, to produce sugars (pentoses and hexoses) and a solid residue enriched in lignin. Five flow rates between 2.50 and 20.00 mL/min and four temperatures between 135.0 and 285.0 ºC were tested in order to maximize the production of sugars, avoiding the formation of degradation products.

Optimum conditions for the extraction of hemicellulose were identified at 185.0 ºC and 5.00 mL/min, leading to a pentoses yield of 67.409 wt%, with 0.702 wt% of degradation products. Almost all the pulp is extracted at 285.0 ºC.

SEM images show very well the changes in the wood structure at different temperatures. A kinetic model was developed, describing the extraction and hydrolysis of hemicellulose and cellulose with absolute average deviations around 10 % for sugar extracted mass.



Keywords

Eucalyptus, hemicelluloses, fractionation, biorefinery, semi-continuous.

1. Introduction

Cellulose and hemicellulose contained in woody biomass can be hydrolysed to monomeric sugars, which can be further fermented to ethanol, or can be converted in higher value products [1-3]. Xylose from hemicellulose, for instance, can be converted to furfural, which is a precursor used in different fields, such as oil refining, plastics, pharmaceutical, and agrochemical industries [4]. L-Xylose can be also hydrogenated or enzymatically transformed to xylitol, which is a sweetening agent and is also used for preventing tooth decay [5]. HMF (Hydroxymethylfurfural) derived from hexose sugars can be oxidized to obtain 2,5-furandicarboxylic acid, which can substitute terephthalic acid (PTA) in the production of polyesters and other current polymers containing an aromatic moiety [6]. The idea of transforming biomass to energy, materials, and chemicals, defines the concept of a biorefinery [7-10], particularly being an interesting topic nowadays, considering the issues related to fossil combustibles and derivatives.

Direct fermentation of biomass to ethanol by enzymatic digestion cannot succeed without a pre-treatment to modify the cross-linked structure between lignin and polysaccharides and reduce the biomass recalcitrance to enzymatic hydrolysis [11, 12] .

A promising, clean and cheap way to fractionate lignocellulosic materials is the so called autohydrolysis, which simply consists of treating biomass with hot pressurised liquid water: during the reaction, most of the hemicelluloses are extracted and hydrolysed to monomers, with a consequent release of acetic acid originated from the cleavage of the acetyl groups bonded to the oligosaccharides; a lower amount of cellulose is released, due to the crystalline structure of the polymer, which makes it more difficult to dissolve and hydrolyse [13]. Moreover, the structure of the cell walls becomes more accessible to

enzymatic attacks. Hydrolysis and degradation of the extracted products will be more severe along with temperature, residence time and low pH.

In comparison with other pre-treatments with mineral acids [14, 15] or bases [16, 17] added to the reaction media, autohydrolisis has a lower environmental impact, as the only reagent is water and no further detoxification treatments are required to neutralize the sludges. Autohydrolysis is also a cost effective process, as the variation of pH in the liquors is very low and there is no corrosion of the equipment [18, 19]. In literature there are several examples of this process, performed with different biomass crops and residues. Agriculture wastes like wheat straw [20, 21], corn stover [22, 23] and rice straw [24, 25] have been intensively explored for this kind of process, due to their abundance and easy availability. Also many woody biomass have been widely used [26-28], as they contain less inorganic substances than agricultural crops [29] and contain more acetyl groups that enhance the catalytic activity of the process (in particular hardwood species) [18].

A profitable biomass for hemicellulose extraction is needed. This raw material should have a high growth rate, a low water consumption and a high content of hemicellulose. *Eucalyptus globulus* has all these characteristics and, in particular, it is the world's most efficient tree for producing pulp. Moreover, eucalyptus wood has a high density enabling the tree to capture large amounts of CO_2 (0.1359 t CO_2 /year/tree) and thus to accumulate more carbon per unit of volume compared to other forest species .

A high biomass yield and a low water consumption (306 L/kg dry material against 400 L/kg dry material for oak trees or 1000 and 2000 L/kg dry material for herbaceous species like corn and soya respectively) [30] make eucalyptus very attractive from an industrial point of view, not only for paper production, but also as a sustainable and carbon-neutral source for liquid fuels and bio-compounds.

In addition, eucalyptus is a tree of considerable importance in the Iberian Peninsula and in the world, due to its wide expansion and its spread use in industrial applications, mainly for the paper industry.

Garrote et al. studied the fractionation of *Eucalyptus globulus* wood [31] , the extraction of hemicellulose and the production of xylose from xylooligosaccharides [32], after pre-treatments in a batch reactor.

While there are several studies dealing with the autohydrolysis in batch reactors [33-39], only a smaller number of articles regards flow-through reactors [11, 40, 41].

In our study, we investigate the autohydrolysis of *Eucalyptus globulus* wood in a semi-continuous reactor, consisting in a tubular reactor loaded with wood chips, constantly through by pressurized hot water. This kind of set-up allows a high solid / liquid ratio and a rapid removal of the products, preventing their degradation. Moreover a continuous supply of fresh water to the system guarantees a high concentration gradient at the solid-liquid interface, thus, enhancing the mass transfer respect to a batch or a semi-batch reactor [29].

Recent studies have been completed using flow-through extractions with corn stover biomass. Authors found that flow-through extraction resulted in higher xylose yield, and greater lignin removal respect to batch reactors [42]. The removal of lignin makes the remaining cellulose after the pre-treatments more digestible by the enzymes [43].

Respect to continuous reactor, where biomass and water are continuously fed into the reactor, in semi-continuous reactor solid pumping and extreme milling is avoided, reducing considerably the costs.

All these characteristics make, in our view, the semi-continuous reactor the best for the pre-treatment of biomass in a future industrial scenery.

Different liquid flow rates (2.50, 5.00, 10.00, 15.00, 20.00 mL/min) and different reaction temperatures (135.0, 185.0, 235.0, 285.0 °C) were tested in order to maximize the yield of polysaccharides extraction avoiding the formation of degradation products that would inhibit a further fermentation step [44].

SEM pictures of the exhausted solid bed were taken to analyse the structure of the wood after the pre-treatments.

In addition to optimizing the temperature, whose effect was already explored in batch systems [28, 31], the liquid residence time for the extraction of hemicellulose from eucalyptus in a semi-continuous reactor was optimized in this work. Effects of the residence time were suddenly checked at temperature not normally suitable for the extraction of hemicellulose.

Moreover an auto catalytic kinetic model developed by our group, and previously validated for another raw material (holm oak), was simplified and implemented. It can be stated that even changing the biomass, the model represent very well the extraction and hydrolysis in a semi-continuous reactor.

2. Materials and Methods

2.1 Materials

Eucalyptus globulus wood used as the main raw material of all the experiments originated from Cantabria (Spain). Wooden branches were cut in slices with a jigsaw, and then reduced to small pellets with an average Feret diameter of 0.6 cm. The composition of the raw material (Table 1) in terms of structural carbohydrates, extractives, ashes, humidity and lignin were determined according to the standard methods published by National Renewable Energy Laboratory (NREL) [45].

The column used for the separation of the compounds was SUGAR SH-1011 Shodex at 50.0 ºC and a flow of 0.80 mL/min, using a solution of 0.01N of sulphuric acid and water Milli-Q as mobile phase. A Waters IR detector 2414 and Waters dual λ absorbance detector 2487 (210 nm and 254 nm) was used to identify the sugars and their derivatives. The calibration reagents used for HPLC analysis were: cellobiose (+98%), glucose (+99%), fructose (+99%), glyceraldehyde (95%), pyruvaldehyde (40%), arabinose (+99%), glycolaldehyde (+98%), 5-hydroxymethylfurfural (99%), lactic acid (85%), formic acid (98%), acrylic acid (99%), mannose (+99%), xylose (+99%), galactose(+99%), levulinic acid (≥ 97%), furfural (+99%), acetic acid (+99%) purchased from Sigma and used without further modification.

For the analysis of sugars sulphuric acid (96%) and calcium carbonate (≥ 99.0%), purchased from Panreac were used.

Table 1. Composition of the raw material

wt% Umidity	wt% Extractives	wt% Ashes	wt% Klason Lignin	wt% Glucans	wt% Xylan	wt% Arabinian	wt% Acetil Groups
6.501	3.088	0.138	25.741	39.742	18.796	2.593	3.401

2.2 Reactor set-up for the experiments

The experiments were carried out in a laboratory-scale fixed bed reactor (R-01, 38 cm length, ½" O.D. SS316 piping, 0.37" I.D.) (as depicted in Figure 1).

The reactor was charged with 5.00±0.01 g of wood pellets, two metallic filters were placed at the top and at the bottom, in order to avoid the loss of solid particles during the experiments. Deionized water was introduced continuously into the reactor, in up-flow, using a PU-2080 HPLC pump.

The feed flow, at room temperature, was preheated by the out-flow of the reactor, through a concentric tube heat exchanger working in counter current (E-02, 70 cm length, 1/4"-3/8"). A preheater (E-01, 200 cm of 1/8" AISI 316 piping) was placed after the heat exchanger and located, together with the reactor, inside a former chromatographic oven HP5680, which could be set at the desired temperature.

Pressure was controlled by a Go-backpressure valve (BPV-01) installed at the liquid outlet. The out-flow pH was measured online through an electronic pH-meter (Nahita model 903).

A heat exchanger allowed to recover between the 70% and 85% of the energy input; the plant, even if in laboratory scale, was designed to operate according to green concepts of energy saving.

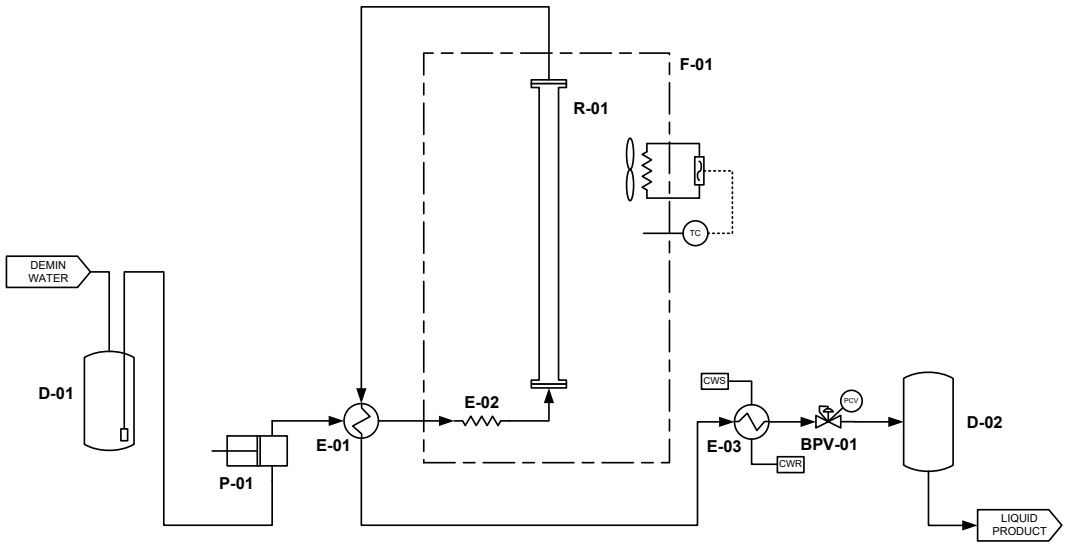


Figure 1. Schematic flow diagram of the experimental system. Equipment: D-01 feeder, P-01 pump, E-01 feed water preheater, R-01 hydrothermal reactor, F-01 reactor air convection oven, E-02 preheat capillary, BPV-01 product depressurization Go-backpressure valve, E-03 product cooler, D-02 liquid product vessel.

2.3 Experimental procedure and analytical methods

A set of 9 experiments was carried out: 5 with changing water flow rate (2.50, 5.00, 10.00, 15.00, 20.00 mL/min), at constant temperature (185.0°C), and 4 with changing temperature (135.0, 185.0, 235.0, 285.0 °C) at constant flow rate (5.00 ml/min). Pressure was kept constant at 100.0 bar, in order to guarantee the liquid phase of the aqueous reaction media at all the operated temperatures. In the initial stage of the experiments the reactor was filled with a constant amount of wood, and then connected to the system. A cold liquid pressure test was made before each experiment, in order to check the presence of leaks in the system, and to ensure the complete wetting of the wood. Water was then heated in a pre-heating capillary and when the reaction temperature was reached the oven was turned on and the pump was set to the desired feed water flow. Time 0 was defined

as the time in which the first drop of liquid left the system, at this point the measurement of pH started and a first sample of liquid was taken.

The total time of each experiment was 90 min, 20.0 mL liquid samples were taken every 10 min, pH was recorded online every 1 minute during the first 30 min, and then every 2 minutes until the end of the tests. After 90 min, the pump was switched off to zero flow and the oven temperature lowered to 20.0 ºC; the system was then slowly depressurised, the reactor untightened and all its content was collected in a beaker and dried for 24h at 60.0 °C. Finally the empty reactor was reconnected to the system and deionized water was flushed to clean all the pipes.

To determine the amount of sugars extracted and the degradation products produced after the autohydrolysis, a posthydrolysis process of the extracted liquor was performed to break all the oligomers in monomers, and allowing the accurate count of the extracted products after a HPLC separation.

10.0 mL of each sample were completely hydrolyzed with 4.0 mL of sulfuric acid 96 %wt. and consequently incubated in an oven for 30 min at 30.0 °C. The mixtures were then diluted with 86.0 mL of deionized water and warmed in an oven for 1 hour at 121.0 ºC. At the end of the acid hydrolysis, the samples were cooled down to room temperature, calcium carbonate was added in order to raise the pH to a value between 6 and 7, the solution was filtered through 0.22 μm nylon filters and the content of sugars was determined by HPLC.

The solid resulting from each experiment was processed as explained by the standard methods published by National Renewable Energy Laboratory (NREL) [45], residue of Klason lignin was determined as well as the amount and quality of soluble compounds not extracted by the thermohydrolysis.

Unprocessed eucalyptus wood was characterized as explained in paragraph 2.1, in order to relate the amount of compounds extracted during the experiments with the raw material composition.

Main peaks and areas were identified and calculated through a band-analysis via fast Fourier transform (FFT) and band-adjustment by Gaussian functions. The adjustment was done by minimizing the quadratic error using a Nelder-Mead algorithm.

2.4 Uncertainty analysis

In order to check the reliability of the experimental data, the uncertainty of all of them was calculated. Regarding the concentration obtained by HPLC Eq. 1 was used to consider the repeatability, the experimental data deviation and the effect of the calibration. Once this value was obtained, a typical propagation expression was used to obtain the uncertainty of the values calculated following the NREL standard methods [45].

$$s_{\mathrm{x}} = \frac{s_{\mathrm{y}}}{\mathrm{m}} \cdot \sqrt{\frac{1}{L} + \frac{1}{N} + \frac{(\overline{y_{\mathrm{x}}} - \overline{y})^2}{m^2 \cdot S_{\mathrm{xx}}}}$$

$$s_{\mathrm{y}} = \sqrt{\frac{\sum(y_{\mathrm{i}} - \hat{y}_{\mathrm{i}})^2}{N-2}} \qquad (1)$$

$$s_{\mathrm{xx}} = \sum_{i=1}^{\mathrm{N}} (x_{\mathrm{i}} - \overline{x})$$

3. Results and discussion

The total amount of sugars and acetyl groups contained in eucalyptus wood was equal to 64.532 wt% of the total mass (sum of glucans, xylan, arabinan abd acetyl groups represented in Table 1).

Semicontinuous extraction/reaction is particular in terms of residence time. In the semicontinuous plant two different reaction times were defined in the system:

- solid residence time τ_{sol}, which was constant for all the tests (always 90 min), and corresponded to the total duration time of the experiments, as the solid was static inside the reactor;
- liquid residence time τ_{liq}, which varied in relation with the operational conditions of the experiment, in particular with the liquid flow rate and with the porosity (void volume of the bed). An initial porosity ε_0 was defined, as the ratio between the volume of the empty spaces in the reactor at the beginning of the experiments, and the total volume of the empty reactor. ε_0 was determined in the lab to be 0.70. A final porosity ε_f was calculated (Eq. 2) taking into account the mass variation of the solid particles inside the reactor between the beginning and the end of each experiment; m_0 and m_f corresponded to the initial and the final mass of the solid in the reactor. Liquid residence time (Eq. 3) was calculated considering an average porosity ε_{av} between the initial ε_0 and the final porosity ε_f of the bed in each experiment. To calculate the final porosity, it was assumed that the density of the wood particles remained constant during time, and that there were only variations in the volume of the particles.

$$\varepsilon_f = \varepsilon_0 + (1 - \varepsilon_0)\frac{(m_0 - m_f)}{m_f} \qquad (2)$$

$$\tau_{liq} = \frac{V}{\dot{V}}\varepsilon_{av} \qquad (3)$$

The liquid residence time is influenced mostly by the liquid flow rate and slightly by the temperature (meanwhile we operated under liquid phase conditions, and the changes in liquid density with temperature are low). The most important variable is temperature, as an increase in temperature leads to a greater extraction of soluble compounds from the

wood (as it will be shown), which means a decrease of the solid particles volumes along with an increase of the porosity of the bed. The density of the solution was considered to be equal to the density of water, as the concentration of soluble compounds was always low, and did not influence the residence time of liquid.

A summary of experimental conditions and results obtained is shown in Table 2. All the results were calculated as cumulative values at the end of each experiment. Figure 2 represent the cumulate values of sugars and acetic acid resulting from the experiment at 185 °C and 5 mL/min, where the mass of the compounds detected by HPLC are represented in function of the solid residence time. Final values of all experiments (calculated at τ_{sol} =90 min) are shown in Table 2.

At first glance, one can see how the major percentage of sugars obtained correspond to xylose, while glucose and arabinose were exceedingly lower. Acetic acid was also measured, which was the ultimately responsibly of the decrease in the pH during the experiments (see pH evolution in Figure 3).

"Yield tot" indicates the fraction of soluble compounds (sugars and degradation products) in the liquid respect to the total amount of soluble compounds in the raw material, detected by HPLC.

Mass balance was checked ($m_{balance}$) by summing up the amounts of soluble compounds in the extracted liquor and in the exhausted solid, detected by HPLC, and then dividing the result to the amount of soluble compound in the raw material. In all the cases the balance can be considered respected. The deviations from 100% may be due, in addition to the uncertainty explained in section 2.4, to possible peak integration errors. Some peaks are in fact slightly overlapping, and the calculation of their area may deviate slightly from the correct value.

The yields of hexoses (so-called C6) and pentoses (so-called C5) are calculated by dividing the mass of pentose and hexose sugars extracted, by the total amount of sugars in the raw material. Lignin percentage in the exhaust solid with respect to the initial weight of the raw material is represented in the last column of Table 2.

Table 2. Experimental table for the study of eucalyptus wood autohydrolysis in a semicontinuous reactor.

°C	mL/min	min	wt% [a]	wt% [a]	wt% [a]
T	**Flow**	**t liq**	**Yield tot**	**Mass Balance**	**Yield C6**
185.0±1.1	2.500±0.010	7.65±0.15	26.042±0.067	101.014±0.214	0.419±0.002
185.0±1.1	5.000±0.010	3.82±0.11	26.237±0.052	100.231±0.205	2.282±0.005
185.0±1.1	10.000±0.010	1.90±0.10	21.695±0.039	100.453±0.202	0.731±0.002
185.0±1.1	15.000±0.010	1.27±0.10	22.931±0.039	100.636±0.202	0.000±0.012
185.0±1.1	20.000±0.010	0.95±0.10	22.043±0.037	98.741±0.196	0.000±0.010
135.0±1.1	5.000±0.010	3.69±0.11	0.313±0.000	98.857±0.221	0.000±0.000
185.0±1.1	5.000±0.010	3.82±0.11	26.237±0.052	100.231±0.205	2.282±0.005
235.0±1.1	5.000±0.010	3.92±0.11	45.476±0.083	98.039±0.189	12.436±0.022
285.0±1.1	5.000±0.010	4.12±0.11	85.684±0.153	101.429±0.177	64.703±0.114

wt% [b]	wt% [b]	wt% [b]	wt% [b]	wt% [b]	wt% [b]	wt% [b]
$w_{glucose}$	w_{xylose}	$w_{acetic\ acid}$	$w_{arabinose}$	$w_{formic\ acid}$	$w_{pyruvaldehyde}$	w_{HMF}
0.167±0.002	9.336±0.040	1.874±0.010	1.092±0.030	0.524±0.005	0.000±0.000	0.570±0.003
0.911±0.003	12.138±0.030	1.460±0.006	1.533±0.005	0.000±0.004	0.000±0.000	0.011±0.001
0.292±0.001	11.994±0.017	1.291±0.005	0.271±0.007	0.000±0.000	0.000±0.000	0.000±0.000
0.000±0.012	12.503±0.004	0.946±0.000	1.184±0.007	0.000±0.000	0.000±0.000	0.000±0.000
0.000±0.010	13.095±0.004	0.713±0.000	0.260±0.010	0.000±0.000	0.000±0.000	0.000±0.000
0.000±0.000	0.200±0.000	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000
0.911±0.003	12.138±0.030	1.460±0.006	1.533±0.005	0.000±0.004	0.000±0.000	0.011±0.001
4.965±0.009	11.318±0.032	1.706±0.006	1.422±0.018	0.537±0.003	0.940±0.010	1.228±0.002
25.831±0.049	14.245±0.044	2.336±0.009	2.461±0.025	1.161±0.003	1.800±0.010	2.691±0.005

wt% [b]	wt% [b]
$w_{furfural}$	$w_{lignin\ residual}$
3.460±0.005	26.122±0.434
0.690±0.004	27.168±0.456
0.000±0.000	27.155±0.443
0.000±0.000	26.670±0.448
0.000±0.000	26.968±0.442

0.000±0.000	25.471±0.448
0.690±0.004	27.168±0.456
6.910±0.003	21.874±0.447
4.160±0.003	12.421±0.679

[a] Percentage of the components in the liquid respect to the weight of the component in the raw material.

[b] Percentage with respect to the total initial weight of the raw material.

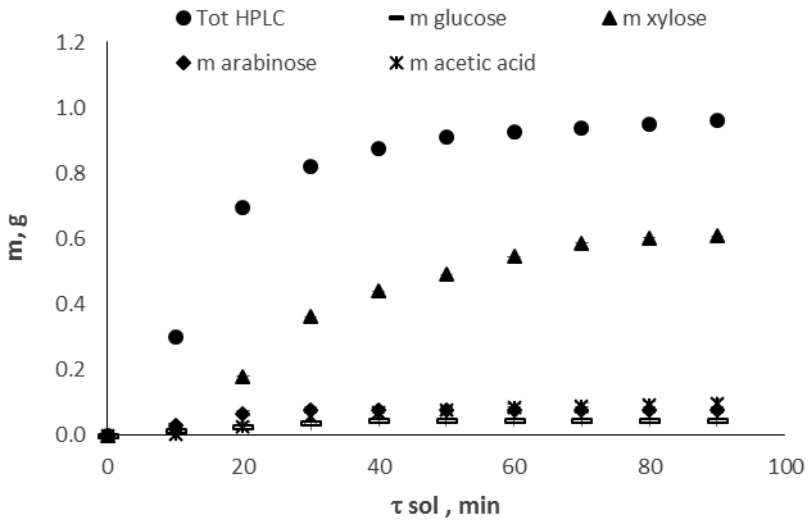
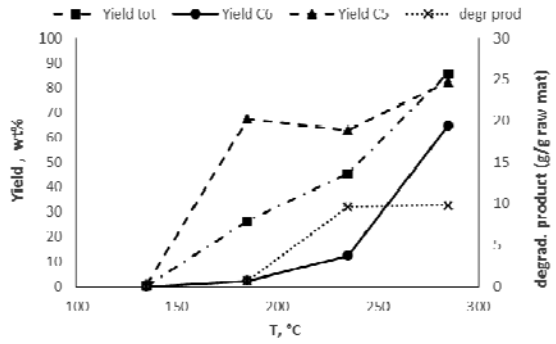
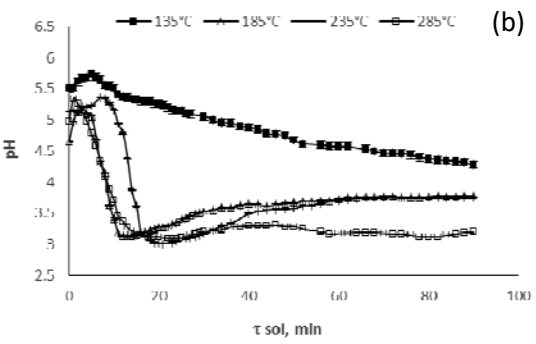


Figure 2. Fraction of soluble compounds in liquid as a function of solid residence time at a constant liquid flow rate 5 mL/min and constant temperature 185.0 ºC.

(a)





(b)

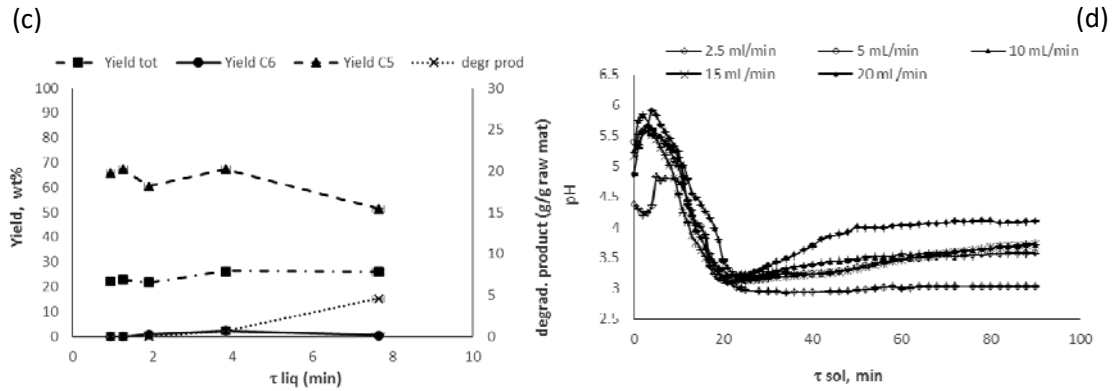


Figure 3. Yield of soluble compounds as a function of temperature at a constant liquid flow rate 5 mL/min (a). pH in function of τ_{sol} at different temperatures and constant flow rate 5 mL/min (b). Yield of soluble compounds as a function of the liquid reaction time at 185.0°C (c). pH in function of τ_{sol} at different liquid flow rates and constant temperature 185.0°C (d).

3.1. Effect of temperature

In figure 3a, the yield of soluble compounds is represented as a function of temperature, with a constant liquid flow rate of 5.00 mL/min. Degradation products, in the second vertical axe, are calculated as the sum of formic acid, furfural, HMF and pyruvaldehide. Acetic acid was not considered as a degradation product becouse it results from the deacetylation of hemicellulose.

At constant flow rate, the amount of solubilised compounds increased linearly with increasing the temperature.

At 135.0 °C, there is not extraction, while 185.0°C were enough to extract hemicellulose with a pentoses yield of 67.409 wt%; cellulose was not depolimerized at these conditions, and degradation products were below 1 wt% with respect to the original weight of the raw material.

At 235.0 °C, cellulose started to be depolymerized and the yield of hexoses increased to 12.436 wt%; pentoses yield decreased to 62.817 wt% while the amount of degradation products reached the 9.613 wt% of the original mass of wood.

Yield of hexoses became 64.703 wt% at 285.0 °C, as the increasing of temperature promoted the depolymerization of cellulose. At the same conditions, hemicellulose extraction reached its maximum point with a yield of 82.369 wt% for pentoses, while the total amount of degradation products only increased slightly with respect to the previous experiment. The sum of C5 sugars with acetic acid furfural and formic acid gives the amount of hemicellulose extracted, resulting in a yield of 91.01 wt%. Cellulose yield, calculated by summing C6 sugars with HMF and pyruvaldehyde, resulted to be 76.10 wt%.

At the beginning of the process, hemicellulose is more easily extracted than after few minutes, as it can be seen in the cumulated curve in Figure 2. Therefore, two kinds of hemicellulose can be distinguished in wooden biomass: one easier to extract, and the other closely associated with cellulose, which can be removed only with more severe conditions [46]. It looks like that at 285.0 °C, the structural modification of the cell walls and the removal of the cellulose, allowed the extraction of the associated hemicellulose. Extraction of cellulose starts at 235.0 °C with a yield of 12.436 wt% for C6, reaching a value of 64.703 wt% at 285.0 ºC. 85.684 wt% of the pulp is extracted at this temperature, leaving a residual solid containing mainly lignin, with a few fibres of cellulose and hemicellulose oligomers.

pH (figure 3b) reached a minimum value and then started to increase slowly: increasing the temperature, pH increased slower after the minimum point, and reached a lower value at the end of the experiment.

High temperatures indeed led to a stronger depolymerisation of hemicellulose, and thus to a greater deacetylation and further reduction to acetic acid. At 135.0 °C pH kept decreasing during the whole reaction, without reaching a minimum value, exhibiting the

lowest deacetylation rate. The kinetic of depolymerisation of hemicellulose was very slow [47, 48], and the amount of acid produced was very low compared with the other experiments.

3.2. Effect of liquid residence time

Liquid residence time plays an important role in the autohydrolysis process, as it is directly related with the residence time of the main reagent (water) and the solubilised compounds inside the system. This drasticly determines the concentration of acetic acid in the liquor, and subsequently the free protons to induce a further hydrolysis.

The yield of pentoses was almost invariable at 185.0 °C under different liquid residence times (figure 3c), with the exception that it slightly decreased at τ_{liq} = 7.79 min. The yield of hexoses was very low in all experiments, as the operational temperature was not sufficiently high to target the cellulose activation energy and depolymerize it; glucose detected derived surely from the hemicellulose.

There was no decomposition of sugars when the liquid residence time was between 0 and 1.91 min, degradation products appeared at τ_{liq} = 3.84 min, at τ_{liq} = 7.65 min the amount of degradation products results to be 4.55 wt% with respect to the raw material mass.

It is well-known that under high temperatures the acetyl groups that bind hemicellulose with lignin are released and reduced to acetic acid [49], leading to the chain reaction called autohydrolysis of hemicellulose [32, 36].

A temperature of 185.0ºC and a flow rate of 5.00 mL/min lead to a yield of C5 sugars of 67.409 wt%, comparable to that obtained by Garrote et al. with *Eucalyptus globulus*

wood, in a batch reactor at 181 ºC (58.4 wt%) [50]. Lower flow rates lead to the decomposition of sugars, while higher flow rates do not improve the extraction, but are not recommended as they would lead to greater difficulty in the separation.

In Figure 3d it can be seen that pH decreased rapidly, starting from a value around 5.5 (pH of deionised water) and reaching a minimum value around pH = 3.0 at about 20 minutes from the beginning of the reaction. After that time, it started to increase slowly, due to the reduction in acetyl groups in the hemicellulose along with time and the continuous incoming water. At constant temperature, with the lowest flow rate (2.50 mL/min) pH was almost constant after τ_{sol} = 20 min; with higher flow rates pH increased more rapidly, and with 20.00 mL/min the velocity of neutralization was the highest, and the highest final pH at τ_{sol} = 90 min is reached (pH = 4.10). The lowest pH value is reached with 2.50 mL/min (pH = 2.93) and the highest with 20.00 mL/min (pH = 3.20).

In accordance with other works [43, 51] it can be stated that powering the mass transfer trough the increase of the flow rate, leads to the higher removal of hemicellulose from the wood. Long liquid residence times enhance the hydrolysis of the oligomers, and thus the production of acetic acid that catalyses the depolymerisation of oligomeric xylans and further the degradation of monomers in the liquid phase.

3.3. Kinetic model for biomass fractionation

A simplified kinetic model was proposed for the overall extracted biomass, considering the variation of temperature and flow rate in the process. This model was based on a preliminary model for holm oak hydrothermal fractionation, which was performed by our research group [52] and has been used here to help in the clarification of the main effects.

In the solid phase, polymers start to depolymerize into monomeric sugars. Therefore, cellulose is fractionated to hexose monomers and hemicellulose to mainly pentose monomers and acetic acid. In parallel, hemicellulose, cellulose and deacetylated hemicellulose are partially solubilized by water, where they also are converted into monomers. The latter, only would be soluble at temperatures greater than 235 ºC. Regarding cellulose, its degradation is only considered at temperatures above 235.0 °C. Finally, acetic acid dissociation in protons and aqueous anions was introduced by an equilibrium constant. Thus, the reaction pathway includes 6 reactions, 3 in solid phase and 3 in the liquid phase (see Figure 4).

The model was obtained applying a mass balance for each compound in both phases [52]. This mass balance includes the mass transfer between both phases, the kinetics of each compound and the effect of the extraction in the bed porosity by the factor φ . The final expressions for the mass balance in liquid phase and in solid phase are Eq. (4) and Eq. (5), respectively.

$$\frac{d(1-\mathcal{E})\cdot C_{S_j}}{dt} = r_j - k_j \cdot a \cdot (C_{L_j}^{*} - \bar{C}_{L_j}) \qquad (4)$$

$$\frac{\delta C_{L_j}}{\delta t} = \frac{1}{\mathcal{E}} \cdot \left[r_j - \frac{u}{L} \cdot \frac{\delta C_{L_j}}{\delta z} - \varphi \cdot C_{L_j} \cdot \frac{dC_t}{dt} + k_j \cdot a \cdot (C_{L_j}^{*} - \bar{C}_{L_j}) \right] \qquad (5)$$

Equilibrium concentration in liquid phase (C_{Lj}*) was obtained by the product of the concentration in the solid and an equilibrium constant (H_j), which represents the solubility of biomass (C_{Lj}*=$H_j \cdot C_{sj}$).

Finally, kinetics (Eq. (6)) of each compound were calculated by an autocatalytic expression because it is useful to reproduce big changes in concentration. In liquid phase, they were multiplied by the proton concentration to include the auto-hydrolysis effect.

$$r_j = \sum_{i=1}^{i=n_{reac}} \Phi_{i,j} \cdot r_i$$

$$r_i = k_i \cdot \prod_{j=1}^{j=N} C_{f_j} \cdot \left(1 - \alpha_{i,j} \cdot \frac{C_{f_j}}{C_{f_t}}\right)^{\beta_{i,j}} \quad (6)$$

Where α is the initial velocity factor and β the acceleration factor. The former is related with the resistance of the material against thermal degradation and its recommended value is 0.99 [53]. The latter represents how fast the degradation is once it has started.

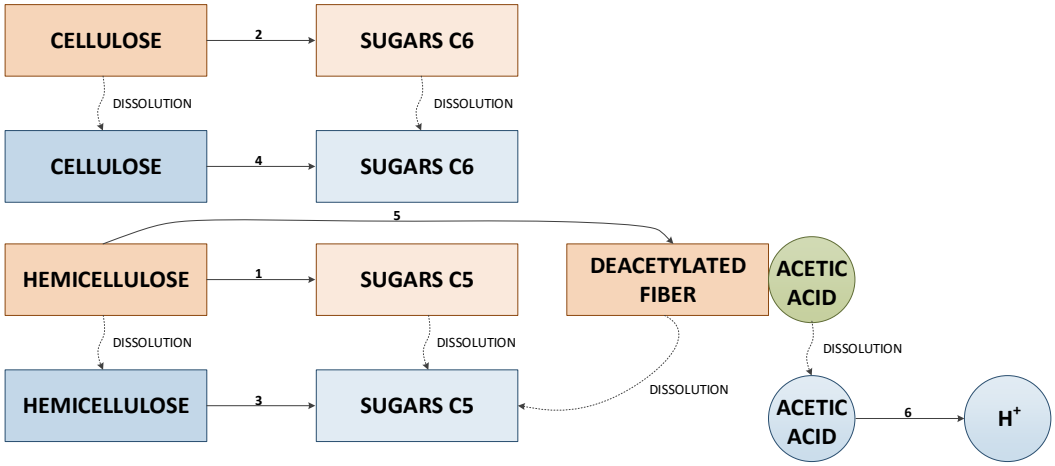


Figure 4. Simplified reaction pathway for eucalyptus hydrothermal fractionation.

3.3.1. Fit to experimental data

The developed model was used to adjust the experimental data of the extracted sugars, pentoses and hexoses and the pH evolution with time. In order to simplify the problem at high temperatures (greater than 185.0 ºC), the total amount of degradation products was assumed as sugars. Therefore, xylose, arabinose, furfural and formic acid were summed to calculate pentoses (C5); while, glucose, HMF and pyruvaldehyde to calculate

hexoses (C6). The data obtained at 135.0 ºC were not used due to the low amount of biomass extracted at this operational condition. These fittings imply an optimization problem which was solved by the Broyden–Fletcher–Goldfarb–Shanno's method [54] . The objective function was the addition of the Average Absolute Deviation (A.A.D., see Eq. (7)) of these three profiles. The calculated parameters are arrayed in Table 4.

$$A.A.D. = \sum_{i=1}^{n} \frac{1}{n} \cdot \left| \frac{X_{exp} - X_{sim}}{X_{exp}} \right| \cdot 100 \qquad (7)$$

For the experiment at 285.0 ºC and 5.00 ml/min, the result of the adjustment is shown in Figure 5. The model was able to reproduce the evolution of pentose sugars and the pH, including the fact that the maximum in sugar concentration and the pH minimum took place at the same time. This agreement shows that the model was able to successfully simulate the hemicellulose deacetylation. In addition, from the adjustment shown in Figure 5c, it can be concluded that the model also could reproduce the behavior of the hexose sugars, whose maximum delated around 60 min with respect to pentoses due to the high resistance of cellulose against hydrothermal degradation. Therefore, pentoses are extracted at the beginning of the operation and hexoses only are recovered at the final stage. However, around 20 % of sugar degradation was found at these operational conditions (Table 2), which is relatively high.

On the other hand, the A.A.D. for each experiment is arrayed in Table 3, being the average deviation

28.57, 39.20 and 5.7 % for pentoses, hexoses and pH respectively. These discrepancies were relative low taken into account the small amount of sample introduced in the reactor, the dilution of the samples, the complexity of the process (which has been simulated only with 8 compounds) and the biodiversity of the wood. In addition, the deviations fall up to 10.49 % and 13.17 % for C5 and C6, respectively, when the simulated and the

experimental extracted mass are compared (Figure 2d and 2e). Therefore, it seems that the proposed model was able to reproduce the biomass fractionation by hydrothermal treatments.

Table 3. A.A.D. of the fitted experiments.

T	Q	A.A.D. (%)[1]		
ºC	mL/min	C5	C6	pH
185	2.5	24.51	*	2.9
185	5	28.91	*	4.23
185	10	35.8	*	1.09
185	15	42.96	*	12.72
185	20	12.92	*	8.43
235	5	36.43	48.58	6.75
285	5	18.46	29.83	2.91
AVERAGE		28.57	39.20	5.57
T	Q	A.A.D. (%)[2]		
ºC	mL/min	C5	C6	
185	2.5	15.80	*	
185	5	9.38	*	
185	10	7.03	*	
185	15	8.22	*	
185	20	8.67	*	
235	5	8.08	14.37	
285	5	16.27	11.97	
AVERAGE		10.49	13.17	

*Due to the low temperature, cellulose fractionation was assumed as negligible.[1] Deviation for the instant concentration. [2] Deviation for the extracted mass.

Table 4. Mass transfer and kinetic parameters obtained from the fitting of the model to experimental data.

FLOW EFFECT

Flow	k·a(min^{-1})						β_{HC}	β_C
mL/min	HC[1]	C[2]	C5[3]	C6[4]	Acetic[5]	DH[6]	dimensionless	dimensionless
2.5	0.006	*	0.019	0.019	0.002	*	15.5	0.0
5	0.009	0.009	0.029	0.029	0.0025	0.01	15.0	0.0
10	0.011	*	0.031	0.028	0.021	*	14.8	0.0
15	0.016	*	0.040	0.028	0.030	*	13.5	0.0
20	0.020	*	0.054	0.054	0.070	*	15.0	0.0
R^2	0.98	*	0.98	0.98	0.91	*	0.96	*

TEMPERATURE EFFECT

T	H(dimensionless)						β_{HC}	β_C
ºC	HC[1]	C[2]	C5[3]	C6[4]	Acetic[5]	DH[6]	dimensionless	dimensionless
185	0.073	000	0.584	0.584	0.20	0.00	15.5	0.0
235	0.400	0.20	0.627	0.627	0.21	0.02	21.0	0.0
285	0.500	0.21	0.650	0.650	0.22	0.23	28.0	4.2
R^2	0.91	-	0.97	0.97	1	-	0.99	-

T	K (min^{-1} · g^{-1})					
ºC	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]
185	0.2	*	0.7081	*	0.0234	0.079
235	1.0	0.0008	1.9821	0.053	0.0238	0.095
285	4.5	0.0220	4.4442	0.248	0.0240	0.120
R^2	0.99	*	0.999	*	0.97	0.98

[1]Hemicellulose. [2]Cellulose. [3] Pentose (Sugar C5). [4]Hexose (Sugar C6). [5]Acetic Acid. [6] Deacetylated hemicellulose.

[a]Hemicellulose breaking in solid phase, [b]Cellulose fractionation in solid phase, [c]Hemicellulose degradation in water, [d]Cellulose degradation in water, [e]Hemicellulose deacetylation, [f]Acetic Acid dissociation, *Parameter not considered due to the low temperature.

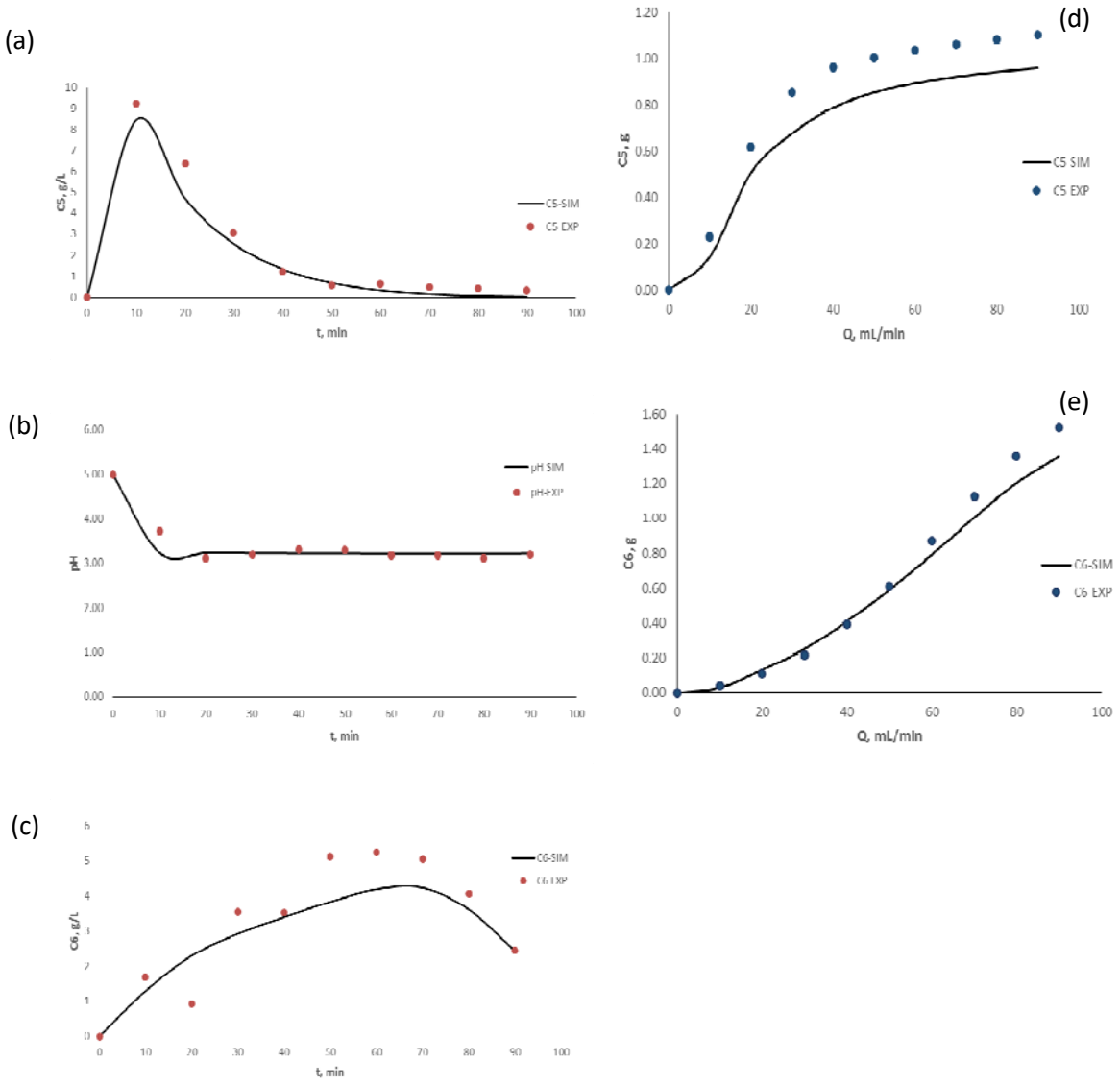


Figure 5. Adjustment of the experiment at 285.0 ºC and 5 ml/min. (a) Sugar C5, (b) pH and (c) Sugar C6 and comparison between the experimental and simulated extracted mass of C5 (d) and C6 (e) . C5-SIM: Simulated concentration of sugars C5. C6-SIM: Simulated concentration of sugars C6. pH-SIM: Simulated pH. C5-EXP: Experimental concentration of sugars C5. C6-EXP: Experimental concentration of sugars C6. pH-EXP: Experimental pH.

3.3.2. Analysis of the model parameters

The physical sense of the parameters listed in the Table 4 was checked in this section. Regarding the mass transfer coefficient ($k_j \cdot a$), it was seen that all of them followed a linear function with flow (regression coefficients greater than 0.91) as it was expected. In the same way, it was calculated that the equilibrium constant or solubility (H_j) was enhanced with temperature linearly (R^2 greater than 0.91), which is a common behaviour in a solid dissolution process. Finally, it was also obtained that the kinetic constants (k_i) followed the Arrhenius' law with R^2 bigger than 0.97.

Furthermore, β increases with temperature and decreases with flow. Temperature is the main variable in this process and it enhances the extraction and justifying the increment in the acceleration factor (β). In contrast, the flow reduces the acceleration factor, although it also enhances extraction. The reason could be that it also affects the residence time of the liquid phase, so a higher flow means less degradation product formation, more dilution and smother liquid profiles. It is also remarkable that the kinetics for cellulose fractionation are always lower than the kinetics for hemicellulose degradation, which agrees with the fact that cellulose is stronger than hemicellulose against hydrothermal degradation.

3.4. Lignin removal and structural alteration

Table 2 shows that at 185.0 °C lignin content in the exhaust solid is almost equal to the one in the raw material. At 235.0 °C and 5.00 mL/min a lignin reduction of approximately 15.00 wt% occurred, while a reduction of approx. 52 wt% at 285.0 °C occurred. A loss in lignin content in wooden biomass during hydrothermal pre-treatments is widely

documented [12, 55], Leschinsky et al. reported that C thermal pretreatment of *Eucalyptus globulus* wood at 170 °C causes a loss of the molecular weight in lignin [56].

When lignocellulosic biomass is subjected to high temperature or mild acidic pre-treatments, lignin and lignin-carbohydrate complexes coalesce creating some spherical formations that migrate out to the wall cells and deposit in the surface of the residual biomass. These droplets have a negative effect on enzymatic hydrolysis of cellulose, affecting the efficiency of enzymatic conversion in a lignocellulosic biorefinery [57].

Figure 6 shows 3 SEM images of the Eucalyptus wood after treatment at 185.0 °C, 235.0 °C and 285.0 °C, with a liquid flow rate of 5.00 mL/min. At 185.0°C no evident changes are visible in the wood structure: the three-dimensional structure of lignin associated with linear molecules of cellulose is visible in figure 6a.

A modification in the structure of the wood is evident at 235.0 °C: lignin-carbohydrate droplets start to appear on the wood surface, as shown in figure 6b. The number of droplets increases significantly at 285.0 °C (figure 3c) where only sporadic fibres of broken cellulose are visible, directly connected through hemicellulose units (associated hemicellulose) as described in chapter 3.1.

While in batch reactors, all the droplets deposit on the surface and harden after the cooling, creating a barrier for enzymatic attach, in semi-continuous reactor this issue is lightened, as the liquid flow carries them constantly out of the system; this is evident from the lignin loss observed at high temperatures. This is another big advantage of using a semicontinuous reactor for the pretreatment of biomass.

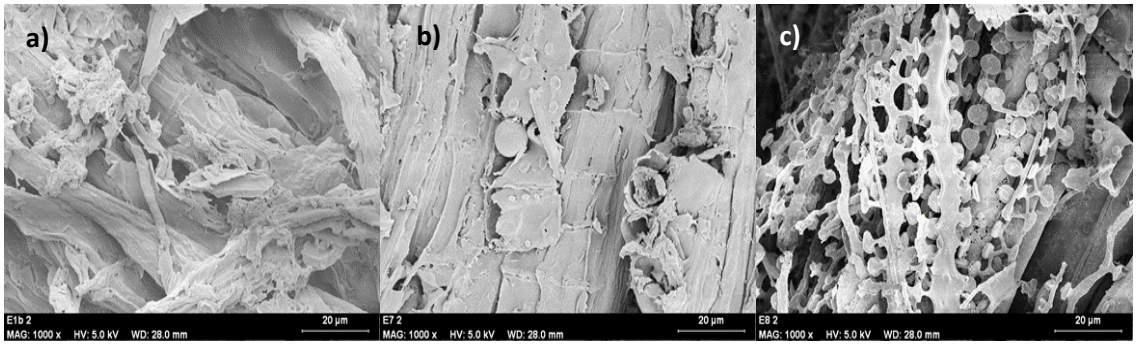


Figure 6. SEM images of Eucalyptus exhausted wood after pretreatment at a) 185.0°C, b) 235.0 °C, and c) 285.0 °C with a liquid flow rate of 5 mL/min.

4. Conclusions

In this study, we have focused in the effect of liquid flow rate on the extraction of hemicellulose from eucalyptus biomass, more specifically in the residence time. Operating in semi-continuous mode has the advantage of separating the residence time of the solid (easy to charge and keep inside a tubular reactor) and the liquid (moving through the bed created). Solid residence times between 20 and 40 min are perfect for extracting hemicelluloses, liquid residence times below 2.00 min avoid by-products with a temperature of 185° C. The influence of the flow is not perceived at 135 ºC, while at higher temperatures, the increase in yield is counterbalanced by the formation of degradation products. A simplified model considering an auto catalytic kinetics represents the operation very well. This work establishes the basis of the scale-up of the semi-continuous hydrothermal reaction.

Acknowledgements

The authors acknowledge the Spanish Economy and Competitiveness Ministry, Project FracBioFuel: ENE2012-33613 and the regional government (Junta de Castilla y León), Project Reference: VA330U13 for funding. MEng. Gianluca Gallina wish to

acknowledge the Spanish Economy and Competitiveness Ministry for the scholarship/predoctoral contract BES-2013-063556. Alvaro Cabeza also wish to acknowledge the Spanish Economy and Competitiveness Ministry for the scholarship/predoctoral contract FPU2013/01516.

Abbreviations and symbols

Greek letters

$\alpha_{i,j}$: Initial velocity factor for the compound "j" in the reaction "i", dimensionless.

$\beta_{i,j}$: Acceleration factor for the compound "j" in the reaction "i", dimensionless.

β_C: Acceleration factor for cellulose, dimensionless.

β_{Hc}: Acceleration factor for hemicellulose, dimensionless.

$\mathcal{E}$: Porosity of the bed, dimensionless.

$\mathcal{E}_f$: Porosity of the bed, calculated at the end of the experiment, dimensionless.

$\mathcal{E}_{av}$: Average porosity of the bed, between the beginning and the end of the experiment, dimensionless.

$\mathcal{E}_0$: Porosity of the bed, calculated at the end of the experiment, dimensionless.

φ: Relation factor between porosity and the total concentration in solid phase, dimensionless.

$\Phi_{i,j}$: Stoichiometric coefficient of the compound "j" for the reaction "i", mg.

τ_{sol} : residence time of solid inside the reactor, min

τ_{liq} : residence time of liquid inside the reactor, min

Symbols

C_{f_j}: Concentration of the compound "j" in the phase "f", mg/L

C_{L_j}: Concentration of the compound "j" in the liquid phase, mg/L

$\bar{C}_{L_j}$: Average concentration of the compound "j" along the reactor in liquid phase, mg/L

$C_{L_j}^*$: Equilibrium concentration of the compound "j" in liquid phase, mg/L

C_{S_j}: Cocnetration of the compound "j" in the solid phase, mg/L

C_t: Total concentration in the solid, mg/L

Q: Liquid flow rate, mL/min

Ea/R: Activation energy, K

H_j: Equilibrium constant between the solid and the liquid, dimensionless

k: Pre-exponential factor of the kinetic constant, $mg^{-1} \cdot min^{-1}$

k_i: Kinetic constant, $mg^{-1} \cdot min^{-1}$

$k_j \cdot a$: Mass transfer coefficient multiplied by the specific exchange area, min^{-1}

N: Number of compounds, dimensionless

n_{rec}: Number of reactions, dimensionless

L: Length of the reactor, m

t: Operating time, min

r_i: Reaction velocity "i", mg/min·L

r_j: Reaction rate of the compound "j", mg/min·L

R^2: Coefficient R^2, dimensionless

T: Operating temperature, ºC

u: Liquid velocity in the reactor, m/min

$x_{i_{EXP}}$: Experimental value of the fitted variable

$x_{i_{SIM}}$: Simulated value of the fitted variable

z: Coordinate along the length of the reactor, dimensionless

s_x: uncertainty of the experimental concentration, ppm

s_y: standard deviation of the calibration patrons, ppm

s_{xx}: deviation of the HPLC areas, dimensionless

L: number of experiment repetitions, dimensionless

N: number of calibration points, dimensionless

m: slope of the calibration, ppm

x_i: experimental HPLC area, dimensionless

$\overline{x}$: average experimental HPLC area, dimensionless

y_i: patron concentration “i”, ppm

$\widehat{y_i}$: calculated patron concentration “i”, ppm

$\overline{y_x}$: average experimental concentration, ppm

$\overline{y}$: average patron concentration, ppm

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CHAPTER 2

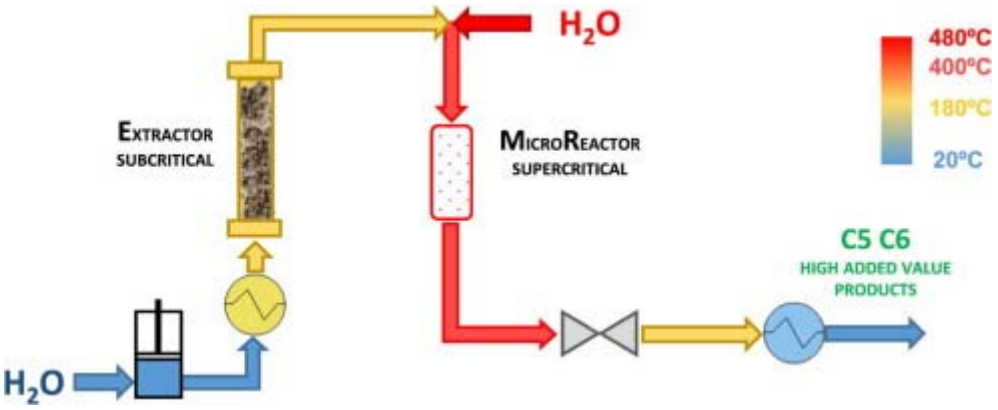
ONLINE INTEGRATED FRACTIONATION-HYDROLYSIS OF LIGNOCELLULOSIC BIOMASS USING SUB- AND SUPERCRITICAL WATER.

ONLINE INTEGRATED FRACTIONATION-HYDROLYSIS OF LIGNOCELLULOSIC BIOMASS USING SUB- AND SUPERCRITICAL WATER

Abstract

A novel process coupling the fractionation and hydrolysis reactors is presented. Holm oak was used as real lignocellulosic biomass to be treated. In the fractionation reactor, hemicellulose and cellulose were solubilized and partially hydrolyzed in different stages with the aim of feeding the hydrolysis reactor with high C5 concentrations or C6 concentrations. The fractionation was performed in two stages: at 180ºC optimizing the hemicellulose extraction and at 260ºC extracting cellulose and hard hemicellulose remaining in the biomass structure. Three water flows were tested: 11, 17 and 26 cm^3/min. Sugar yields from 71 to 75% were reached, mainly composed of xylose and glucose oligomers and lower amounts of other chemicals, like retro-aldol products, acetic acid or 5-HMF. The outlet stream from the fractionation reactor was directly mixed with sub or supercritical water at the inlet mixer of a SHR where the reaction time was precisely controlled. The temperature, pressure and reaction time were modified to get an insight of their effect on the yield of retro-aldol condensation products. Yields of 24% for glycolaldehyde, and pyruvaldehyde were found at 8.3 s, 350ºC and 160 bar (hydrolysis reactor conditions). In other hand, 25% of lactic acid was found at 0.23 s, 400ºC and 245 bar. A discussion based on a known reaction pathway is proposed. Moreover, a kinetic model for the hydrolysis reactor was proposed, being able to reproduce the experimental data with deviations lower than 10 % for sugars and other products extracted. This combined process performs a selective valorization of real lignocellulosic biomass,

avoiding the costly process of extreme grinding needed for the fluidization in a continuous hydrothermal process.



Keywords: Glucose, Glycolaldehyde, Kinetics, Process Development, Xylose, Holm Oak Wood

Introduction

Even if it is reasonably assumed that biomass from plants will be the main carbon source in the future, the choice of which reaction medium should be used to depolymerize and valorize biomass has not been taken yet. Pressurized fluids, especially sub and supercritical water (Tc=374ºC and Pc= 22.1 MPa), can be pointed as a promising alternative to depolymerize and valorize biomass [1-5]. Physical and chemical properties of water can be modified by adjusting pressure and temperature around the critical point, making water a reaction medium able to favor different kind of reactions [1]. Because of this reason, hot pressurized water has been used as reaction medium for fractionation [6-9] , hydrolysis [10-12] and valorization of biomass [13-16].

The composition of lignocellulosic biomass is highly dependent on the plant species and growth conditions. However, it can be considered that the average composition of lignocellulosic biomass is approximately: cellulose (40% wt.), hemicellulose (25% wt.), lignin (25% wt.), extractives and ashes (10% wt.) [17]. Although biomass is composed by diverse and complex molecules, it can be fractionated principally into C6 sugars (mainly glucose), C5 sugars (mainly xylose) and lignin [3]. These three fractions can be further modified to produce a wide range of products like: ethanol, hydrogen, glycolaldehyde, pyruvaldehyde, lactic acid and 5-HMF among others [3, 18-25].

The fractionation of biomass can be defined as the selective separation of C5 sugars, C6 sugars and lignin from the original biomass matrix. This process was studied under hydrothermal conditions in different ways of operation: batch, semi batch and continuous [3, 26]. Semi batch and continuous processes allow obtaining higher yields of sugars and chemical compounds than batch reactors, because it is possible to control the temperature (T) and the residence time (t_r) more accurately than in batch processes. Continuous

processes are the most appropriate to control the reaction conditions (T and t_r), however, in most cases it is necessary to apply expensive pretreatments to the raw material before the fractionation+hydrolysis process, for example: exhaustive size reduction [27]. On the other hand, the continuous process can be performed at different operating conditions in order to separate the C5 sugars from the C6 sugars.

The extraction of hemicellulose from woody biomass can be carried out at temperatures between 130 ºC and 260 ºC, solid reaction times between 20 and 60 min and liquid residence times inside the reactor between 0.1 min and 1 min. At those conditions, hemicellulose can be both extracted and hydrolyzed. After the extraction at 180ºC, two products are usually obtained: a liquid composed mainly of C5 sugars and a solid composed of C6 sugars and lignin. These two products can be separated by filtration. Then, the cellulose in the solid can be hydrolyzed at supercritical conditions to obtain a water solution of C6 sugars and a solid enriched in lignin. These processes can be carried out in two reactors with a filtration operation between them. Another option which allows the intensification of the process is using one fixed bed reactor. In such a case, the biomass is loaded in the reactor and the hydrolysis temperature is changed in order to hydrolyze C5 or C6 sugars [28]. The semi batch process allows high performances on the yields of C5 sugars hydrolysis. However, when the reaction temperature is increased to hydrolyze the recalcitrant cellulose and hemicellulose, the yield of recovered sugars decreases because of the increment of the sugars further reactions [10, 11, 29].

The continuous reactors have been employed in many applications for the valorization of sugar streams allowing a precise control over the reactions [19-21]. These reactions can be managed using pressurized water and choosing the adequate reaction conditions. For

example, at temperatures between 200ºC and 300ºC (25 MPa) the water molecules are highly dissociated favoring the ionic reactions, like the production of 5-HMF from fructose and glucose [1]. On the other hand, at 400ºC (25 MPa) the water molecules are highly associated favoring the non-ionic reactions, like the retro aldol condensation reactions [1].

In this article, a novel integrated fractionation-valorization process was designed and built using wooden biomass as raw material and water (subcritical and supercritical) as reaction medium. The wooden biomass was fractionated in a fixed bed reactor at different temperatures. The solubilized products were directly injected to a continuous near critical water reactor to efficiently convert C5 and C6 sugars into valuable products, like glycolaldehyde, pyruvaldehyde and lactic acid avoiding a further hydrolysis to organic acids. In addition, a kinetic analysis of the biomass hydrolysis was done in order to study the differences in the process when subcritical and supercritical conditions were used.
The objective of this research paper was to design a novel process capable of converting lignocellulosic biomass into valuable products eluding the excessive milling of biomass and decreasing the number of reactors.

1. Experimental

2.1 Materials

Deionized water produced by Elix® Advantage purification system was used as reaction medium to run the experiments. The standards used in a High Performance Liquid Chromatography (HPLC) analysis were: cellobiose (≥98%), glucose (≥99%), xylose (≥99%), galactose (≥99%), mannose (≥99%), arabinose (≥99%), glyceraldehyde (≥95%), glycolaldehyde dimer (≥99%), lactic acid (≥85%), formic acid (≥98%), acetic acid

(≥99%), acrylic acid (≥99%), furfural (99%) and 5-hydroxymethylfurfural (≥99%) purchased from Sigma. 0.01 N solution of sulfuric acid (HPLC grade) in Milli-Q® grade water was used as the mobile phase in the HPLC analysis. Sulfuric acid (≥96%) and calcium carbonate (≥99%) supplied by Panreac, Spain, were used as reagents for the quantification procedure of structural carbohydrates and lignin [30]. Also, Milli-Q® water was used in this determination. Holm oak wood employed as raw material was collected in Spanish forests. The wood was milled obtaining chips with average width of 2 mm and average length of 5 mm, as it is shown in Figure S1 of Supplementary Material.

2.2 Analytical methods

The composition of the holm oak wood raw material, exhausted solid and extracted liquor was determined through two Laboratory Analytical Procedures (LAP) from NREL [30, 31]. The procedure for solid samples consists in quantifying the structural carbohydrates and lignin in the biomass as follows. A) The biomass was weighted before and after being dried in an air driven oven at 105 °C for 24 hours in order to calculate the moisture content. B) Dried biomass was treated in a Soxhlet equipment with n-hexane, leaving a solid free of oils and other extractives. C) 300 mg of dried and free-extractives solid from step (b) were hydrolyzed in 3 ml of 72% wt sulfuric acid solution at 30 °C for 30 min, in order to break the bonds between biopolymers and the main solid structure. D) The mixture of oligomers obtained in step (c) is diluted using 84 ml of deionized water and heated at 120 °C for 60 min with the aim of hydrolyzing hemicellulose and cellulose to obtain their correspondent monomers [32]. E) The solid is separated from the solution by vacuum filtration. F) The total mass of solubilized sugars was quantified as the difference in weight between the original solid and the exhausted solid after oven drying at 105 °C in oven for 24 hours. G) The exhausted solid is placed in a muffle at 550 °C for 24 h and

the remaining residue was weighted before and after this step to calculate the insoluble lignin and the ash content of the sample. H) A liquid aliquot was analyzed with UV-Vis spectrophotometer at 320 nm with extinction coefficient of 34 $Lg^{-1}cm^{-1}$ [33] to calculate the amount of soluble lignin. I) Another liquid aliquot was neutralized to pH range 6 to 7, then it was filtered using a 0.2 μm membrane and analyzed by HPLC determining the carbohydrates composition. This procedure is performed using a column SUGAR SH-1011 (Shodex) with a 0.01 N of sulfuric acid solution as a mobile phase. To identify the soluble products, two detectors were used: Waters IR detector 2414 (210 nm) and Waters dual λ absorbance detector 2487 (254 nm). In order to calculate the amount of carbohydrates, each chromatogram was integrated numerically by decomposing it into a sum of 9 to 13 Gaussian peaks, minimizing chi squared function of a Levenberg-Marquardt-Flecher algorithm [34]. Glycolaldehyde and Pyruvaldehyde resulted to be overlapped, since the retention time of their standards is extremely close (11.99 vs 12.24 minutes, respectively). So we refer to them as glycolaldehyde-pyruvaldehyde.

The raw material contained 1.6 % wt. extractives, 1.8% wt. moisture, 0.2% wt. ashes, 24.2% wt. Klason lignin (from which 4.0% corresponds to soluble lignin), 45.7% wt. of hexoses, 23.9% wt. pentoses. The sum of all the components represents the 97.4% of total weight, the discrepancy is due to experimental errors like the loss of solid material after the recovery at the end of the experiments, or the inhomogeneity of the material which can have slightly different compositions depending on the analyzed aliquot; in any case, it is inside the acceptable experimental error.

The amount of C6 was calculated as the sum of glucose, cellobiose and fructose concentrations. Xylose was the only C5 detected. Acetic acid was considered to come from the deacetylation of xylan during the extraction process or, as explained in the next

sections, from the hydrolysis of pyruvaldehyde. The hydrolysis products from hexoses and pentoses were mainly glyceraldehyde, glycolaldehyde, pyruvaldehyde, lactic acid 5-hydroxymethylfurfural and in some cases acrylic acid were detected in very low concentration.

The procedure followed to analyze liquid samples consists in the steps (C), (D) and (I) described above. In this case, the carbon content liquid solutions was determined by total organic carbon (TOC) analysis using a Shimadzu TOC-VCSH equipment. Every sample was previously filtered using a 0.2 μm syringe filter and diluted 1:10 times with Millipore water.

The pH of the outlet stream was measured online using an electronic pH-meter (Nahita model 903).

2.3 *Experimental setup and operation procedure*

The setup used in this work is shown in Figure 1. The system consisted in two reactors online integrated: 1) the fractionation reactor, where the C5 and C6 are solubilized and partially hydrolyzed; 2) the supercritical hydrolysis reactor (SHR), which converts the soluble compounds into added value products. The fractionation line is composed of a water deposit (D.1), downstream an American Lewa EK6 2KN high pressure pump (P.1, maximum flow rate 1.5 kg/h) propels water through a pre-heater (H.1, 200 cm of 1/8" SS 316 pipe, electrically heated by means of two resistors of 300 W) which ensures an uniform temperature at the reactor inlet. The reactor (R.1), a tube of SS 316, 40 cm length, 1.27 cm O.D., is heated by three flat resistors of 300 W each, placed axially along a machined aluminum bar with 5.08 cm O.D. Both, preheater and the reactor are located inside a former chromatographic oven HP5680. The out-flow stream from the extraction line is mixed with the supercritical water stream, entering in a second reactor (SHR) (R.2).

The supercritical water line is composed of a heater (H.2), a tube of 18 m, 1/8 in O.D. SS316 wrapped around a brass cylinder and heated by two cartridges and two flat resistors, which provided adjustable power of up to 10 kW, in order to control the temperature of this stream. The water flow was generated by a Milton Roy XT membrane pump (P.2, maximum flow rate 6 kg/h). The SHR allows a fast heating of the biomass stream, which is mixed almost instantaneously with the supercritical water stream, and a rapid cooling of the products, which takes place through a sudden expansion which efficiently stops the hydrolysis. In this way, the reaction time could be precisely calculated, as the reactor works isothermally. Pressure was controlled Micro Metering valve 30VRMM4812 from Autoclave Engineering (V.4). The setups of the two reactors were presented in detail in previous works [29, 35].

An average amount of 6.12±0.03 gr of holm oak biomass was placed inside the fractionation reactor for each experiment. Two metallic filters were used, located on the top and bottom of the reactor, avoiding the release of the solid during the experiments. A pressure test with cold pressurized water was carried out before every experiment, with the aim to check the presence of leaks in the system. Then, the supercritical line was heated ensuring the functioning of the system at required operating conditions. Once these conditions were stable, the pumps were switched off and both, the preheater and the fractionation reactor, were heated up until the temperatures reached the respective set values. Afterwards, both pumps were switch on again and the flow and pressure were set to the desired conditions, zero time is considered when pressure reached the desired value. A total of 11 experiments were performed (3 fractionations and 8 coupled reactions), obtaining a total of 130 liquid and 11 solid samples, characterized with the methods described above. Six experiments were performed varying the temperature in the SHR

from subcritical (350 ºC) up to supercritical (400 ºC) conditions, maintaining the pressure at 25.0±1.0 MPa. The reaction time in this reactor was modified by varying the water flow-rate and changing the reactor volume (2.2 or 12.4 cm^3); reaction times between 0.25 s and to 12 s were tested. Three different water flows (11, 17, 26 cm^3/min) were tested in the fractionation line, maintaining constant the ratio with the flow of supercritical water stream, to get the desired conditions during the further hydrolysis. The feed composition to the SHR was analyzed by carrying out three fractionations without the second hydrolysis stage, at the same conditions of temperatures, flow-rates and pressure tested with the coupled reaction.

The fractionation in the fixed bed reactor was performed in two stages marked by two distinct temperatures: 180 ºC to extract the hemicellulose and 260 ºC to remove most of the cellulose fraction from the biomass. The heating time between both setpoints was in the range of 5-10 min, while the flow was temporarily stopped for the experiment running at 26 cm^3/min. In order to follow the reaction evolution, the pH of the outlet stream was measured online sampling every 1 minute. Liquid samples (30-40 cm^3) were taken according the pH variations every 5 to 20 min for the experiment at 11 cm^3/min, and every 2 to 8 min for the other experiments. The overall experiment time varied from 110, 60 and 45 min for the runs at 11, 17, 26 cm^3/min, respectively (called here as (1), (2) and (3)). After the last sample was grabbed, the heating was turned off and the fractionation reactor was let to cool down to room temperature with air flux. Both pumps were set to zero flow and the system was depressurized. The solid was removed from the reactor, filtered and dried 24 h at 105ºC for further analysis. After cleaning, the fixed bed reactor was placed back, tightened and the system was washed out with deionized water.

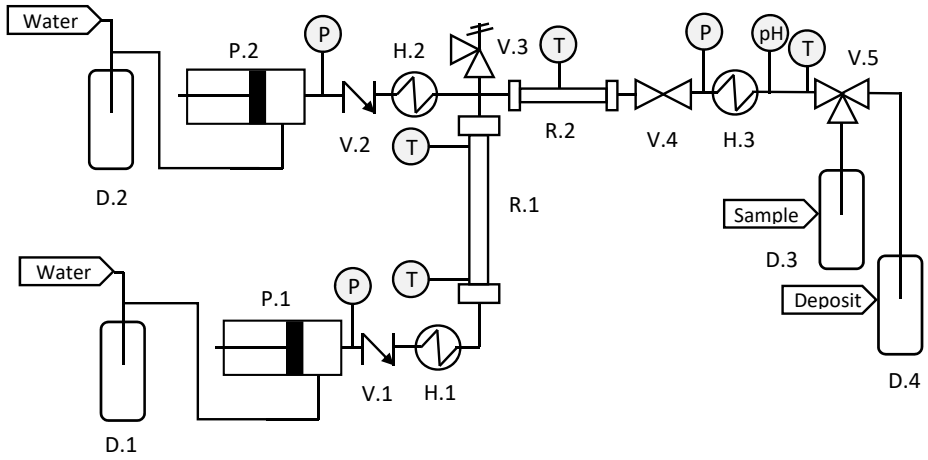


Figure 1. Experimental setup coupling fractionation and hydrolysis reactors.

D.1, D.2: Deionized water deposits. P.1: American Lewa EK6 2KN High pressure piston pump. P.2: Milton Roy XT membrane pump. V.1, V.2: Parker check valve. H.1: Electric low temperature heater, 100 cm of 1/8 in SS316 piping and 2 kW resistor. H.2: 1800 cm of 1/8 in SS316 piping and, high temperature heater and 10 kW resistor. R.1: Fractionation reactor, 40 cm length, ½ in O.D. SS316 piping. V.2: Parker relief valve. R.2: Second Reactor (SHR) built with ¼ in O.D. SS316 tubing. Two reactors sizes were used 11 cm and 100 cm of length. V.3: Parker relief valve. V.4: high temperature valve Autoclave Engineers 30VRMM4812. IE: 200 cm of concentric tube heat exchanger ½ in- ¼ in. V5: Three way Parker valve. D.3: Falcon® flasks. D.4: 25 L polyethylene products deposit.

3. Results and Discussion

3.1. *Biomass fractionation*

From the analysis of the raw holm oak, the amount of soluble material was 4.65 ± 0.03g, corresponding to 72.1% of the biomass weight. 3.02 ± 0.02g of this soluble mass were composed of hexoses (C6) and 1.58 ± 0.01g of pentoses (C5). The spatial time of the liquid (τ_l), is determined using the liquid flow rate, the reactor volume and the average porosity of the bed (ε_{i0}=0.457±0.01, ε_f=0.948±0.019). The latter was calculated by means

of Eq. (1), taking into account the initial and the final fraction of void volume in the bed, due to the shrinking size of the biomass particles, and also considering a constant density for water [35] (since its variation with temperature is less than 2%) and a constant density of the holm oak wood (800 kg/m^3, dry based for holm oak species). In this sense, residence time for the liquid inside the fixed-bed reactor was in the range of 1.0 min $<\tau_l<$2.1 min.

$$\varepsilon_f = \varepsilon_0 + (1-\varepsilon_0)\frac{(m_0-m_f)}{m_0} \qquad (1)$$

Figure 2 shows the cumulated mass of total soluble materials, oligomers and monomers of C5 and C6, as well as products deriving from the further reaction of sugars. These values were determined using TOC and HPLC analysis of the products. The different conditions were obtained by changing water flow-rates in the fractionation line for the experiments 1, 2 and 3. The break points shown in the curves signals present the transition between the two temperature stages. The mass of soluble compounds detected by TOC was calculated by dividing the value of total organic carbon concentration recognized by the equipment by a factor 0.42 (Eq. (2)).

$$r = \Sigma r(i) = \sum \frac{m(i)\,(RM)}{m_{sol}tot\,(RM)} \frac{Mw(i)}{Mw\,\Sigma C(i)} = 0.42 \qquad (2)$$

The factor *r* is the ratio between the molecular weight of the soluble compounds extracted from holm oak raw material to the molecular weight of the atoms of carbon in the same compounds. This value is an approximation that allows comparing the mass obtained by TOC analysis (total amount of C) with the mass quantified by HPLC (total amount of soluble compounds).

This approximation is based only on the sugar contents and it is used as a general value for all the experiments. It does not consider the effect in the carbon ratio of the

condensation and dehydration reactions happening during the extraction and hydrolysis. In addition, it does not take into account the amount of soluble lignin since it is relatively low (only a 4% in the raw material, see section 2.2). However, in all the experiments, the mass balance matched with a maximum error around 20%.

The overall material balance was calculated by summing the mass of the solid recovered from the reactor at the end of the experiment to the mass of the soluble material estimated using the quantified amount by TOC and the assumed factor showed in equation 2; and to the mass of insoluble lignin flushed by the water stream. For experiments 1, 2 and 3, this mass balance was equal to 103.8, 93.7 and 84.9% related to the amount of biomass fed to the reactor.

The mass of soluble material obtained by TOC with the same values obtained by HPLC for each sample are compared in the first row of graphs in Figure 2 (a). The values plotted in Figure 2 are the yields of soluble compounds obtained from each technique related to the same amount in the raw biomass. The discrepancy between both values is reduced when the water flow-rate through the extraction reactor is increased from 11 to 26 cm^3/min (23.6, -4.1 and -3.2% for the three flows, respectively). This fact could be explained by the increasing production of compounds derived from the sugars hydrolysis (mainly organic acids) not identified by HPLC or whose value is so low that it cannot be detected. From HPLC chromatographs of experiment 1, some peaks do not fit with the retention time of the 17 standard compounds identified in this column (e.g. Figure S2 in Supplementary Material). Besides, some other peaks were not completely resolved. The amount of sugars and soluble oligomers of C5 and C6 obtained from fractionation are displayed in the second line of graphics in Figure 2 (a). Most of the hemicellulose was hydrolyzed to oligomers, in fact hemicellulose is highly soluble in water because of the

abundance of acetyl groups in its amorphous structure[36], and after the first breaking leads to the production of soluble oligomers.

The yield of C5 at the end of second stage of temperature was 87.3, 89.8 and 93.1%, for experiments 1, 2 and 3, respectively. On the contrary, the crystalline nature confers to cellulose a water insoluble character, so, the oligomers with only very low molecular weight would be water soluble. The ratio between the amounts of hexoses oligomers to monomer is near to one for the experiment 1 and this ratio is enlarged with the flow increase (experiments 2 and 3). The oligomers quantification was obtained from the difference between glucose, cellobiose, fructose and xylose of the liquid containing all the soluble sugars and the same compounds obtained from the total acid hydrolysis of this liquid [35]. So, high liquid spatial times enhanced the hydrolysis and solubilization of hexoses. This distribution could be related to the difference in the activation energy of the cleavage of the hydrogen bonds between celluloses and the α1-4 glycosidic bond hydrolysis, which is known that is favored at subcritical conditions [10, 29]. The last row of graphs in Figure 2 (a) displays different amounts of products from the hydrolysis of xylose, glucose and fructose. These amounts are depreciable at the first stage and are increased after temperature is raised. However, these compounds are one order of magnitude lower than the soluble sugars during the extraction. An example is 5-HMF, which is produced mainly in the second stage of fractionation temperature where the conditions make the water a highly ionic medium in the fractionation reactor. The main components in the output stream were 5-HMF, pyruvaldehyde, glycolaldehyde and lactic acid. The decrease of the reaction time of liquid inside the reactor diminished the further transformation of sugars.

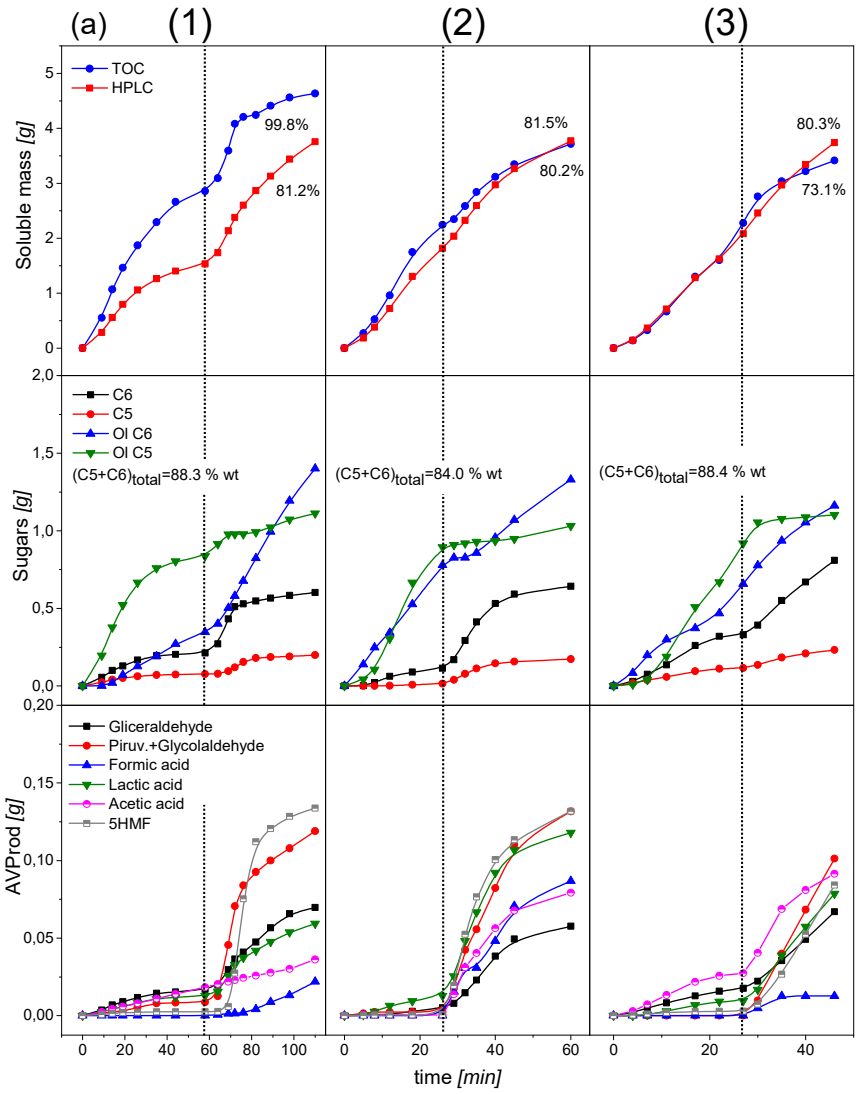


Figure 2. Product distribution and mass balance in the biomass valorization.

(a) Results from the fractionation without further hydrolysis for 11, 17 and 26 cm^3/min (1, 2 and 3) in the extraction line. The first line of graphs represents to the percentage of soluble compounds identified by total organic carbon (TOC) and HPLC. The yields, calculated by the obtained soluble fractions [g]/(4.65 g), are showed above each curve. Second row of graphs in Figure 2 shows the amount of carbohydrates in the form of sugars and oligomers. The last row displays the time evolution of the products derived from the hydrolysis of sugars in the fixed bed reactor.

(b) Products distribution after hydrolysis with supercritical water at 380ºC.

(c) Product distribution after hydrolysis with supercritical water at 350ºC.

(d) Product distribution after hydrolysis with supercritical water at 400ºC and short residence times. In experiment 11, the absence of water flux in Reactor 1 makes heating process faster (5 min). We choose this option in order to avoid a large transition state.

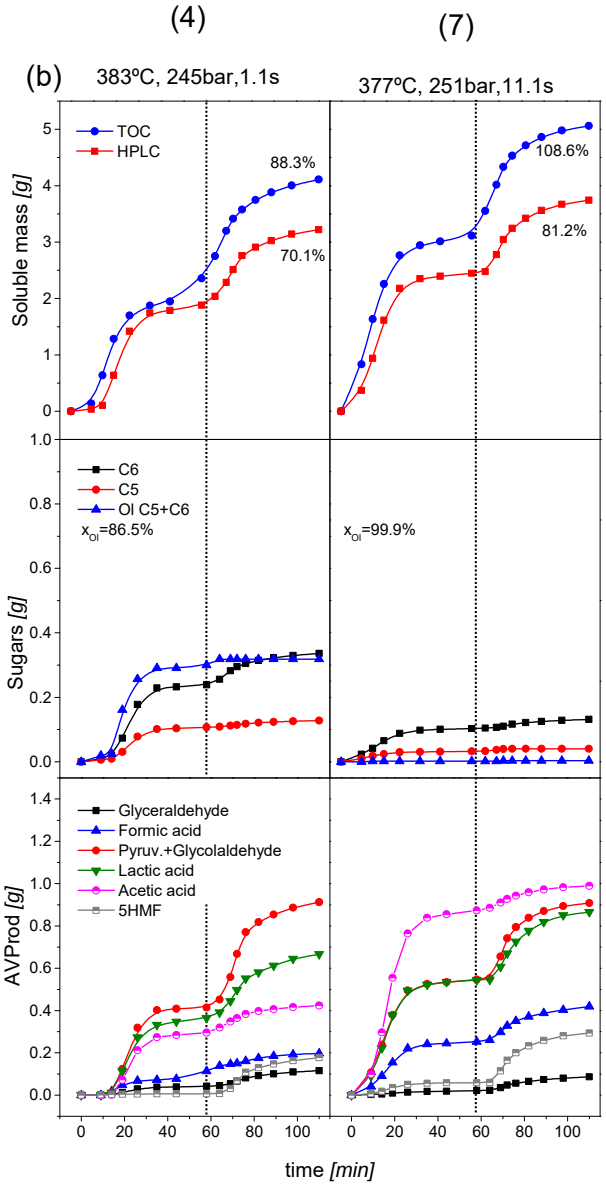


Figure 2. (Continued)

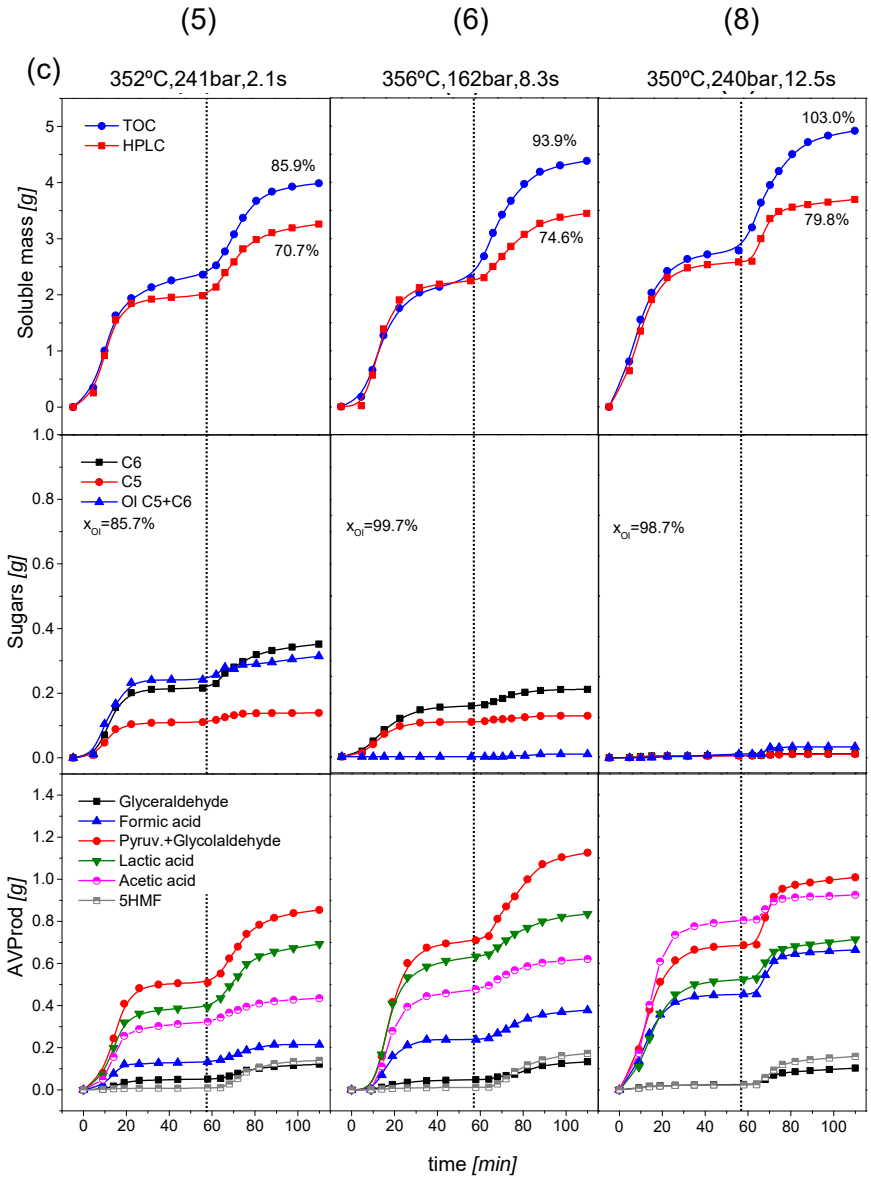


Figure 2. (Continued)

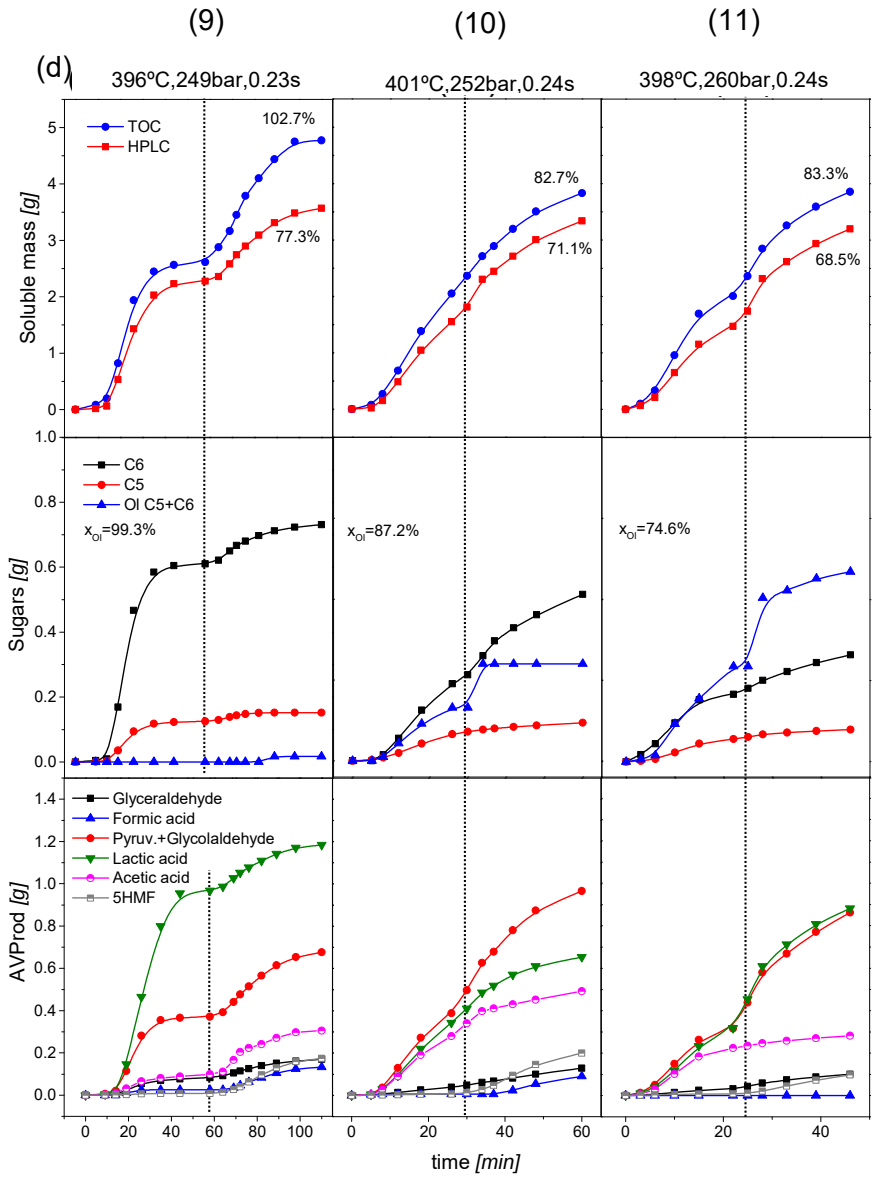


Figure 2. (Continued)

3.2 *Biomass valorization with sub and supercritical water hydrolysis*

The outlet stream from the fixed bed reactor was fed to the SHR together with a water stream at temperatures and pressures near to its critical point. The aim was to obtain a fast and selective hydrolysis of the oligomers and sugars extracted from the biomass. The reaction time in the supercritical reactor (t) was varied in order to modify the selectivity to different chemicals.

The optimum temperatures and flow-rates for the extraction have been identified in extraction step, as they lead to the maximum yield. For this reason, a flow rate of 11 cm^3/s and temperatures of 180º and 260ºC (for the two stages, respectively) were chosen for most of the experiments. Only the experiments 10 and 11 were performed with the same liquid flow-rates used in the fractionation experiments 2 and 3 (17 and 26 cm^3/s, respectively).

This approach also allows knowing the composition of the stream entering in the second reactor. Eight experiments were performed, as shown in Table 1. Three temperatures and 6 reaction times were tested, keeping constant the temperatures of water through the first reactor.

Three reactions (6, 7 and 9) were performed in a longer reactor, aiming to increase the reaction time in the second reactor (*t)*. A lower pressure was used in reaction 6 (161 bar) to observe the influence of water density in the products distribution.

Overall mass balance, calculated as described in section 0.53.1, is presented Table 1. The values (avg=96.9 %, sd=6.5 %) indicates that no significant gasification takes place in the supercritical reactor. First row of graphs in Figure 2 (b), (c) and (d) presents the mass of the soluble materials quantified in the outlet stream of SHR by TOC and HPLC. The percentage yield of each experiment respect to the soluble mass in the raw holm oak is presented as the number in the each graph. Some differences between these two procedures are observable, mainly increasing t_e in the second stage of fractionation or at higher sugars conversion. These findings could be related to the production of small organic acids, ketones and aldehydes (levulinic and acrylic acids, dihydroxyacetone, formaldehyde) and other compounds not identified by HPLC, see Figure S2 in Supplementary Material. This hypothesis agree with the decrease of the mass difference

observed by both techniques when a high water flow is involved (as commented in section 3.1), indicating an over breaking and oxidation of the products of interest at higher *t*.

Table 1. Temperatures, pressures, residence times, mass balance and oligomers conversions of the experiments coupling Fractionation+Hydrolysis reactors.

[a] Residence times were calculated based on the concepts drawn in reference [29].

[b] Mass balance accounts the amount of solid recovered from the Extraction reactor adding the mass of insoluble lignin flushed in the water stream and the total mass solubilized measured by TOC.

[c] Oligomers conversion involves the mass of oligomers quantified by HPLC outcoming from the Fractionation related to the mass of oligomers in the outlet stream of the sub-supercritical reactor.

Exp	T [ºC]	P [MPa]	t^1 [s]	$MB_{TOC}{}^2$ [%]	$X_{Olig}{}^3$ [%]	$Y1_{AVP}{}^4$ -	$Y2_{AVP}{}^5$ -
4	383.7 ± 5.1	245.7 ± 4.6	1.06	92.2	86.5	0.008	0.079
5	352.5 ± 4.4	241.3 ± 3.7	2.10	89.3	85.7	0.004	0.109
6	355.9 ± 5.7	161.8 ± 1.1	8.31	97.3	99.7	0.006	0.067
7	377.2 ± 3.5	251.9 ± 5.9	11.15	105.9	99.9	0.247	0.281
8	349.9 ± 2.4	239.6 ± 4.2	12.50	103.1	98.7	0.233	0.132
9	396.1 ± 3.6	249.1 ± 5.1	0.23	103.6	99.3	0.440	0.254
10	401.2 ± 2.8	252.2 ± 3.9	0.24	93.0	87.2	0.481	0.278
11	398.3 ± 3.0	259.9 ± 3.4	0.24	91.2	74.6	0.530	0.228

[1] t: reaction time in hydrolysis reactor, [2] Global mass balance of the coupled process, [3]Conversion of oligomers from hemicellulose and cellulose, [4,5] Yields of added value products in the time period of the first and second stage of temperature during fractionation Y_i=$mass_i$ / mass soluble material in raw biomass

3.2.1 Oligomers and sugars conversion

Oligomer conversion to C5 and C6 monomers was calculated by difference between the stream entering to the SHR (composition obtained in experiment 1 corresponding to reactions 5 to 9 and composition of experiment 2 and 3 for experiments 10 and 11, respectively), and the stream leaving the reactor after the hydrolysis.

The conversions of oligomers to C5 and C6 monomers are reported in the sixth column of Table 1. In all the runs, oligomers conversion was higher than 85%. the exception was

experiment 11, in which the small *t* and high dilution could be the cause of the low conversion. C5 Sugars (xylose) and C6 (cellobiose, glucose and fructose) are intermediate compounds in the reaction pathway. Comparing the amount of C6 and C5 in experiments 4 and 5 (see Figure 2 (b) and (c)), it can be seen that the conversion of sugars is faster than cleavage of oligomers to monomers at subcritical temperatures and even at a temperature a little higher than the water critical point (e.g. 380ºC). A different behavior is detected near to 400ºC, like in experiment 9, where oligomers conversion seems to be faster than sugars hydrolysis, in agreement with the observations reported in the literature for oligomers originating from microcrystalline cellulose [5]-[11]. Surprisingly the time needed for a complete conversion of sugars is quite larger than the pure cellulose hydrolysis at the same temperature (eg. 350ºC: 2 s in ref. [29] vs 12 s in this work). This could be related to the hydrolysis of C5 and C6 contained inside the porous structure of fluidized microparticles of biomass coming from the fractionation reactor. Also the presence of other ions or compounds could be linked to this attenuation.

3.2.2 Added value products (AVP) from the sugars hydrolysis

The third row of graphs in Figure 2 (b), (c) and (d) displays the amount of added value chemicals (AVP) (glyceraldehyde, glycolaldehyde, pyruvaldehyde, lactic acid, formic acid, acetic acid and 5-HMF) produced from hydrolysis of cellobiose, glucose, fructose and xylose. The reaction pathway of cellulose hydrolysis involving oligomers and cellobiose as intermediaries was reported in the literature [29]. Xylose hydrolysis in near critical and supercritical water was analyzed by several authors [37, 38].

The combined pathway is presented in Figure 3. Not all the products involved in this scheme were identified by the liquid chromatography. The cellulose pathway shown in Fig. 3 involves two consecutive hydrolysis, the first one in which the oligosaccharides

are hydrolyzed to glucose and xylose and the second one in which glucose and xylose are involved in two possible pathways: isomerization and dehydration or retro-aldol condensation [18, 38]. Glucose can follow a reversible isomerization to produce fructose, however, the reverse reaction is almost inhibited at the same conditions [19, 20]. Glucose can also be transformed into 1,6 anhydroglucose and fructose can be transformed into 5-hydroxymethylfurfural through a dehydration reaction [39]. The other alternative of glucose conversion is the retro-aldol condensation producing glycolaldehyde and erythrose [29]. Erythrose is further transformed into glycolaldehyde by the same reaction mechanism [18]. The retro-aldol condensation reaction of fructose produces glyceraldehyde and dihydroxyacetone. These molecules are further isomerized into pyruvaldehyde [19], which is transformed to lactic acid by an extra oxidation. Hemicellulose hydrolysis is quite similar; the first step is the depolymerization to produce xylose and xylose oligomers. After that, xylose can be isomerized to D-xylulose, assuming that D-xylulose as an intermediate for furfural and retro-aldol products (glyceraldehyde, pyruvaldehyde, glycolaldehyde, lactic acid, dihydroxyacetone, formaldehyde) [37, 38]. This reaction pathway consists of a retro-aldol reaction (Lobry de Bruyn-Alberta van Ekenstein (LBET)) from D-xylose and D-xylulose, similar to that involving D-glucose and fructose.

In the experiments, a considerable amount of glycolaldehyde-pyruvaldehyde and lactic acid was observed. The distribution of these chemicals was similar for experiments 4 and 5 (Figure 2 (b) and (c)) in spite of the difference of water properties at both temperatures. At 352ºC and 241.3 bars, the water density and Kw are 614.7 kg/m^3 and 5.10^{-6}, respectively. On the other hand, at 383ºC and 245.7 bar, those properties take a value of 319.7 kg/m^3 and 1.10^{-8}, both calculated as developed in literature [40]. A little difference

in the amount of glycolaldehyde-pyruvaldehyde as well as in the 5-HMF is perceived, principally at times corresponding to the first stage of temperature of the extraction step. This behavior could be explained by the presence of large amounts of H^+ ions coming from the acetylation taking place in the fractionation, in addition to the H^+ produced by the water ionization. Also, considering the nature of raw biomass, other ions in solution could be present. Under 352 ºC and pressure, isomerization of glucose to fructose and further dehydration is favored for pure cellulose hydrolysis [39]. But, in the present cases, the high yields of glycolaldehyde-pyruvaldehyde and lactic acid are evidences that the retro-aldol pathways take place as well. Experiments 4 and 7 were performed at similar temperature and pressure but involving different residence times (t=1.06 vs 11.15 s, respectively). For experiment 7, the acetic acid amount was highly increased, mostly in the time period corresponding to the first stage of temperature of fractionation. This acetic acid exceeded the amount produced in the hemicellulose deacetylation. The retro-aldol pathway coming from xylose by means of glyceraldehyde route, could explain the difference of acetic acid obtained directly from lactic acid decarbonylation. This extra amount of acetic acid could not be considered only from the hemicellulose source, since there is also a large concentration of C6 in the first fraction of the feed stream (see Figure 2 (a)). This C6 portion could also contribute to the glyceraldehyde route. Figure 4 displays the pH of the output stream after the fractionation stage (experiment 1) and the coupled process fractionation+hydrolysis (experiments 4, 5, 7 and 8). The pH in the stream after the second reactor was lower to that corresponding to the fractionation step itself, comparing the H^+ concentration of the experiments during the time period of the first stage during the extraction. This observation agrees with the fact that extra amount of acetic acid was produced when a deeper hydrolysis was performed (see experiments 7

and 8). After this time period, no difference in the pH can be detected. Similar behavior was observed from experiments 5 and 8, however, in this case, larger amount of formic acid was observed compared to the experiments above mentioned (see Figure 2 (c)).

The pressure change in the studied range had no effect on the chemicals distribution (see Figure 2 (c), experiments 5 and 6). This means that the variation of density as well as of Kw, do not modified largely the selectivity between isomerization-dehydration and retro-aldol pathways like it does in the pure cellulose hydrolysis. In this last, isomerization of glucose to fructose is highly inhibited by decreasing density [29]. The results of experiment 6 are in agreement with that commented above for experiments 4 and 5. The highest yield of glycolaldehyde-pyruvaldehyde (calculated as mass of product/mass of soluble material in raw biomass) was obtained for experiment 6 (24.4%), probably due to the combination of low temperature and long *t*.

A different distribution is observed in the experiment 9, where lactic acid is the most abundant product and acetic acid is depleted compared to the experiment 8 (see Figure 2 (d)). This finding could be explained by the short *t* of the mixture at high temperature condition in which the reactions are stopped before lactic acid production in the retro-aldol route, inhibiting the acetic acid formation. This selectivity seems to take place mainly during the time period of the first stage of the fractionation, because after that, the formation of lactic acid as well as of glycolaldehyde-pyruvaldehyde is lower. The highest yield of lactic acid was found at Experiment 9 (25.5%). The water flow increase in the first reactor has no clear effect on the production of retro-aldol compounds (see reactions 10 and 11 in Figure 2 (d)). Under these conditions, the oligomers breakup seems to become slower, since their amount is enlarged related to the monomeric sugars. In both

cases, the retro aldol pathways are followed producing glycolaldehyde-pyruvaldehyde and lactic acid in similar yield.

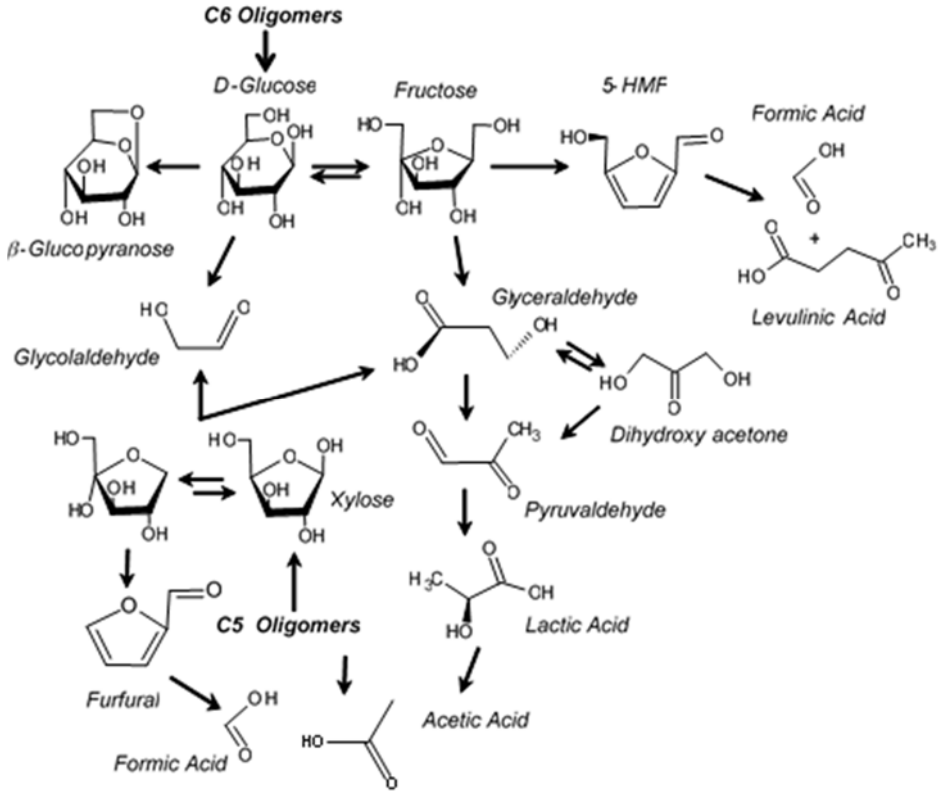


Figure 3. Combined reaction pathway of oligomers C5 and C6 including the glucose and xylose further reactions in hot pressurized water.

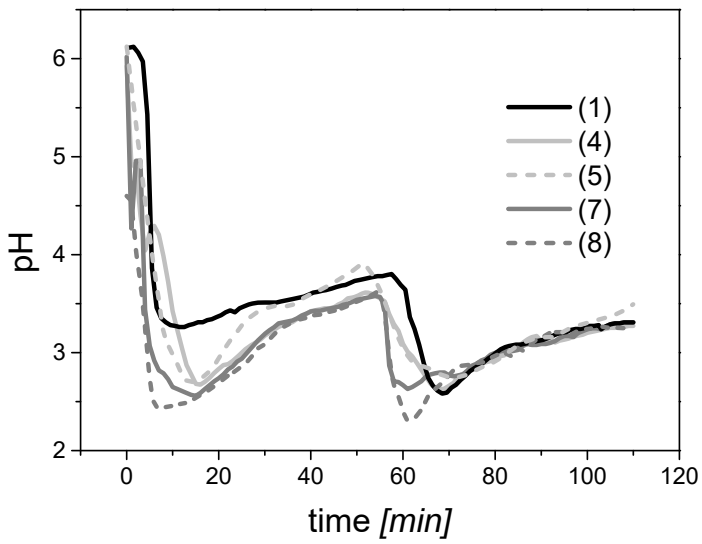


Figure 4. pH of the liquid product vs time. Comparison of the reactions at low (experiments 4 and 5) and high conversions (experiments 7 and 8) at sub and supercritical conditions of water.

3.3 Hydrolysis kinetic model

Aiming to analyze further the results obtained by the coupled system, a kinetic model for the second reactor is proposed in this section. This model takes into account the solubilized biomass composition fed to the second reactor. It was specially focused on the time period corresponding to the first stage of the solubilization (at the conditions of experiment 1) since this step produces an outlet stream with higher amount of the chemicals of interest (see last two columns of Table 1). The reaction pathway proposed in this case, showed in Figure 5. It is a simplified version of the real hydrolysis described in Figure 3. The modelling was done by the transient regime mass balances for each compound in the fluid: oligomers, sugars and products (Eq. (3)). Moreover, the following assumptions have been considered: (1) the reaction order for all the kinetics is 1 for the biomass compound and proton concentration in water, (2) there are no diffusional effects in fluid phase, (3) kinetic constants follows Arrhenius' law and (4) the reactor works at the same temperature at any point. Regarding kinetics, a conventional expression was used including the effect of the concentration of water proton since it is a hydrolysis process (Eq. (4)).

$$\frac{\delta C_{L_j}}{\delta t} = r_j - \frac{u}{L} \cdot \frac{\delta C_{L_j}}{\delta z} \qquad (3)$$

$$r_j = \mathrm{C}_{H^+} \cdot \sum_{i=1}^{i=N} \propto_{i,j} \cdot K_{L_i} \cdot C_{L_i} \qquad (4) \qquad (4)$$

3.3.1 Numerical resolution

Eq. (3) is a set of 6 partial differential equations (PDE) which has to be discretized to obtain a set of ordinary differential equation (ODE). The resolution of this set of ODEs was performed by the Runge-Kutta's method with a 8th convergence order and the discretization by coupling orthogonal colocation method on finite elements [41]. The fitting of the experimental data constitutes an optimization problem. Due to its complexity, it was previously seeded by manual iteration, and then, optimized by the Nelder-Mead-Simplex method. Moreover, as the inlet concentration of the hydrolysis reactor was variable and the oligomer properties changed with extraction time [42], the problem was optimized at every experimental point. Finally, the solution was reviewed in order to ensure the physical meaning of the parameters. The objective function was the minimization of the Absolute Average Deviation (A.A.D., Eq. (5)) for oligomer, sugar and products concentration at the SHR output.

$$A.A.D. = \sum_{i=1}^{n} \frac{1}{n} \cdot \left| \frac{X_{exp} - X_{sim}}{X_{exp}} \right| \cdot 100 \qquad (5)$$

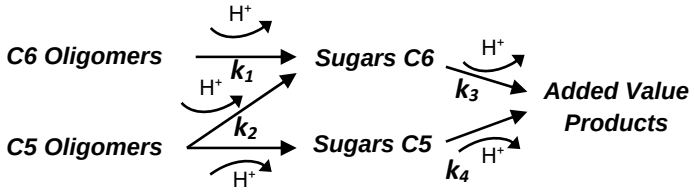


Figure 5. Simplified reaction pathway for solved biomass hydrolysis.

3.3.2 Experimental data fittings

In order to validate the model, only experiments 4, 5 and 9 were used because they were carried out at similar residence times and three different temperatures (see Table 1). For experiment 4, the data at extraction time of 9 and 14 min were not considered because they do not follow the tendency fixed by the set of the three experiments used (4, 5 and 9). Moreover, as each experiment was carried out independently, the inlet for the reactor was assumed to have the same composition that experiment 3 but with TOC profile of the fitted experiment (4, 5 and 9). The deviation between the model and the experimental data is arrayed in Table 2 and for experiment 5 it also can be seen in Figure 6. The model was able to reproduce successfully the hydrolysis of solubilized biomass, being the average A.A.D. 21.14 %, 37.37 %, 18.41 % and 7.24 % for instant hemicellulose and cellulose oligomers, sugars C6, sugars C5 and their degradation products (the added value products respectively or AVP). These discrepancies changed to 27.46 %, 7.61 %, 9.31 % and 3.99% respectively when cumulated values were used. Taking into account these last values, it can be checked that the highest error is in the estimation of the oligomers mass, which can be caused by the fact that the experimental data were obtained by the difference between the TOC and the sum of the other compounds (sugars and AVP). Moreover, the deviation between the experimental and simulated TOC was also calculated in order to check that the mass conservation law is followed. For all the cases, this mass balance deviation result in zero percent with three significant figures (0.00%). The kinetic constants and the stoichiometric coefficients (K_{L_i} and $\propto_{i,j}$ in Eq. (4), respectively) had to be obtained from fitting. Regarding to $\propto_{i,j}$, it was always 1 less for the final products coming from hemicellulose oligomers since they are composed by pentoses and hexoses [43, 44]. These fitted parameters, which are shown in Table 3, require a deeper analysis and they are discussed in the next section.

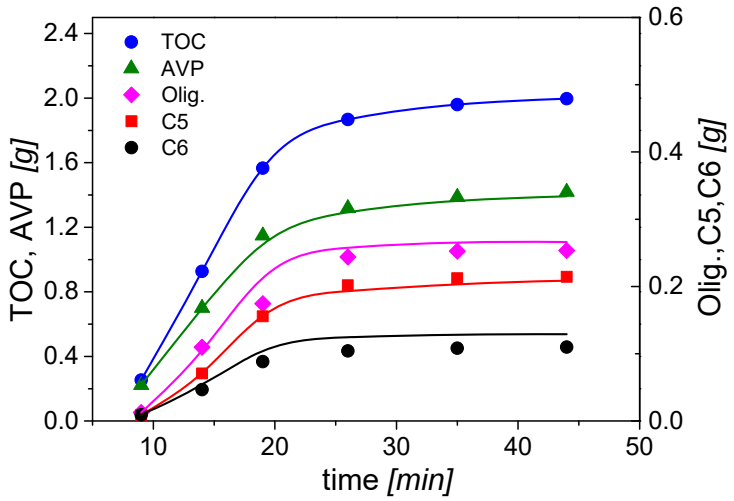


Figure 6. Experimental and simulated amounts of soluble material by TOC, C5+C6 Oligomers, sugars C6, sugars C5 and added value products obtained in experiment 5.

Symbols represents the experimental values and continuous lines represents the results of the kinetic model using the optimized kinetic parameters.

Table 2. Average absolute deviation of the fittings (experiments 4, 5 and 9) and simulations (experiments 7 and 8).

	ADD %							
	Instantaneous				Cumulated			
Experiment	Olig.[1]	C6[2]	C5[3]	AVP[4]	Olig[1]	C6[2]	C5[3]	AVP[4]
4	21.70	21.88	21.92	7.38	26.85	10.78	4.98	7.52
5	20.58	29.11	22.16	6.27	6.99	2.15	14.82	1.78
9	*	61.04	11.15	8.07	48.53	9.90	8.15	2.66
Average	21.14	37.34	18.41	7.24	27.46	7.61	9.31	3.99
7	*	*	*	5.77	*	*	*	4.94
8	*	*	*	0.93	*	*	*	1.02
Average	*	*	*	4.65	*	*	*	3.32

*[1] Oligomers from hemicellulose and cellulose, [2] Sugars C6, [3] Sugars C5,[4] degradation products. * Compound not detected. ADD% of total organic content was 0.0.*

Table 3. Fitted parameters used to estimate the kinetic constants depending on the extraction time.

k_1[a]	ln(k)[e]	Ea/R[f]	k_2[b]	ln(k)[e]	Ea/R[f]

	-	[K]		-	[K]
A	1.04	1.03	A	1.08	1.08
B	0.04	0.06	B	0.03	0.05
C	106	46543	C	109	48553
k_3[c]	ln(k)[e] -	Ea/R[f] [K]	k_4[d]	ln(k)[e] -	Ea/R[f] [K]
A	3.26	2069	A	2.85	1834
B	22.05	24.22	B	21.25	20.15
C	1.35	4.01	C	1.70	3.58
D	87.32	35238	D	89.63	36081

[a] Cellulose oligomer breakup constant, [b] Hemicellulose oligomer breakup constant, [c] Sugars C6 hydrolysis constant, [d] Sugars C5 hydrolysis constant, [e] Natural logarithm of the Arrhenius' pre-exponential factor, [f] Activation energy.

3.3.3 Analysis of kinetic parameters

The dependence of the kinetics parameters with temperature was proved. The regression coefficient (R^2) according the Arrhenius' theory was higher than 0.84 for all the cases (see Table 4). No change in the kinetic behavior was observed through the critical point (see Table 4) like does in the hydrolysis of microcrystalline cellulose [45] according to the commented in section 3.2.2, since there is not simultaneous solubilization in the SHR. However, a dependence of the kinetic behavior was observed with the extraction time, since the rates of production of valuable chemicals is decreased after the maximum of solubilized mass is reached (see Figure 7 (a) and (b)). This observation could be related with the influence of some of the chemicals produced by the further hydrolysis of sugars on the hydrothermal hydrolysis. In addition, it is also interesting that after this change, the kinetics of the sugar transformation tend to their initial value while the kinetic of the oligomer breakdown grows exponentially. This difference would be originated by the changes in the molecular weight of the extracted oligomers and the fact that they would be transformed more quickly if the molecular weight is lower. Moreover, it can be seen that temperature can compensate this negative effect, being negligible for oligomers at 400ºC (Figure 7 (c)).

Other interesting result is the evolution of the ratio between the four kinetic constants. In section 3.2.1 it was indicated that sugar transformation is faster than oligomer cleavage in subcritical conditions and lower in supercritical water. This behavior agrees with the obtained from the fittings, but only before the time of maximum of extraction (Figure 7 (a) and (c)). So, from this point, the changes in molecular weight and the raw material transformation makes the oligomer cleavage always greater. As the oligomer composition changes with extraction time, the kinetic cannot be reproduced by a typical Arrhenius' kinetic. So, two equations function of this time (t_e) are proposed, one for the pre-exponential factor (Eq. 4) and other for the activation energy (Eq. 5).

$$ln(k) = C \cdot \left|t_{e_{max}} - A \cdot t_e\right|^B \qquad (4)$$

$$Ea/R = D + \frac{A}{1+e^{(C\cdot(t_e-B))}} \qquad (5)$$

In Eq. 4, the parameter *C* is the natural logarithm of the pre exponential factor at the maximum extraction time ($t_{e_{max}}$) and parameters *A* and *B* introduce the effect of the changes in the structure and reaction medium. A would be related with the strong of the compound against its degradation by hydrolysis. B would be a measure of how structure or reaction medium can accelerate or restrain the degradation. In Eq. 5, *D* is the activation energy at the time where the biggest solubilization takes place, *E* and *F*, are the parameters that consider the role of the structure and reaction medium and *G* is the time when the maximum extraction is reached. In this case, E would represent how the medium or the structure can enhance the hydrolysis or hinder it. F would be the compound resistance against degradation.

Finally, the evolution of the hexoses content in hemicellulose oligomers is represented in Figure 7 (b). It can be observed that the ratio between these values grows with time. This

result was expected because hexoses would make the dissolution more difficult and would explain the fact that in experiment 5 the extraction was faster than 4 and 9 experiments (see Figure 7 (c)). Moreover this result agree with the data reported by other authors [46, 47].

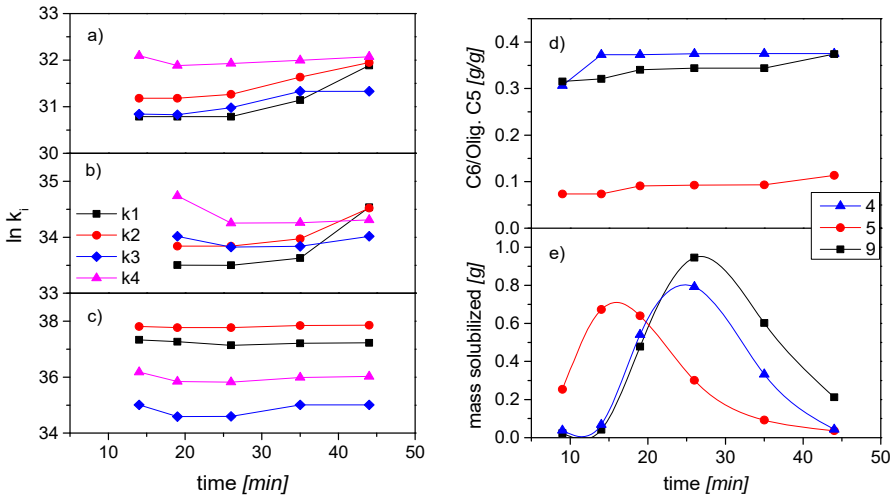


Figure 7. Evolution of the logarithm of the kinetic constants for experiment 5(a), 4(b) and 9 (c). Ratio C6/OligC5 (d). Solubilized mass with the extraction time for the experiments 5, 4 and 9 (e). k_1: Cellulose oligomer breakup kinetic constant, k_2: Hemicellulose oligomer breakup kinetic constant, k_3: Sugars C6 hydrolysis kinetic constant, k_4: Sugars C5 hydrolysis kinetic constant.

Table 4. Regression coefficient (R^2) of the Activation Energy with temperature for the kinetic constants showed in Figure 5.

t_e[1][min]	k_1[a]	k_2[b]	k_3[c]	k_4[d]
		R^2		
19	0.88	0.87	0.99	0.999
26	0.88	0.86	0.99	0.98
35	0.87	0.85	0.99	0.97
44	0.93	0.89	0.9999	0.96
Average	0.89	0.87	0.99	0.97

[a] Cellulose oligomer cleavage constant, [b] Hemicellulose oligomer cleavage constant, [c] Sugars C6 hydrolysis kinetic constant, [d] Sugars C5 hydrolysis kinetic constant. [1] Fractionation time.

3.3.4 Simulated experiments

As it was mentioned in section 3.3.2, only experiments 4, 5 and 9 were used to validate the model. Experiments 7 and 8 were not considered because their reaction time were much higher, which implies almost a total conversion at the reactor outlet. However, it checked if the model was able to reproduce their behavior. The result of the simulations are presented in Figure 8, being the absolute deviation around 4% for both experiments. Therefore, the model can predict successfully the hydrolysis at both low (0.2 – 1.0 s) and high (11.1-12.5 s) residence times.

3.3.5 Model limitations

From the results showed in the three previous sections, the model was able to successfully reproduce the experimental behavior of the set-up. In fact, this model can be used for any other lignocellulosic biomass. However, it is limited to processes where soluble lignin is low and when the aim is to reproduce the overall behavior of a solubilized biomass stream hydrolysis instead of an analysis of each individual compound. Furthermore, this model can be also adapted to processes where the inlet stream is variable in time.

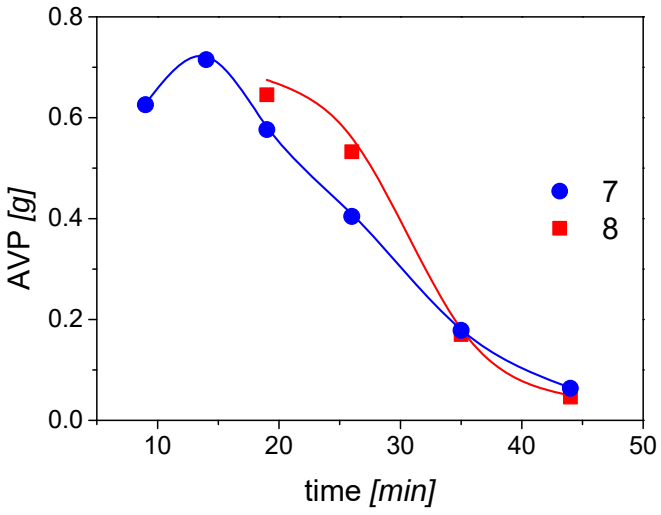


Figure 8. Comparison between the experimental and simulated data for AVP in experiment 7 and 8. Symbols are the experimental data and full lines shows the prediction of the model with optimized kinetic parameters for each data.

Conclusions

A new process coupling fractionation and hydrolysis steps was developed. By means of this process, up to 64.2% of feed Holm oak wood was solubilized mainly as oligomers of hexoses and pentoses and sugars with a small fraction of retro-aldol compounds. The low ratio of the amount of oligomers to monomeric sugars in the outlet stream could be explained by a similar behavior than in the case of pure cellulose hydrolysis: the rate of monomers hydrolysis is higher to the oligomers break up in subcritical conditions, but this tendency is reverted at supercritical temperatures.

The main products of the further hydrolysis in the second reactor were glycolaldehyde, pyruvaldehyde and lactic acid. Yield (related to the amount of soluble sugars in the raw biomass) of 24 wt% of Glycolaldehyde-Pyruvaldehyde was found at long reaction times (350ºC, 160bar and 8,6 s) and 25 wt% of lactic acid was found at short reaction time but high temperature (400ºC, 250 bar and 0.23s). An increasing amount of acetic acid was observed at the highest residence times (e.g. 12 s).

The distribution of products is related with a combined reaction hydrolysis pathway of cellulose and hemicellulose involving oligomer cleavage to monomers, isomerization steps and two competing paths: Retro-aldol condensation and dehydration. The influence of the water density and the amount of ions H^+ coming from the dissociation process is not clear as it is in the case of the hydrolysis of pure cellulose, in which the glucose

dehydration is highly inhibited and retro aldol pathways clearly favored at temperatures and pressures above the water critical point. In the present work, products coming from retro-aldol paths as well as products of dehydration are observed in both conditions: sub and supercritical. Finally, a general kinetic modelling for the hydrolysisreactor was proposed. This model could reproduce the experimental data for sugar and added value products with deviations lower than 10%. Besides, the calculated kinetic parameters reproduced the changes in oligomer and sugar conversion when the hydrolysis is performed in supercritical conditions instead of in subcritical water. This model can be applied to any other lignocellulosic biomass with a low content of soluble lignin.

The main advantage of this combined process consist in providing a liquefied biomass stream to a selective hydrolysis reactor giving the valorization of the raw material avoiding the costly grinding of particles from several millimeters to less than two hundred microns needed to pump it in a water stream belonging to a high pressure process.

Acknowledgements

The authors acknowledge the Spanish "Ministerio de Economía y Competitividad (MINECO)" and FEDER funds, Project BioFraHynery CTQ2015-64892-R and the regional government (Junta de Castilla y León), Project Reference: VA330U13 for funding. MEng. Gianluca Gallina wishes to acknowledge the Spanish "Ministerio de Economía y Competitividad (MINECO)" for the scholarship/predoctoral contract BES-2013-063556. MEng. Alvaro Cabeza would like to thank to the Spanish "Ministerio de

Educación, Cultura y Deporte", training program of university professors (reference FPU2013/01516) for the research training contract.

Abbreviations and symbols

Acronyms

A.A.D.: Average absolute Deviation.

Olig: Hemicellulose and cellulose oligomers.

C_6/OligC_5: ratio hexoses to hemicellulose oligomers.

Greek letters and symbols

A-*G*: Parameters for kinetics constant estimation.

$\propto_{i,j}$: Stoichiometric coefficient of the compound "j" for the reaction "i", dimensionless.

C_{H^+} : Concentration of the protons, mg/L.

C_{L_j} : Concentration of the compound "j", mg/L.E_a/R: Activation energy, K.

ε: Porosity of the bed, dimensionless.

ε_f : Porosity of the bed, calculated at the end of the experiment, dimensionless.

ε_{av}: Average porosity of the bed, between the beginning and the end of the experiment, dimensionless.

ε_o: Porosity of the bed, calculated at the end of the experiment, dimensionless.

K_{L_i} : Kinetic constant, min^{-1}.

k: Pre-exponential factor of the kinetic constant, $mg^{-1} \cdot min^{-1}$.

L: Length of the reactor, m.m_0: initial mass of the solid in the reactor, g.

m_f: final mass of the solid in the reactor, g.

m(i) (RM): total amount of component (i) in the raw material, extracted by acid hydrolysis and detected by HPLC analysis, g.

Mw(i): molecular weight of component i, g/mol.

$Mw(i)\sum C_{(i)}$ *:* molecular weight of the sum of the atoms of carbon in component *i*, g/mol.

$m_{sol}tot(RM)$*:* total amount of soluble compounds in the raw material, extracted by acid hydrolysis and detected by HPLC analysis, g.

N: Number of compounds, dimensionless.

n: Total number of experiments, dimensionless.

R^2: Coefficient R^2, dimensionless.

r: ratio between the molecular weight of the soluble compounds extracted and the molecular weight of the atoms of carbon, dimensionless.

r(i): ratio between the molecular weight of the soluble compounds extracted and the molecular weight of the atoms of carbon for compound *i*, dimensionless.

r_j : Reaction rate of the compound "j", mg/min·L.

u: Liquid velocity in the reactor, m/min.

t: Residence time in the SHR, s.

t_e: Extraction time, min.

$t_{e_{max}}$: Maximum extraction time, min.

$x_{i_{EXP}}$: Experimental value of the fitted variable.

$x_{i_{SIM}}$: Simulated value of the fitted variable.

z: Coordinate along the length of the reactor, dimensionless.

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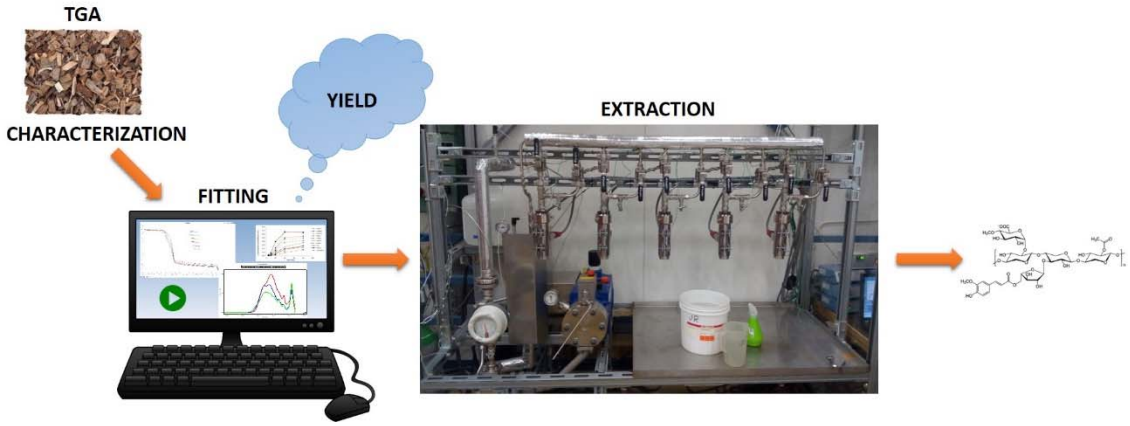
CHAPTER 3

RAW MATERIAL EFFECT ON HEMICELLULOSE EXTRACTION YIELD AND MOLECULAR WEIGHT DURING HOT PRESSURIZED WATER PRETREATMENT BY AUTOHYDROLYSIS.

RAW MATERIAL EFFECT ON HEMICELLULOSE EXTRACTION YIELD AND MOLECULAR WEIGHT DURING HOT PRESSURIZED WATER PRETREATMENT BY AUTOHYDROLYSIS

Abstract

A comprehensive study on the hemicellulose extraction from 10 different tree species was performed at 160 ºC using a novel cascade reactor. The aim was to identify which wood species were best candidates to obtain a high concentration, yield and/or molecular weight of hemicelluloses. Hydrothermal extractions at several times (from 5 to 80 min) were performed. We demonstrated that there is a relation between extraction yield (between 9.7 and 40.3%), composition of the raw material and initial structure determined via TGA data. Additionally, a new empirical equation able to estimate the hemicellulose extraction yield from initial composition data was developed. The highest yield was obtained with eucalyptus wood. Molecular weight of the oligomers varied from 3.4 to over 100 kDa. Three trends were observed: molar mass decay with time, maximum and minimum of molar mass. In general, the higher the extraction yield, the lower the molecular mass of the hemicelluloses.



Keywords: Hemicellulose; subcritical; TGA; biomass; hydrothermal pre-treatment; hydrothermal carbonization

Introduction

Lignocellulosic biomass, the most abundant on earth, can be obtained from various sources, such as wood, agricultural and municipal waste and other raw materials which do not compete with edible crops for direct human or animal consumption.

Lignocelluloses primarily consist of three pseudo-components, lignin, cellulose and hemicelluloses, combined in a resistant structure; however, the versatile composition, enables the production of various fuels and high value chemicals [1]. Among the various lignocellulosic biomasses, wood is an important renewable resource because of the huge availability of trees and their non-seasonal character; moreover trees do not require intensive use of fertilizers and pesticides to grow, and they generally contain a minor amount of inorganic substances compared to crops [2].

Extraction and applications of hemicellulose

Hemicelluloses can be isolated from biomass in molecular weights above 3 kDa and can be used for multiple applications. The production of films for packaging applications made with hemicelluloses to replace synthetic plastics has been widely studied [3-6]. Another important application is the production of hydrogels used as drug carriers [7, 8] and to adsorb heavy metal ions from aqueous solutions [9] . According to other studies, it seems also that xylans have the potential to be used in medicine as cholesterol depressant, HIV inhibitor and dietary fibers although these studies are still very preliminar [10].

An effective and clean way to extract hemicellulose is to pretreat the lignocellulosic biomass with hot pressurized water [11-15]. At temperatures above 100 ºC, water is able to extract hemicellulose from biomass. The extracted oligomers may undergo hydrolysis

in aqueous medium, catalyzed by hydronium ions and acetyl groups originated from hemicellulose [14, 16].

Depending on the type of raw material used, hemicellulose has a different composition: partially acetylated xylans are the predominant hemicelluloses in hardwoods, while galactoglucomannans are the predominant hemicelluloses in softwoods; hardwoods are therefore an important raw material for obtaining a hemicellulose rich in xylose.

In a previous work carried out by our group, we studied the efficiency of extraction with water at a temperature of 250 ºC from 9 different species of typical trees in the Castilla y Leon region (Spain). In particular, the total yield of extracted sugars, and the inhibitory effects of lignin in the efficiency of the reaction was evaluated [17]. Such temperature was too high, and both hemicellulose and cellulose were co-extracted and degraded.

Optimization and modelling of the extraction process

In this paper, we will focus on the extraction of hemicellulose alone, using optimal conditions defined in other experiments for maximizing yields, without incurring the degradation of sugars [11, 18]. The main hypothesis that we wanted to test was whether there is a clear relationship between biomass structure and composition and the quality of the hemicellulose extracted. Biomass structure was indirectly investigated via TGA model analysis. Both processes (thermal and hydrothermal degradation) involve similar phenomena, like oligomer cleavage [19]. There is therefore a similarity between the change of the structure of the biomass due to hydrothermal and thermal degradation. Furthermore, TGA is a cheap and quick technique that only requires a little amount of sample to be performed (10 mg). Fractionation of wood from 10 different tree species was carried out in a batchwise operated cascade reactor at a constant temperature of 160

ºC with total recirculation. The concentration of hemicellulose extracted from the species was analyzed at different extraction times by calculating and comparing the yields of the extractions. The molecular weights of the oligomers obtained during various extraction times were measured and a direct correlation with the pH of the extracted solution was identified. The content of lignin and cellulose in the various species was also determined to understand if the composition had an influence on the extraction process. With the help of this methodology, an empirical equation for yield of hemicellulose extraction was proposed.

1. Materials and methods

1.1 Materials

Lignocellulosic raw materials used in this study came from urban trees located in Castilla y Leon (Spain).

The tree species studied were: walnut (*Juglans regia*), large leaved linden (*Tilia platyphyllos)*, field elm (*Ulmus minor*), plane (*Platanus x acerifolia*), eucalyptus (*Eucalyptus globulus*), sour cherry (*Prunus cerasus*), catalpa (*Catalpa bignonioides*), maple (*Acer saccharum*), almond (*Prunus dulcis*) and cedar (*Juniperus oxycedrus*). Nine of the wood species were hardwoods, while cedar was the only softwood.

Trees had an approximate age of 30-35 years, with an average height of 18-20 m. During a seasonal pruning, the top of the trees was cut and trunk sections with a diameter of about 20 cm and a height of 5 cm were picked up as a raw material for our experiments. Disks were cut in slices and bark was manually removed in order to reduce the content of extractives [20, 21] . The wood was dried, chipped, milled with a Fritsch Universal

Cutting Mill Pulverisette 19 (Germany) and sieved to a particle size between 1.25 and 2.00 mm with a Retsch Vibratory Sieve Shaker AS 200 basic.

2.2 Experimental procedure and analytical methods

2.2.1 Determination of pH

The pH of the extracted solution (hydrolysate) was measured with a Phenomenal pH meter using a refillable glass electrode model 221 with a built-in PT 1000 temperature sensor. The measurement was performed at ambient temperature, mixing the solution with a magnetic stirrer.

2.2.2 Liquid samples chemical composition

Liquid samples collected from the experiments were subjected to acid methanolysis [22]. Resorcinol and sorbitol were used as internal standards. First, a certain amount of liquid containing about 0.1 mg of carbohydrates was freeze-dried in vacuum. A methanol-based sugar monomer solution containing a known amount of the sugar monomers, was used to prepare calibration samples.

An equivalent of 2 mL of 2 M HCl/MeOH anhydrous were added to the experimental and calibration samples, heated subsequently to 100 ºC for 3h. After cooling at ambient temperature, 170 µL of pyridine was added to neutralize the excess of acid, together with 1 mL of sorbitol (0.1 mg/mL in MeOH) and 1 mL of resorcinol (0.1 mg/mL in MeOH). The solution was dried under nitrogen gas at 50ºC and then using a vacuum desiccator. The samples were finally silylated using 150 µL of pyridine, 150 µL of hexamethyldisilazane (HMDS) and 70µL of trimethylchlorosilane (TMCS). The

derivatised samples were analysed by a gas chromatograph with flame ionization detection [23].

About 1 µL of each silylated sample was injected through a split injector (250 ºC, split ratio 1:25) into a column coated with dimethyl polysiloxane (HP-1, Hewlett Packard). The column length, internal diameter and film thickness were 25 m, 200 µm, and 0.11 µm, respectively. Hydrogen was used as carrier gas with a flow rate of 45 mL/min. The following temperature programme was applied: 100 ºC, 2 ºC/min, 8 min at 170 ºC, 12ºC/min and 7 min at 300 ºC. The identification and quantification of sugars were accomplished through the injection of standard samples and proper calibration.

2.2.3 Molecular weight analysis

Molecular weights of the hemicelluloses extracted were determined by high-performance size-exclusion chromatography (HPSEC) equipped with multiangle laser-light scattering (MALLS) and refractive index (RI) detectors. The columns employed were Ultrahydrogel TM Column, Linear, 10 µm, 7.8 mm X 300 mm, 500 – 10M. The eluent was 0.1M $NaNO_3$ with a flowrate of 0.5 mL/min at 40ºC. Calculations were performed with the software Astra, Wyatt Technology.

2.2.4 TGA analysis

TGA analysis of the raw materials was carried out in a TGA/SDTA RSI analyser of Mettler Toledo. Samples of approximately 10 mg were heated at a rate of 20 ºC/min under N_2 atmosphere (60 N mL/min flow) from a temperature of 50 ºC up to temperatures around 800 ºC.

2.2.5 Raw material characterization

The total amount of extractives, lignin and structural carbohydrates in the raw materials was determined according to the standard methods published by the National Renewable Energy Laboratory (NREL) [24]. Dried biomass was treated with n-hexane in a Soxhlet equipment, in order to remove the extractives. 300 mg of dried and free-extractives solid were hydrolyzed in 3 mL of 72% wt. sulfuric acid solution at 30 °C for 60 min. The mixture obtained was diluted using 84 mL of deionized water and heated at 120 °C for 60 min to hydrolyze oligosaccharides and obtaining their correspondent monomers. Solid was separate from the liquid solution by vacuum filtration, placed in a muffle at 550 °C for 24 h and the remaining residue was weighted before and after this step to calculate the insoluble lignin and the ash content of the sample. A liquid aliquot was analyzed with UV-Vis spectrophotometer at 320 nm with extinction coefficient of 34 $Lg^{-1}cm^{-1}$ [25] to calculate the amount of soluble lignin. Another liquid aliquot was neutralized to pH range 6 to 7, then it was filtered using a 0.2 μm membrane and analyzed by HPLC to determine the carbohydrates composition.

The column used for the separation of the compounds was SUGAR SH-1011 Shodex at 50.0 ºC with a flow rate of 0.80 mL/min, using a solution of 0.01N of sulphuric acid and water Milli-Q as the mobile phase. The sugars and their derivative were identified with Waters IR detector 2414 and Waters dual λ absorbance detector 2,487 (210 nm and 254 nm).

Carbohydrates composition of hemicelluloses contained in the raw material was measured through GC analysis, after subjecting the solids to acid methanolysis: 2 mL of 2M HCl/MeOH anhydrous were added to 10 mg of dry solid and heated to 100 ºC for 5h [22]. Next steps were the same described in paragraph 2.2.2 for liquid samples. As acid methanolysis, at the conditions used in this paper [22], is not strong enough to break cellulose, glucose identified by GC is assumed to proceed from hemicellulose hydrolysis.

Cellulose content in the raw material was calculated related to the glucose content by substracting the glucose detected by GC to the total glucose detected by HPLC.

The calibration reagents used for analysis were: cellobiose (+98%), glucose (+99%), fructose (+99%), glyceraldehyde (95%), pyruvaldehyde (40%), arabinose (+99%), glycolaldehyde (+98%), 5-hydroxymethylfurfural (99%), lactic acid (85%), formic acid (98%), glucuronic acid (99%), mannose (+99%), xylose (+99%), galactose (+99%), rhamnose (+99%), galacturonic acid (+99%), furfural (+99%), acetic acid (+99%), 4-O-methylglucuronic acid (4-O-MeGlcA) (98%) all of them purchased from Sigma-Aldrich and used without further modification.

2.2.6 Experimental set-up and procedure

The experiments were carried out in a batchwise operated cascade reactor composed of five Parr units connected in series, designed and developed at Åbo Akademi [18, 26]. The main advantage of this system was that, during the same experiment, multiple liquid and

solid samples at different resident times could be collected, unlike classic batch reactors where only one sample could be collected from one experiment. The nominal volume of each Parr unit was 200 mL. The experimental device is depicted in Figure 1.

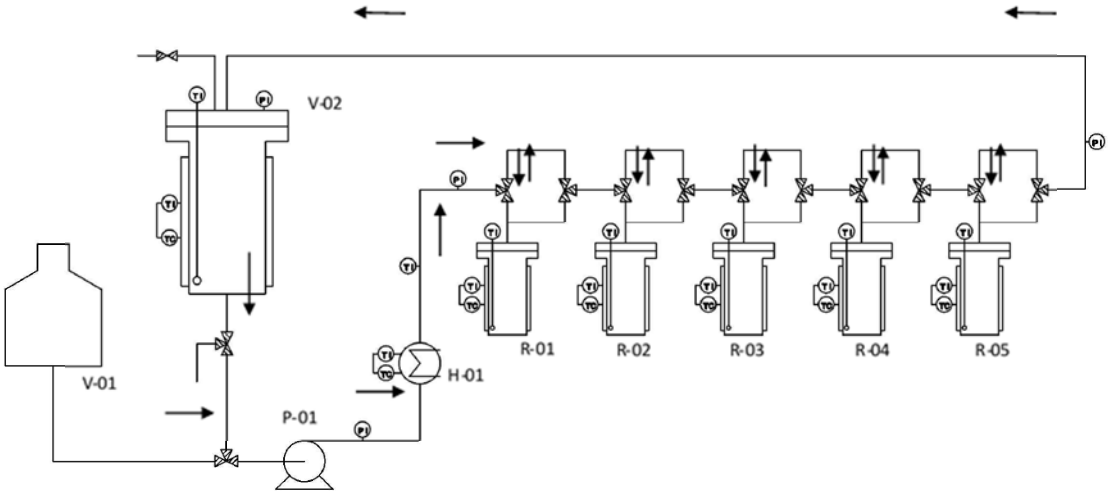


Figure 1. Simplified scheme of the batch cascade reactor used in the experiments. Equipment: V-01 Water tank, V-02 Collector vessel, P-01 Pump, H-01 Heater, R-01/R-05 Reactors.

A flowrate set at 150 $L \cdot h^{-1}$ was fully recirculated through the units and through a buffer vessel with a volume of 2 L, in order to mimic and maintain a constant mixing inside the system. The liquid/solid ratio was approximately 160. A metallic net was located on the top of each of the 5 units to keep the solid particles inside, avoiding them to be washed away by the liquid during the operation.

The pressure was maintained constant at 9 bara (2.9 bar higher than the thermodynamic phase equilibrium of water at the reaction temperature to assure liquid phase) and it was measured before the first reactor and after the last reactor.

Each reactor unit was equipped with an individual heating jacket and the temperature was measured continuously inside and outside each reactor unit and regulated by PID controllers.

The temperature inside the reaction system was fixed for all the experiments to a constant value of 160 ºC. Each reactor unit was loaded with 5 g of dry wood (25 gr in total), filled with distilled water and kept overnight to pre-wet the raw material (swelling); pipes and the 2 L buffer vessel were also filled with a known amount of water. Before starting the experiments, water was recirculated through the pipes and the 2 L vessel, by-passing the 200 mL units and preheated until reaching the desired reaction temperature (160 ºC).

After the desired temperature was reached in the bypass mode, the heating was turned on also for the five reactors heat jackets and hot water was let to enter into the units, stabilizing rapidly the temperature in the system.

At pre-established sampling times (5 min, 10 min, 20 min, 40 min and 80 min) reactors were sequentially by-passed, quenched rapidly and detached from the system. Figure S1 in supplementary material (Appendix 2) represents the temperature profile inside the system during the experiments. The system took about 4 minutes to reach a constant temperature of 160 ° C, once the set reaction temperature was reached, temperature variations were less than ±2 ºC. The time needed to stop the reaction by cooling down each unit from 160 ° C to 85 ° C was less than one minute.

A liquid sample was obtained from every single reactor unit, with a total of 5 per experiment. The samples were then analyzed as explained in section 2.2. A total of 10 experiments were performed, at constant operational conditions; wood from a different species of tree were tested in each experiment.

2. Results and discussion

The hypothesis that we wanted to demonstrate was that there is a relationship between the initial raw material and the extraction of hemicelluloses from wood using water as a solvent. Furthermore, we wanted to demonstrate that the differences occur not only between hardwood and softwood, but even among different hardwoods the difference is clear. For that we tested 10 different tree species in the reactor (9 hardword and 1 softwood). Temperature was fixed at 160 °C because it both guarantees high hemicellulose yields and minimizes degradation and undesired side products [18].

2.1 Raw materials characterization

Table 1 represents the composition of the raw materials in dry basis, determined as explained in paragraph 2.2.5.

Table 1. Composition of wooden biomass from 10 different tree species dry basis.

Species	**Extractives** g/g	**Cellulose** g/g	**Lignin** g/g	**Hemicellulose** g/g	**Acetic Acid** g/g	**Mass balance** % Error
Almond	0.071	0.353	0.306	0.261	0.049	-4.0%
Cedar	0.052	0.314	0.398	0.228	0.058	-5.1%
Sour Cherry	0.021	0.431	0.241	0.304	0.060	-5.6%
Elm	0.022	0.541	0.190	0.248	0.030	-3.1%
Eucalyptus	0.013	0.462	0.252	0.260	0.072	-5.9%

Linden	0.014	0.420	0.278	0.215	0.053	1.9%
Maple	0.012	0.299	0.456	0.239	0.046	-5.2%
Plane	0.023	0.341	0.388	0.243	0.058	-5.3%
Walnut	0.005	0.415	0.330	0.254	0.023	-2.7%
Catalpa	0.002	0.495	0.213	0.251	0.073	-3.4%

The species with the highest content of lignin was the maple (0.46 g/g of wood), while the lowest amount of lignin (0.19 g/g of wood) was contained in elm tree wood. Maple amount of lignin was high respect to other studies [17, 27].

In the experiments carried out in this work, only bark was removed from the wood samples, but sapwood and heartwood were not separated. Also knots, formed when removing branches from the trunk were present in the raw material. It is known that heartwood and branches contain a higher amount of lignin compared to sapwood [28, 29] and this may be the reason of the differences in composition between our work and others in literature. Also the age of the tree is an important factor, as mature trees contain a higher amount of lignin respect to younger trees [30].

The decision of using all the parts of the wood (except the bark, to minimize the extractive content) was made to have a raw material as close as possible to what could be used in a real biorefinery process.

The highest amount of cellulose was found in elm wood (0.54 g/g wood), while maple wood contained the lowest amount (0.30 g/g of wood).

Table 1 shows that the percentage errors in the mass balance are relatively small, always below 5.9%, comparable with other studies [15, 31].

Compounds constituting hemicelluloses from the different raw materials, determined by GC analysis, are compared in Figure 2. Sour cherry contained the highest amount of hemicellulose (0.30 g/g of wood). Xylose was the most abundant component in all tree species analyzed, with a maximum amount in sour cherry wood (0.19 g/g of wood) and a minimum in Cedar (0.07 g/g of wood).

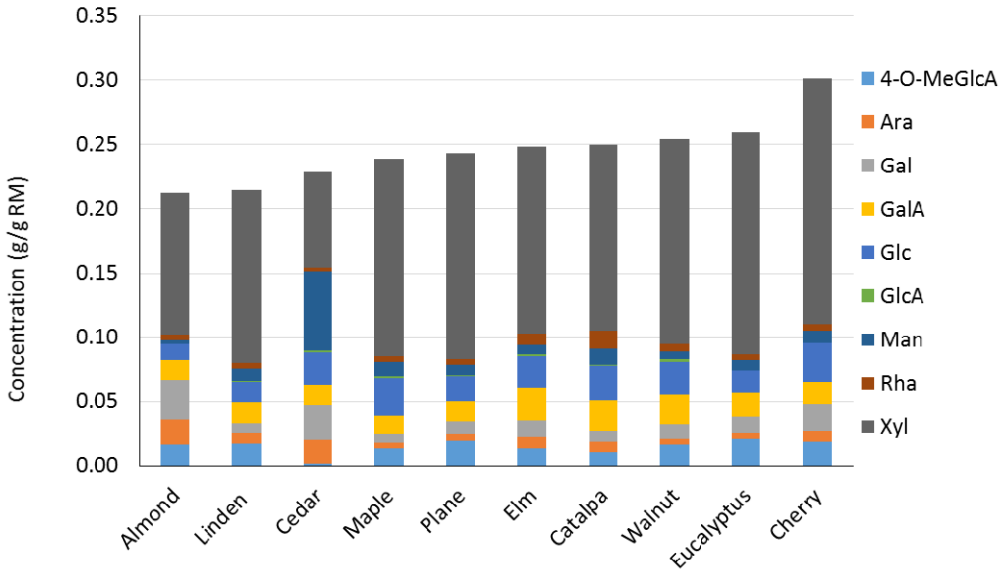


Figure 2. Concentration of 4-O-MeGlcA (4-O-methylglucuronic acid), Ara (arabinose), Gal (galactose), GalA (galacturonic acid), Glc (glucose), GlcA (glucuronic acid), Man (mannose), Rha (rhamnose), Xyl (xylose) in hemicelluloses from 10 different tree species, expressed in g compound/g raw material dry basis.

2.2 Hemicellulose extraction

The yield of hemicellulose extracted after the experiments is represented in Figure 3 as a function of the extraction time. Detailed calculations for the determination of yields and concentration are reported in the supplementary material (Appendix 1): Volumetric

concentrations of hemicellulose extracted in liquid phase are represented in Figure S2a in Appendix 2, while a graph representing extracted hemicellulose vs total hemicellulose content is represented in Figure S2b.

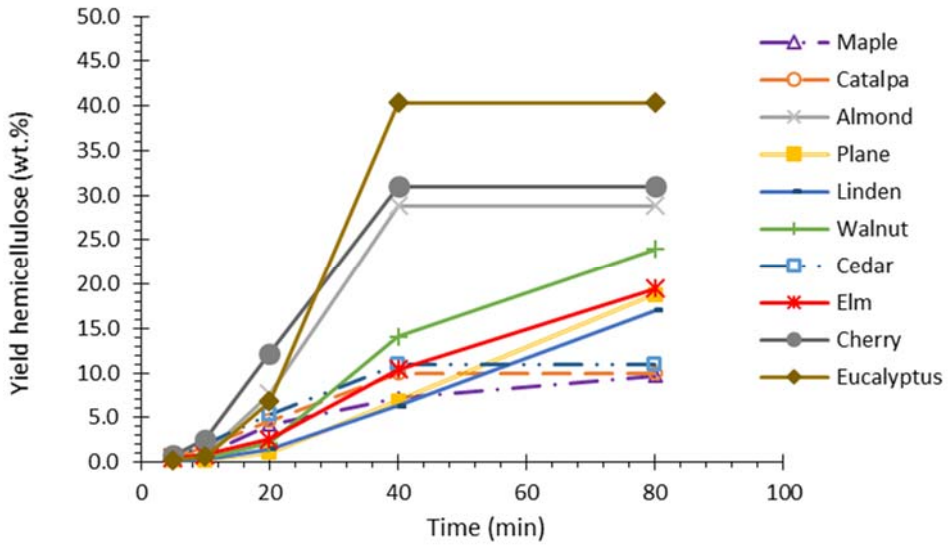


Figure 3. Yield of hemicellulose extracted at different extraction times from the different raw materials.

We found two different behaviors in the extraction curve: (1) walnut, elm, linden and plane showed a continuous almost linear extraction curve during the 80 min, (2) the other species showed slow extraction in the first 20 min, then a rapid increase in the concentration of hemicellulose between time 20 min and 40 min, and finally a plateau from time 40 min till the end. This behavior could be related to the initial sample characteristics and it will be discussed in section 3.4.

The highest yield of hemicellulose extracted and dissolved in the liquid phase was reached using eucalyptus as raw material (40.3%), while the lowest was obtained with maple wood (9.7%). Figure 4 shows that the lowest hemicellulose yields were obtained with

species that contained respectively a high amount of cellulose or a high amount of lignin. In particular, catalpa and elm wood contained over 49% of cellulose, and hydrothermal extraction allowed to extract respectively only 9.9% and 12.4% of the whole hemicellulose. On the other hand, maple, cedar and plane wood contained over 38% of lignin, which leaded to hemicellulose yields of 9.7, 10.9 and 18.8%.

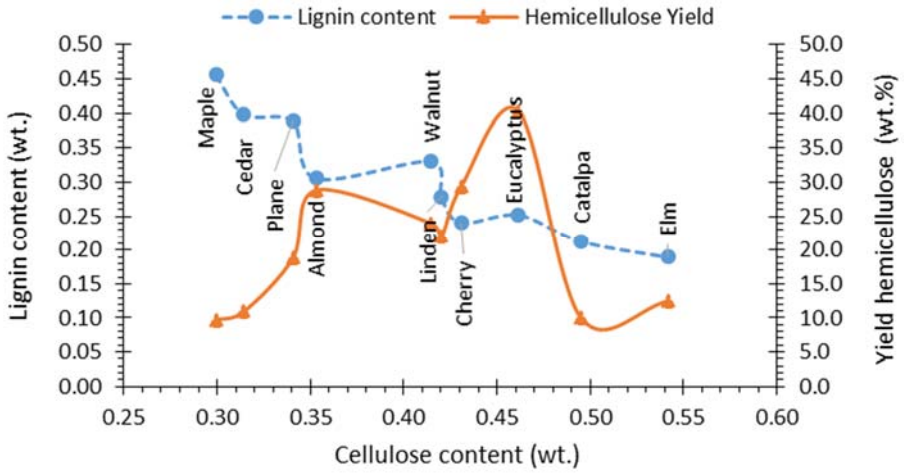


Figure 4. Lignin content in function of the cellulose content; Hemicellulose yield obtained through hydrothermal extraction from 10 tree species with different amount of cellulose and lignin.

It is therefore reasonable to think that the species of trees that contain high amount of lignin or towering amounts of cellulose do not allow achieving high yields in hemicellulose. Lignin and cellulose thus would carry a shield effect that protects hemicellulose and, at the experimental conditions used here, prevents them from breaking through a hydrothermal treatment. Cellulose shield effect was previously observed during the kinetic analysis of biomass isothermal TGA [19], observing that hemicellulose kinetics were enhanced when cellulose degradation started. Regarding lignin, this

assumption was confirmed by previous studies, e.g. Yedro et al. [17] also found that a high amount of lignin drove to a reduction of hemicellulose yield. Moreover, two different kinds of hemicelluloses can be recognized in lignocellulosic biomasses, which differ according to the difficulty of being extracted: one hemicellulose is easy to extract while the other is difficult to recover due to its strong interactions with cellulose and lignin [32-34]. The species that contained intermediate values between those of cellulose and lignin indicated, however, did not show a linear trend in the extraction of hemicellulose, there were indeed fluctuations in the yield values. It can be stated that the composition of the biomass affects the hemicellulose extraction only partially.

It is therefore necessary to study the effect of structure of the plant on the fractionation process, and that is how the three constituent polymers are combined with each other. The study of the histology of the plant is worth studying, although was out of the scope of this study, and for that purpose we envisioned it via a specific modelling tool using thermogravimetric analysis (TGA), as described in section 3.6 [19].

2.3 pH evolution

The evolution of the pH during hot water pretreatments has been analyzed in other works at various temperatures [11, 13, 14, 17]. In this study, we compare the pH evolution in the liquid hydrolysate solutions with hemicellulose extracted from 10 different tree species at 160 ºC. As indicated before, a similar study was carried out by our group, at 250 ºC, where both hemicellulose and cellulose were extracted and in that case degraded [17]. It is well-known that the increase of acidity is directly related to the cleavage of hemicellulose polymers and the release of the structural acetyl group that form acetic acid in the bulk liquid. Futhermore, the protons from acetic acid dissociation catalyze the

hydrolysis of hemicellulose oligomers, triggering a chain reaction called autohydrolysis [35, 36].

Figure 5a represents the concentration of H_3O^+ ions in the extracted solution as a function of extraction time, for all the experiments. The hydronium concentrations grew rapidly during the first 40 minutes of the process, and then exhibited only minor changes (it is worth remembering that the system works under total recirculation, so the acetic acid released goes through the system during the remaining experiment time).

The highest acidity values were achieved by processing wood from catalpa and eucalyptus; the rate of increase in acidity was faster when using catalpa wood. From these results, it can be indirectly deduced that the hemicelluloses of eucalyptus and catalpa contained a high number of acetyl groups, even if the removal from catalpa wood was slightly faster.

Acid liberation due to hemicellulose extraction

In this process, we need to consider two different phases, the solid phase and the liquid phase. Many authors have demonstrated that mineral acids enhance hemicellulose hydrolysis. This means that pH of the liquid phase is intentionally decreased (protons increase) by adding an external acid. On the other hand, the solid phase has its own acidic groups, i.e. acetyl groups. We believe, that for our case, where no mineral acid was added, an increase in the amount of hydronium anions in the solution due to the acetyl group cleavage of the extracted hemicellulose cannot be directly correlated to an improvement in the extraction. However, Figure 5b shows very clearly that for every tree species, an increase in the amount of hemicellulose dissolved lead to an increase in the acidity of the solution. This indicates that the increase of acidity in the solution was a result of

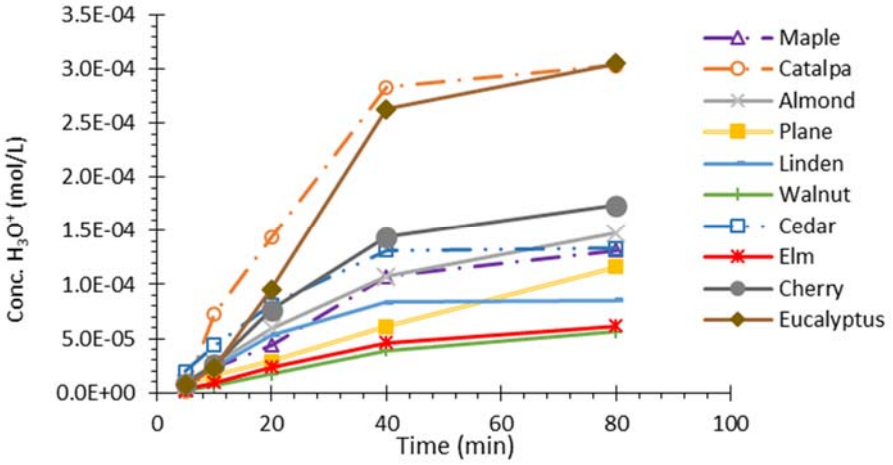
extraction and hydrolysis of hemicelluloses [26], as acetyl groups bounded to hemicelluloses were cleaved and converted into acetic acid. From our point of view, this indicates that the bonded acetyl groups (still attach to the wood) have a positive effect on the extraction, rather than the protons in solution.

To better understand the hydrolysis mechanism, the concentration of acetic acid detected after the characterization of the raw material (representing the total amount of acetyl groups attached to the hemicelluloses) was represented in Table 1 in terms of raw material composition.

It is evident that species containing hemicelluloses with the highest amount of acetyl groups (eucalyptus and catalpa) produced a more acidic solution after extracting the hemicelluloses. Conversely, walnut and elm contained the least amount of acetyl groups and released the smallest concentration of H_3O^+ ions after extracting hemicellulose, later we will see that this also affects the molecular weight of dissolved hemicelluloses.

The phenomenon may be due to the fact that the presence of acetyl groups in solid phase catalyzed the hydrolysis of hemicellulose oligomers as long as they solubilized in the liquid phase. Together with the hemicellulose oligomers, also the acetyl groups were detached from the solid, solubilized and converted into acetic acid, lowering the pH of the solution.

a)



b)

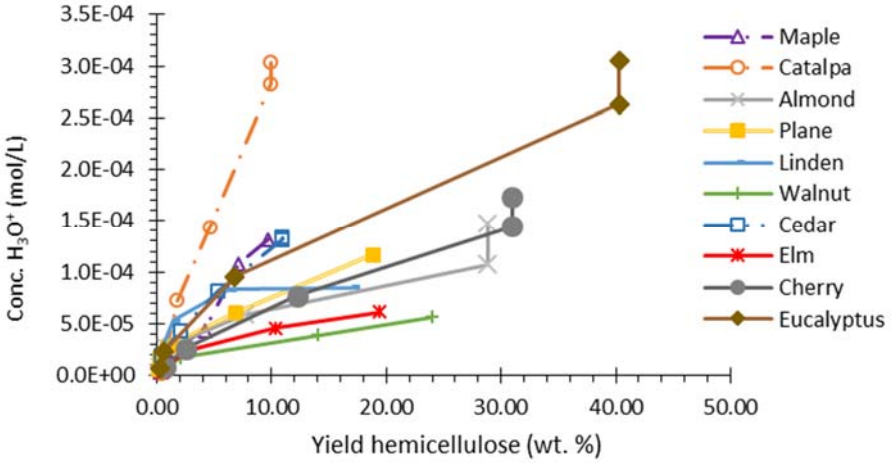


Figure 5. a) Concentration of H_3O^+ ions in the extracted solutions, at different extraction times. b) Hemicellulose yield in function of the concentration of H_3O^+ ions in the extracted solutions.

2.4 Molecular weight distribution of the extracted hemicelluloses

Regarding the molar mass of the extracted hemicelluloses we found three different trends, as we demonstrated in Figure 6.

First trend: molar mass decay with time.

This indicates that from the beginning to the end of the process, hemicelluloses were continuously hydrolyzed. Depolymerization was faster during the first 20 minutes and

then slowed down (Figure 6a). Hemicellulose yields, on the other hand, increased slowly during the first 20 minutes, then more rapidly between 20 and 40 min and finally tended to stabilize in the last 20 minutes.

The possible explication is that, at the beginning of the process, extracted oligomers were long (above 30 kDa) and partially soluble (yields below 3%) while, due to the temperature, insolubilized hemicelluloses started to break and became more soluble (first 20 minutes), until they reached a sufficiently low length that they were rapidly solubilized without further hydrolysis (20 to 40 minutes). In the last 20 minutes, almost all the hemicellulose that could be extracted at 160 ºC were already solubilized and its molecular weight decreased slowly due to the protons in the liquid phase.

Second trend: maximum of molar mass.

At the beginning, very low molecular masses were produced (below 1 kDa), but as the extraction evolves, the molar mass increases, with a maximum at 20 minutes (Figure 6b). Then hemicellulose chain lengths were reduced until the end of the run.

Hemicellulose yields were very low during the first 10 to 20 minutes (a kind of lag time) and then increased linearly.

It seems that short molecules had to be removed before large oligomers could be extracted and then depolymerized.

This tendency could be related to the fact that the hemicelluloses of these species had a stronger and more complex structure [32, 37], needing a preliminary cleaving to achieve a sufficiently small size to be solubilized.

For this reason, only a few molecules with small molecular weight were solubilized at the beginning of the reactions. Later on, temperature effect led to the cleavage of the longest

oligomers that were subsequently hydrolyzed and solubilized, increasing the concentration in the liquid phase.

The extraction yield was relatively low in all the cases (below 20% bottom-line).

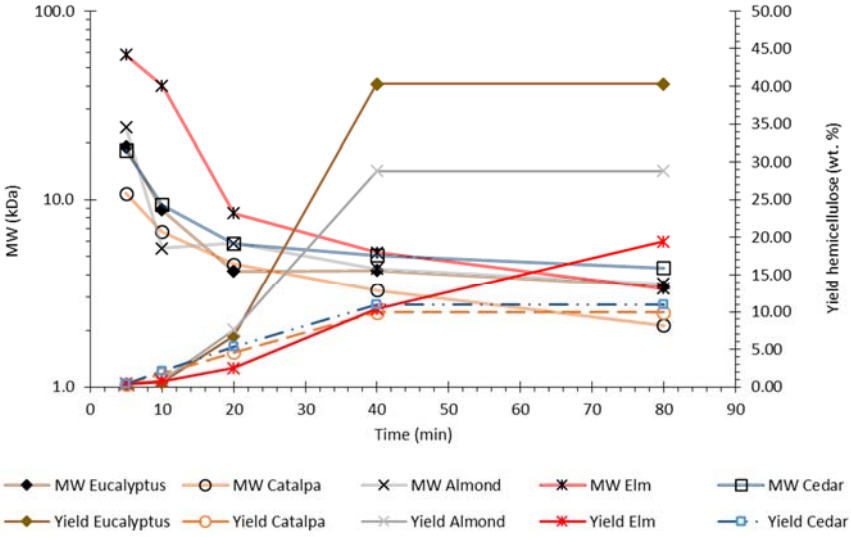
Third trend: minimum of molar mass.

Walnut and cherry revealed a third different behavior (Figure 6c): the molar mass decayed rapidly during the first 20 minutes down to a minimum of around 10 kDa. The curiosity is that from 20 to 80 minutes the average molecular weight grew slightly up to aprox. 15 to 20 kDa.

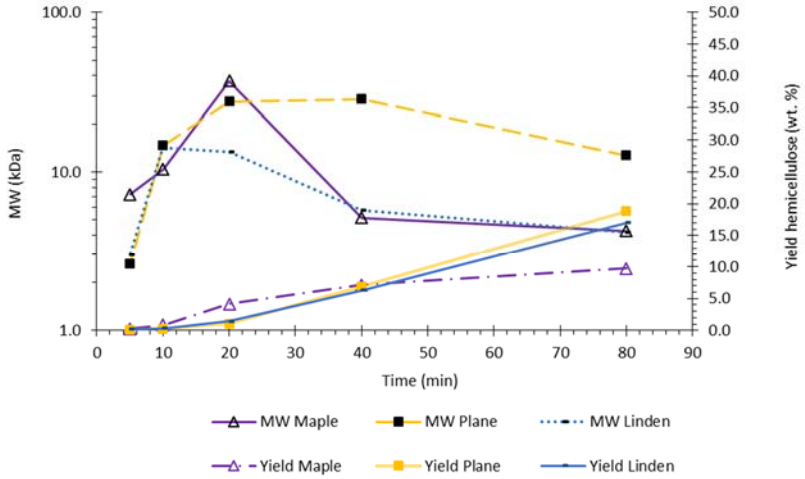
During the first 10 minutes the extraction yield was very low (below 2-3%), then increased rapidly, and in the final 40 minutes tended to stabilize.

Thus, the behavior was similar to that of Figure 6a at the beginning of the reaction: small amounts of long oligomers were extracted until the longest chains (still attached to the matrix) were hydrolyzed and solubilized. The increase in the molecular weight after 20 minutes could be associated to the presence of non-acetylated hemicelluloses in the matrix, more difficult to hydrolyze and solubilize [37], which appears after the removal of the acetylated hemicelluloses [38-40].

a)


100.0
10.0
1.0
MW (kDa)
50.00
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Yield hemicellulose (wt. %)
0
10
20
30
40
50
60
70
80
90
Time (min)
MW Eucalyptus
MW Catalpa
MW Almond
MW Elm
MW Cedar
Yield Eucalyptus
Yield Catalpa
Yield Almond
Yield Elm
Yield Cedar

b)


100.0
10.0
1.0
MW (kDa)
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Yield hemicellulose (wt. %)
0
10
20
30
40
50
60
70
80
90
Time (min)
MW Maple
MW Plane
MW Linden
Yield Maple
Yield Plane
Yield Linden

c)

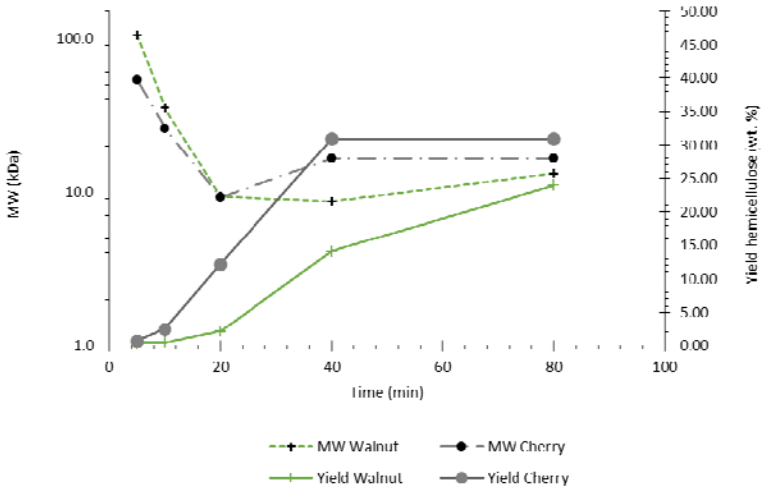


Figure 6. Molecular weight of hemicellulose oligomers extracted from the different species according to curve shape: a) continuous decay, b) maximum c) minimum

The hydrolysis process took place initially in the solid phase, where the effect of the temperature broke the bonds between the sugars oligomers, allowing to achieve a sufficiently short length to be solubilized and further hydrolyzed [11].

The length of the solubilized oligomers depends on the temperature but also by the concentration of acetyl groups contained in the wood, that catalyze the reduction of hemicelluloses lengths before their solubilization [11].

Walnut and elm gave the oligomers with the highest molecular weights and, at the same time, they were the species that contained the least amount of acetyl groups (Table 1). This fact clearly explains how the hydrolysis of hemicelluloses proceeding from these species was not very intense in the solid phase, producing oligomers of large dimensions that were solubilized and subsequently hydrolyzed in the liquid phase.

The hemicelluloses belonging to eucalyptus and catalpa, however, were rapidly hydrolyzed in solid phase by the numerous acetyl groups, producing small oligomers that

were then solubilized. By the time that we measured the first liquid sample (after 5 min), the hemicelluloses were already short.

The size reduction of oligomers in liquid phase was influenced by the increase in the acidity of the extracted solution and the reaction time, as anticipated in paragraph 3.3.

Oligomers polydispersity

Dispersity (formerly polydispersity) of extracted oligomers were divided into three groups and represented in Figure S3 in Appendix 2.

Hemicelluloses extracted from cedar, cherry and eucalyptus (Figure S3a) displayed the maximum polydispersity at the beginning of the process. In the cases of eucalyptus and cedar, values decreased rapidly during the first 5 minutes and then assumed constant values between 1.5 and 2. Dispersity of cherry also decreased rapidly during the first 5 minutes (from a value of 6 to 2), it grew to a value around 3 after 20 minutes and then returned to decrease to a value around 1.7.

Dispersity of hemicelluloses extracted from catalpa, almond, plane, linden, walnut and elm (Figure S3b) had a maximum peak at 10 minutes from the beginning of the process, while polydispersity of maple (Figure S3c) had a maximum peak at 20 minutes from the beginning of the process.

Molecular weight distribution of hemicelluloses extracted from all the species are represented in figure S4 in Appendix 2.

2.5 Hemicellulose extraction yield estimation tool development

There is no denying that the initial biomass composition affects hemicelluloses extraction yield as it was demonstrated with the different species in Figure 4. Also molecular weight seems to have a role in this process, as it was explained in section 3.4.

In order to develop an estimation tool, the individual effect of each main biopolymer (hemicelluloses, cellulose and lignin) on the yield must be studied. To do so, the hemicellulose yield was represented versus hemicellulose, cellulose and lignin content (Figure S5 in Appendix 2).

For hemicellulose, a R^2 coefficient of 0.28 was obtained (Figure S5a), which means that there was a weak or no relation between yield of hemicellulose extracted and hemicellulose content in the raw material. However, for cellulose (Figure S5b) and lignin (Figure S5c) the R^2 increased respectively to 0.74 and 0.82 when *catalpa* and *elm* were not considered: these two species are those with the highest content of cellulose.

It appears that the lignin content negatively affected the hemicellulose extraction, while cellulose content promoted it, except when the cellulose content was extremely high.

There should be a relation between extraction yield and the cellulose/lignin content ratio ($w_{C/L}$) as confirmed in Figure 7a. Therefore, the empirical expression to estimate the extraction yield for $w_{C/L}$ would be Eq. 1.

$$y_{HC} = a \cdot ln\left(W_{C/L}\right) + b \quad (1)$$

Nonetheless, this expression was obtained without including the results for *elm* and *catalpa*. To take into account them, Eq.2 was proposed since these two samples had a $w_{C/L} > 2$, which seemed to produce a negative effect on the extraction.

Lignin negative trend could be explained by the fact that it involves the whole biomass, protecting hemicellulose and making the extraction difficult [17].

However, a high amount of cellulose implies a low lignin content, promoting the extraction of hemicellulose. Nevertheless, if lignin content is much low, the hemicellulose

could be incorporated and protected among the cellulose fibers [32], which prevent hemicellulose extraction.

$$y_{HC} = a \cdot ln\left(W_{C/L}\right) + b - \frac{c_y}{1 + e^{e\cdot\left(d - W_{C/L}\right)}} \quad (2)$$

Finally, the differences between the experimental and simulated yields were minimized by the Solver Excel tool to obtain the parameters c_y, d and e. The final values were a=23.33 (dimensionless), b=19.54 (%wt), c_y=31.58 (%wt), d=2.25 (%wt) and e=34.45 (dimensionless). The average error was 12.7 % with a R^2 of 0.83. The comparison between the simulated and experimental values are depicted in Figure 7b. Additionally, the individual discrepancies are arrayed in the Table 1S in appendix 3. It is worth mentioning that the highest errors between calculated and the experimental data were obtained for: *cedar* (28%), *eucalyptus* (16%), *linden* (32%) and *almond* (20%).

Since these discrepancies cannot be explained only by considering differences in composition, molecular weight or protons releasing, another parameter should be considered: the biomass structure.

Other studies based on SEM analysis demonstrated that when monomers and oligomers are detached from the biomass, the number of cavities in the matrix increases [13, 41, 42], promoting the removal of further carbohydrates. This kinetics can be assumed as autocatalytic as demonstrated in previous studies [13, 32]. The same considerations can be applied for the slow pyrolysis process since biomass thermal degradation follows a slow rate until a certain point is reached, where the mass variation becomes abrupt. This behavior can be checked in Figure S6 in Appendix 2, where the thermal degradation of almond wood during a TGA is showed. It can be seen that there is a slow mass change in

the sample until, when a temperature of 250 °C is reached, 50% of mass is suddenly lost. This change in tendency was associated to the cleaving of the strongest biopolymer structures of biomass (i.e. cellulose and lignin). Thereby, our assumption is that the structure modifications showed during a TGA can give important information about what happens during the hydrothermal treatment.

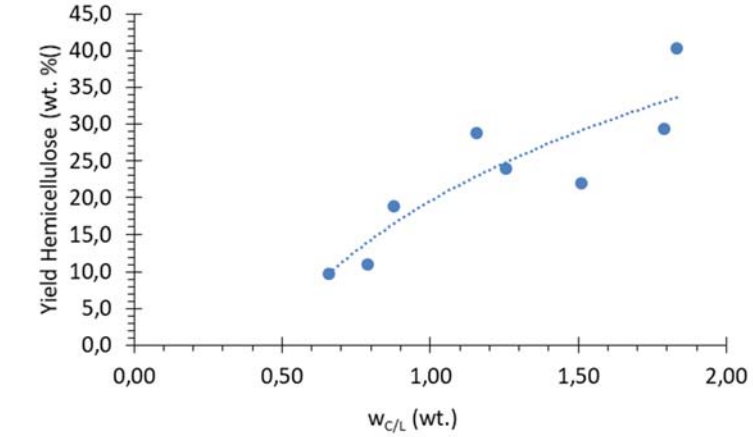
Table 2. Cellulose and lignin content

	$w_{C/L}$[a]	w_T[b]
	-	%wt
Almond	1.16	0.66
Cedar	0.79	0.71
Sour Cherry	1.79	0.67
Elm	2.86	0.73
Eucalyptus	1.83	0.71
Linden	1.51	0.70
Maple	0.66	0.75
Plane	0.88	0.73
Walnut	1.26	0.75
Catalpa	2.33	0.71

[a] Ratio between the cellulose and lignin content: w_C/w_L

[b] Total amount of lignin and cellulose: $w_C + w_L$

a)



b)

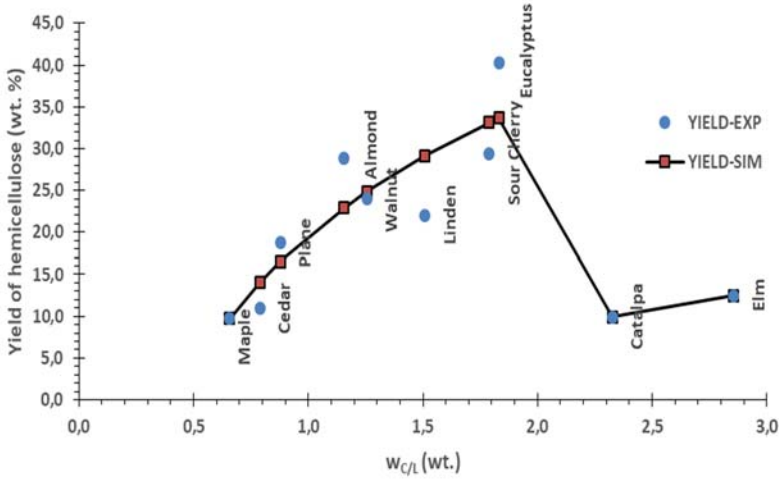


Figure 7. a) Relationship between the hemicellulose extraction yield and the $w_{C/L}$; b) comparison between the experimental and simulated extraction yield.

2.5.1 Estimation tool validation

Once the estimation tool has been developed, it was interesting to evaluate its effectiveness with data obtained from experiments carried at the same temperature and residence time as the one presented in this manuscript.

- Holm oak

The yield and composition data were taken from *Yedro et al.*[43]. In this work, holm oak samples were treated in the same cascade reactor that has been used in our study, obtaining at the same conditions a hemicellulose final yield of 28.50 %. In this case, the value of $w_{C/L}$ was 1.74, which drove to an estimated yield of 32.57 % (deviation estimation of +14.2%).

- Extracted grape seeds

In this test the data were collected from *Yedro et al.* [44] who hydrothermally treated grape seeds after a previous extraction with a mixture of ethanol-water. Final hemicellulose yield reached was 3.56%. For this sample $w_{C/L}$ was 0.51 and the estimated yield was 3.95% (deviation estimation of +10.9%).

- Sugarcane bagasse

Data for the hydrothermal extraction of sugarcane bagasse (final yield of 40.4%) were picked up from *Santucci et al.[45]*. The value for $w_{C/L}$ was 1.91 and the calculated yield was 34.67% (deviation estimation of -14.2%).

- Corn straw

In this case the data were taken from [46], being the experimental yield 6.53% ($w_{C/L}$ = 2.41) and the estimated value 8.61% (deviation estimation of +31.9%).

- Rice straw

Finally, data for hydrothermal extraction of rice straw were obtained from [47], being the experimental yield 6.25% ($w_{C/L}$ = 2.84) and the estimated value 12.32% (deviation estimation of +97.1%).

In summary, the proposed equation can reproduce the hemicellulose extraction yield at 160 º C for 80 min for several types of biomasses like seeds, woods and agricultural wastes. However, when samples very different from wood are used, bigger discrepancies

are obtained, such as for corn and rice straw. Therefore, the proposed expression provides a good estimation of the expected hemicellulose extraction just by using the rough content of lignin and cellulose from the routine analysis.

2.6 Structural effect

As it was mentioned in the previous section, structural differences seem to be the main reason why there was not a perfect relationship between composition and hemicellulose extraction yield. Additionally, it was also introduced the idea that TGA data can be used to understand this structural effect.

With that purpose, TGA was performed for all the biomass samples and fitted by a kinetic model previously developed by our research group [19]. This model (Eq. 3) was obtained applying a transient mass balance for each compound in both phases, liquid and solid. The liquid refers to the water and organic substances that can be present in the sample. The reaction pathway was based on a modification of the Waterloo's mechanism [48-50]. Therefore, it was assumed that each compound present in the sample (e.g. hemicellulose, cellulose and lignin) decomposed into charcoal and gases during the slow pyrolysis process. The charcoal can in turn be volatilized.

$$\frac{dm_j}{dt} = r_j = \sum_{i=1}^{N_r} g_{ij} \cdot r_i \qquad (3)$$

Regarding kinetics, two different types were used: one for liquid phase and another for the solid. For the liquid, it was calculated by a conventional mass transfer expression modified to consider the effect of the sample mass reduction (Eq. 4). In this equation, C_j^*

is the equilibrium concentration in the gas phase, which was obtained by the assumption of ideal gas behavior and using a modified Antoine's pressure vapor expression.

$$r_i = h \cdot \left(C_j^*\right) \cdot m_j^{nl_i} \tag{4}$$

For the solid, a first order autocatalytic expression was considered (Eq.5). The parameter α_i represent the initial velocity factor and reflects how difficult it is to degrade the sample. Its value was fixed to 0.99.

β_i is the acceleration factor and represents how fast degradation is after it has started. This equation was modified for cellulose with another parameter (c) to consider the effect of the heating rate in thermal degradation (Eq. 6). In this work, the value for parameter "c" was fixed at 0.006 because it was the obtained value for those samples where extractives were also present [19], making this study as general as possible.

$$r_i = k_i \cdot e^{-\frac{E_{a_i}}{R \cdot T}} \cdot \mathrm{m_j} \cdot \left(1 - \alpha_i \cdot m_j\right)^{\beta_i} \tag{5}$$

$$r_i = k_\mathrm{i} \cdot e^{-\frac{E_{a_i}}{R \cdot T} + c \cdot T + \ln(T)} \cdot \mathrm{m_j} \cdot \left(1 - \alpha_i \cdot m_j\right)^{\beta_i} \tag{6}$$

The fitting was done by the Simplex Nelder-Mead's method, solving the system of ordinary differential equations (ODEs) by the Runge-Kutta's method with 8th order of convergence [51]. The objective function selected was the Absolute Average Deviation (AAD) defined as follows:

$$AAD = \sum_{i=1}^{N} \frac{1}{N} \cdot \frac{\left|x_{i_{EXP}} - x_{i_{SIM}}\right|}{x_{i_{EXP}}} \tag{7}$$

The ADD and the kinetic parameters for each experiment are included in the supplementary material (Appendix 3), respectively in the Table 1S and in the Table 2S. The model was suitable to reproduce the experimental behavior observed during the TGA with an average error of 1.2 %. Since the discrepancy between the experimental and simulated behavior is low, this calculated kinetics can be used to study how hydrothermal extraction yield is affected by sample characteristics (composition and structure).

2.6.1 Analysis of the TGA kinetics

Slow pyrolysis is affected by a huge set of different variables like the solid and gas residence time, the temperature range, the heating rate, the final temperature, the sample size and the atmosphere type [52, 53]. Moreover, biomass internal structure can also affect it. A good example can be found in *Cabeza et al.* [19] where the pyrolytic behavior of alkaline lignin and lignin extracted from a real biomass were compared. The result was that, while their qualitative response was similar (degradation between 200 °C and 500 °C), the mass variation and the kinetics were completely different, relating this changes to the structural (chemical or physical) differences between the two samples. Following this idea, the kinetics of the samples that in figure 7b showed the highest deviation from the prediction made by the composition (*cedar, eucalyptus*, *linden* and *almond*) were studied in order to check the effect of the biomass structure.

The changes in the individual kinetics (volatilization, char production and char volatilization) for these 4 samples were analyzed. Their values were compared with those other biomasses with a similar cellulose-lignin ratio (wC/L) but that were better simulated by the proposed model. Defining a percentage difference as: $\Delta K=(K_{Studied}-K_{Reference})/K_{Reference}\cdot 100$.

Kinetic of eucalyptus ($w_{C/L}$ = 1.83) was compared with kinetics of cherry ($w_{C/L}$= 1.79); kinetic of cedar ($w_{C/L}$ = 0.78) was compared with kinetic of plane ($w_{C/L}$= 0.88); kinetics of linden ($w_{C/L}$ = 1.51) and kinetic of almond ($w_{C/L}$= 1.16) were compared with the kinetic of walnut ($w_{C/L}$= 1.25).

Only reactions with significant deviations have been considered and represented.

Figure 8 shows that for cherry and eucalyptus, the main differences are in the kinetics of cellulose and lignin volatilization as eucalyptus presents higher values in both cases: lignin and cellulose of eucalyptus are thus easier to volatilize respect to the ones of cherry.

Regarding cedar and plane (Figure 9), the highest differences were in lignin volatilization: lignin belonging to cedar is easier to volatilize.

Therefore, it seems that the easier lignin volatilization, the bigger hydrothermal extraction yield was obtained.

However, this statement was not fully true for *almond* (Figure 10.a) since it had lower volatilization kinetics than *walnut* and *linden* but its yield was slightly higher (around 5-6% more). This discrepancy may be justified by the char production kinetics, being much lower for *almond* (Figure 10b).

Char production means that the sample is going to have a higher content of carbon, making it very difficult to volatilize during a pyrolysis [53]. Thus, a slower production of char would imply a larger amount of compounds with a lower carbon ratio in the biomass, promoting volatilization although the volatilization kinetics are lower, and explaining why *almond* had a higher yield.

A similar behavior can be observed between *eucalyptus* (maximum yield, 40.30%) and *maple* (minimum yield, 9.70%). In this case, the lignin volatilization for maple was up to

95% higher but char formation kinetic was also 85% bigger, which are similar results to the kinetic differences of *walnut* and *linden* with *almond*.

On the other hand, it should be mentioned that in all the cases the differences between the kinetics were lower and lower when temperature was raised. A behavior that may be explained by the exponential dependence of the kinetics with temperature.

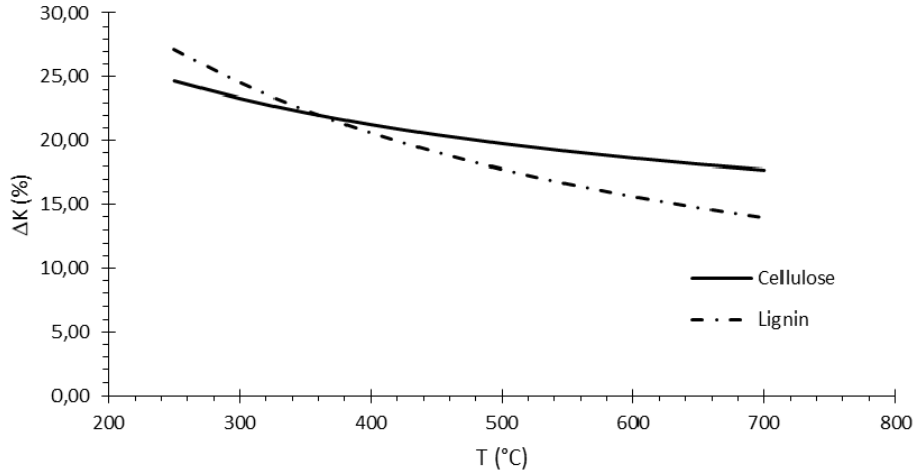


Figure 8. Kinetic differences between *eucalyptus* and *cherry* for cellulose and lignin volatilization. ΔK defined as the difference between the kinetics of eucalyptus and cherry for cellulose and lignin volatilization.

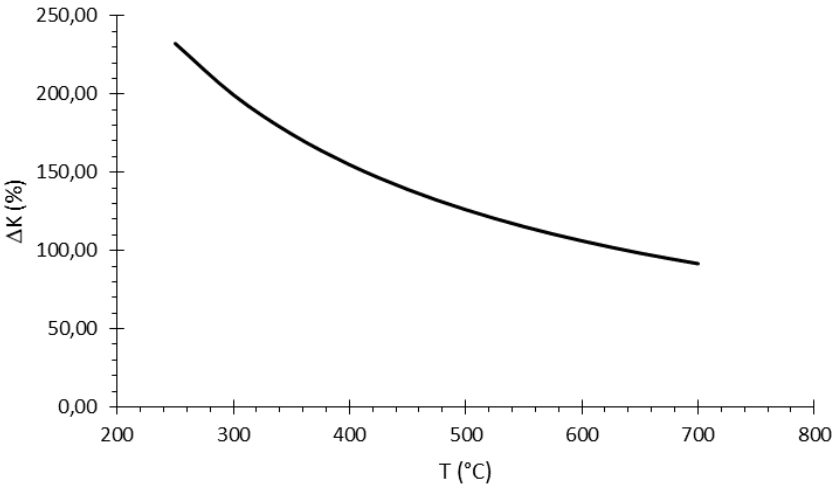


Figure 9. Kinetic differences between *cedar* and *plane* for lignin volatilization. ΔK defined as the difference between the kinetics of *cedar* and *plane* for lignin volatilization.

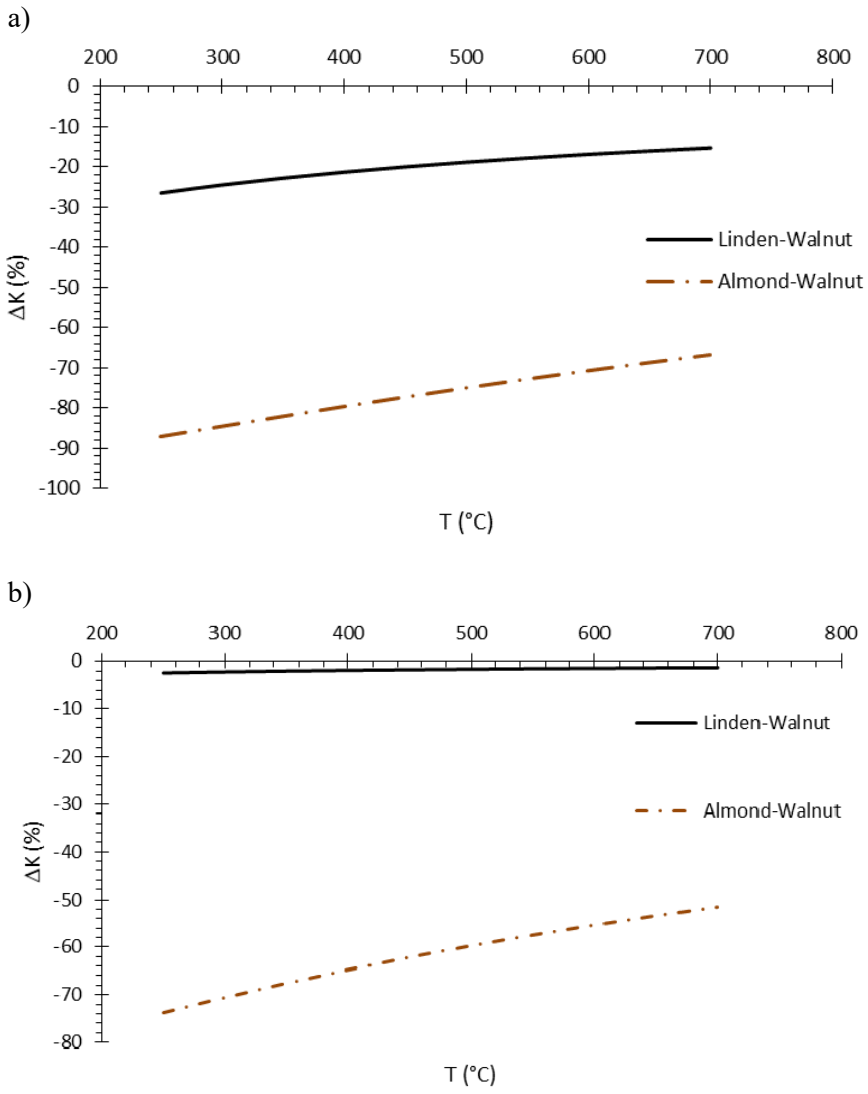


Figure 10. Kinetic differences between linden and walnut and almond and walnut for lignin volatilization (a) and lignin char production (b) ΔK defined as the difference between the kinetics of linden and walnut; and of almond and walnut for lignin volatilization (a) and lignin carbonization (b).

4. Conclusions

The influence of composition and structure on the hemicellulose extraction yield was confirmed not only by experimental results but also by the analysis of the kinetics from a TGA.

We have demonstrated that the acetyl groups contained in the wood attached to the hemicelluloses cause hydrolysis both in the solid and in the liquid phase. When the acetyl groups are still attached to the solid cause severe degradation of the hemicellulose polymer chain and only short hemicelluloses are produced (e.g. catalpa). On the contrary, cases like walnut and cherry with very low initial acetyl groups release the hemicellulose with higher molecular weight. Thus, hemicelluloses with molecular weights higher than 60 kDa can be obtained from walnut wood after 5 min of extraction; while lower molecular weights than 10 kDa, more feasible for the conversion into monomers, can be obtained from the fractionation of woods such as catalpa and eucalyptus. Additionally, 3 different trends (decreasing, maximum and minimum) for molecular weight were observed.

With the method use in this work, species with a high content of lignin or a towering cellulose content led to low hemicellulose extraction yields (around 10%). Structural effects were noticed in some species, like eucalyptus, where the extraction yield (40%) is far greater than the expected taking into account the composition alone.

Additionally, a tool capable to estimate the value of the extraction yield from initial composition data was proposed (average deviation of 12.7%). Moreover, it could be concluded that those sample with higher volatilization kinetics for lignin during a TGA would imply a bigger hemicellulose extraction if lignin char formation kinetics is not also promoted. In general, what has been said so far about the kinetics obtained through the

TGA, while not providing visual data, helps to understand the behaviour of the various constituents of the biomass and their interdependence during the fractionation process.

Acknowledgements

The authors acknowledge the Spanish Economy and Competitiveness Ministry (MINECO) and FEDER Funds, Project CTQ2015-64892-R (BioFraHynery). MEng. Gianluca Gallina wish to acknowledge the Spanish Economy and Competitiveness Ministry for the scholarship/predoctoral contract BES-2013-063556. Alvaro Cabeza also wish to acknowledge the Spanish Economy and Competitiveness Ministry for the scholarship/predoctoral contract FPU2013/01516.

Abbreviations and symbols

Acronyms

AAD: average absolute deviation

ODE: ordinary differential equation

TGA: thermogravimetric analysis

Greek letters

α_i: initial reaction rate factor, dimensionless

β_i: acceleration factor, dimensionless

Symbols

a, b, c_y, d, e: parameters of the empirical equation to estimate the extraction yield

c: heating rate correction factor, dimensionless

C_j^*: equilibrium concentration of the compound "j" in the gas phase, mol/L

$\frac{E_{a_i}}{R}$: activation energy for the reaction "i", K

g_{ij}: stoichiometric coefficient of the compound "j" for the reaction "i", $g \cdot g^{-1}$

h: mass transfer coefficient between the liquid and the gas phases, $g \cdot m \cdot min^{-1} \cdot mol^{-1}$

k_i: Arrhenius' pre-exponential factor for the reaction "i", min^{-1}

K_i: Arrhenius' kinetic constant min^{-1}

m_j: mass fraction of the compound "j", g/g

N: number of experiments, dimensionless

N_r: reaction number, dimensionless

nl_i: mass transfer order for the reaction "i", dimensionless

r_i: reaction rate for the reaction "i", $g \cdot g^{-1} \cdot min^{-1}$

r_j: reaction rate of the compound "j", $g \cdot g^{-1} \cdot min^{-1}$

t: operating time, min

T: operating temperature, K

w_C: cellulose content, $g \cdot g^{-1}$

$w_{C/L}$: ratio of cellulose-lignin content, dimensionless

w_L: lignin content, $g \cdot g^{-1}$

w_T: lignin and cellulose composition, $g \cdot g^{-1}$

$x_{i_{EXP}}$: experimental value of the variable "X" in the experiment "i"

$x_{i_{SIM}}$: experimental value of the variable "X" in the experiment "i"

y_{HC}: estimated hemicellulose extraction yield, %wt

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CHAPTER 4

HYDROTHERMAL EXTRACTION OF HEMICELLULOSE FROM LAB TO PILOT SCALE.

HYDROTHERMAL EXTRACTION OF HEMICELLULOSE FROM LAB TO PILOT SCALE

Abstract

A flow-through reactor of 27.5 mL for the extraction of hemicelluloses with hot pressurized water was scaled up to 2000 mL (factor=72). Experiments were conducted with the two systems using catalpa wood as raw material at 160 and 170 ºC, yields of extracted hemicellulose and monomeric xylose were compared to determine the effectiveness of the scale-up.

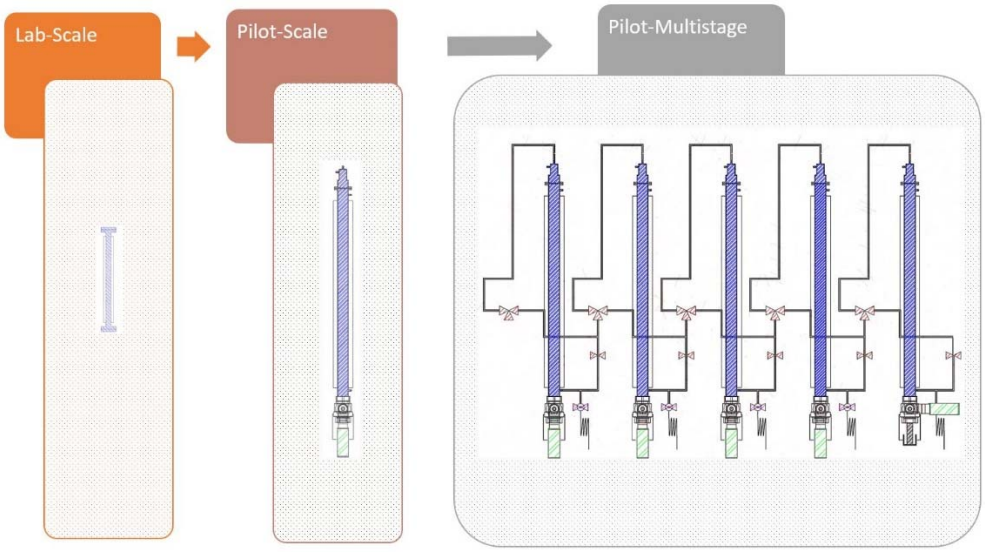
The one pilot reactor system was subsequently upgraded by designing and building a manifold system composed of 5 flow-through reactors capable of working in series. Technological innovations implemented, permitted a continuous operability, minimizing downtime when replacing the biomass during loading and unloading phases.

The system also allowed to collect entirely the spent solid biomass in an orderly way after the extraction, and to work with different feedstocks without limitations due to the particle size.

A study was performed to determine the evolution of the composition and molecular weight of the extracted solution, by varying the residence time of the liquid phase and the solid phase within the system. Results showed that the plant worked homogeneously and there were no deviations in the characteristics of the liquid product flowing from one unit to the next.

Extractions were carried out at 140, 150, 160 and 170 ºC with 3 of the 5 reactors connected in series; liquid samples were collected at different times from each of the 3 units and analyzed.

The main objective of this work was to verify the possibility of using the plant set-up for an industrial process by comparing two scales.



Keywords: Pilot plant, hemicelluloses, fractionation, biorefinery, hydrothermal.

Introduction

Hemicellulose is a biopolymer naturally synthetized by most of the existing trees and plants; its presence in all lignocellulosic biomasses with percentages between 15 and 35% makes hemicellulose the second polysaccharide with the highest abundance in nature after cellulose [1]. Unlike cellulose, which has technological interest in many fields such as plastic, pharmaceutical and paper industry, hemicellulose applications are being investigated and the interest for this polymer is growing in recent years.

It was demonstrated that hemicellulose polysaccharides have interesting properties in the manufacturing of food additives [2], emulsifiers [3], plastic film for the protection of foods [4] or superabsorbent hydrogels [5]. The monomers that constitute hemicellulose are also of great interest. Xylans, present mainly in hardwoods, grasses and straws, are mainly composed by xylose, precursor of high-value products such as xylitol or furfural. Xylitol is produced by hydrolysing xylans and subsequently hydrogenating the xylose monomers. The use of xylitol as a substitute to traditional sugar can help in preventing tooth decay and promote remineralisation of small lesions [6]. It can even be of help for osteoporosis prevention [7]. Furfural, a dehydration product of xylose, is used as a solvent in petrochemical industry to extract dienes (such as those used to produce synthetic rubber) from other hydrocarbons and it is also an intermediate product in the production of solvents such as furan [8].

Because of its non-crystalline morphology, in contrast to that of cellulose, hemicelluloses are generally broken and degraded during conventional pulping processes. However, thanks to biomass pre-treatments and novel separation processes it is possible to obtain and isolate hemicellulose oligomers with suitable characteristics to fabricate several specialised materials.

Methods for extraction of hemicelluloses

Traditional methods used for the extraction of hemicelluloses from biomass involve the use of mineral acid solvents. For instance, acid hydrolysis with concentrated H_2SO_4 or HCl is an effective way to extract and convert approximately 100 % of cellulose and hemicellulose into monomers. Yet, special equipment resistant to corrosion and a recover of acid after hydrolysis are required under such conditions.

Dilute acid hydrolysis is a wide used method. H_2SO_4 or HCl with concentration in the range of 2-5 % are generally used (pH close to 0), at 160 to 230ºC. This treatment extracts hemicellulose from biomass effectively and hydrolyses it into monomeric sugars and often into degradation product such as furfural or 5-hydroxymethylfurfural [9]. These processes are suitable if the main purpose is to obtain a high yield of monosaccharides. Nonetheless, if the aim is to obtain oligomers of hemicellulose for fabrication of specialised materials such as gel or plastics, other pre-treatments are required.

Hydrothermal treatment is a technique with several advantages over the methods previously mentioned. Depolymerisation of oligomers and degradation of monomers is notably reduced. Furthermore, as the unique solvent employed is water, the environmental impact is reduced. Pressurized water at temperatures above 120 ºC is subjected to an ionization process forming H_3O^+ ions that induce the partial depolymerisation of hemicellulose. Hemicellulose has acetyl groups alternatively embed in its pentose-hexose structure. Cleavage of acetyl groups consequently leads to the further formation of H_3O^+ ions that catalyses the depolymerisation of hemicellulose. This process, called autohydrolysis, fits very well when the main purpose is to obtain hemicellulose oligomers with higher selectivity over monomers formation and monosaccharide degradation [10].

Hydrothermal extraction plants

There is a huge number of examples regarding hydrothermal hydrolysis of biomass at laboratory scale. In most cases, the process is carried out in batch reactors [11-14], being a minor number of researches conducted with semi-continuous flow-through reactors [15-17] or continuous reactors [18, 19].

Flow-through pre-treatments were recognised to be the best in terms of extraction efficiency and energy economy: thanks to a more effective mass transfer, they allow highest extraction yields respect to batch reactors, and they do not require extreme milling of biomass and suspension pumping such in the case of continuous-flow reactors.

Pilot plants for hydrothermal extraction of hemicellulose are not many. One example is the Integrated Biomass Utilization System (IBUS) which converts biomass into sugars and lignin, and then to ethanol and energy, by using three reactors [20-22]. This system uses a particle pump to move the biomass into the high-pressure systems.

In a previous project developed by our research group, a laboratory-scale flow-through reactor was developed, operated and optimised [16, 17, 23]. That rig, similarly to most of the existing lab plants, including pilot plant reactors [24, 25], consisted of a stainless steel tube filled with biomass, axially crossed by a constant flow of pressurized hot water.

One of the main difficulties in this kind of system was to properly load the raw material and discharge the spent solid contained in the reactor once the extraction process was completed. In fact, the wet biomass swells and agglomerates inside this kind of reactors, forming a compact structure difficult to remove without stopping the operation and being necessary to open the reactor.

Sometimes brute force methods, such as drilling, are necessary to extract the compacted-swollen spent solid. In an industrial context, this implies long periods of inactivity and consequently an economic disadvantage. An example can be found in delayed coking, where the coke vessels operate in semi-batch and high pressure water cutting is needed to recover the final coke product [26].

A good technique to solve this problem is to introduce biomass into a cartridge, which can be inserted and removed quickly, as in the system designed by Smirnova et al. [25, 27, 28].

Results obtained with our laboratory-scale installation, drove our group to design a semi-continuous pilot plant. Initially, a scaled-up reactor was built, with a volume 73 times bigger than our laboratory unit, where biomass could be replaced thanks to the insertion and removal of a cartridge through a ball valve placed on the bottom of the reactor.

The system was then integrated with some characteristics of a batch-wise cascade reactor located in Åbo Akademi (Finland) [29, 30]. That system consisted of 5 Parr units containing biomass, in which a flow of water circulated in closed loop. Each unit could be excluded from the system thanks to a valve system that allowed to deviate the water flow.

After further improvements, we designed a plant consisting of five semi-continuous reactors, each one working in series with the others or with the option of being excluded from the system. A manifold of valves and cartridges, which will be explained later in this work, made possible to extract hemicellulose from biomass without needing to stop the plant. It was even possible to cool down and disassemble the reactors separately during the loading and unloading phases, thus minimizing downtime. The extraction

process could be carried out in a pseudo-continuous way, replacing rapidly the raw material and entirely recollecting the spent solid.

In the first part of this paper, we will focus on the scale-up of a laboratory equipment, we will discuss the scale-up criteria and how we verified the efficiency of the scale up.

Subsequently, we will explain how the pilot reactor was implemented to become a multistage flow-through reactor (Figure 1).

The system was then comprehensively tested by a temperature study, analysing the yield of hemicellulose extraction and its molecular weight. This operation allowed to study the variations in the characteristics of extracted hemicelluloses varying the residence time of the liquid product within the system. It was also studied the depolymerisation and degradation of oligosaccharides varying their residence time inside the plant. The objective of this work was to create a system able to operate in a continuous and fast way, allowing to obtain a product with constant characteristics and composition, and being considered for an industrial production of hemicellulose by hydrothermal extraction.

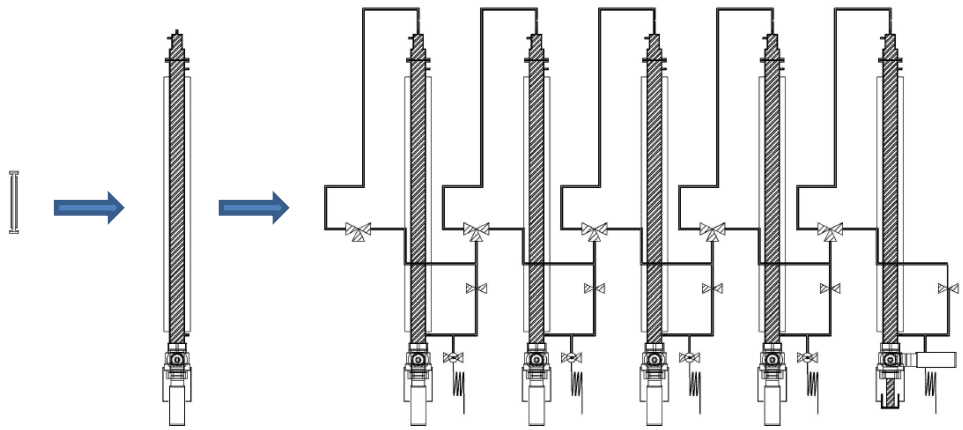


Figure 1. Scale-up from a) laboratory scale reactor to b) single-stage pilot reactor to c) multistage pilot reactor.

2. Experimental

2.1 Raw material characterization

Catalpa bignonioides wood used as the main raw material in all the experiments was originated from Valladolid (Spain). This species was chosen for its abundance in the Castilla y Leon area and the easiness to find pruning residues.

Dry wooden branches were grinded with a chipper, obtaining wood chips with variable particle size between 0.6 and 3.5 cm (showed in Figure S1 of the Supporting Information). They were kept in a dry room inside closed bags until the day of the tests. No bark removal was carried out, since the goal was to start off from low cost biomass, which had undergone a minimum number of pre-treatments.

The composition of the raw material in terms of structural carbohydrates, extractives, ashes, humidity and lignin were determined according to the standard methods published by National Renewable Energy Laboratory (NREL) [31-34]. Dried biomass was treated with water in a Soxhlet equipment, in order to remove the water soluble extractives, and lately with ethanol to remove remaining extractives. 300 mg of dried and free-extractives solid were hydrolysed in 3 mL of 72% wt sulphuric acid solution for 60 min at 30 °C. The mixture was diluted using 84 mL of Milli-Q water and heated in autoclave at 120 °C for 60 min. Solid was separated from the liquid solution by vacuum filtration and placed in a muffle at 550 °C for 24 h. The remaining residue was weighted before and after this step to calculate the insoluble lignin and the ash content of the sample. A liquid aliquot was analysed with UV-Vis spectrophotometer at 320 nm with extinction coefficient of 34 $Lg^{-1}cm^{-1}$ (Sun, Cao, Li, Xu, & Sun, 2014) to calculate the amount of soluble lignin. Another liquid aliquot was neutralized to pH range 6 to 7, filtered using a 0.2 μm membrane and analysed by HPLC to determine the carbohydrates composition.

2.2 Analytical methods

2.2.1 Analysis of liquid samples composition

The total number of compounds contained in the liquid samples was determined by hydrolysing oligomers extracted during the process. 0.8 mL of sulphuric acid (72%) and 15 mL of Milli-Q water were added to 5 mL of liquid samples. This solution was autoclaved at 121 °C for 1h. Prior to the HPLC analysis, liquid samples were filtered (Pore size 0.22 m, Diameter 25 mm, Nylon; FILTER-LAB) [35].

Original liquid samples (before acid hydrolysis) obtained by the extraction process were also filtered and analysed with HPLC.

The column used for the separation of the compounds was SUGAR SH-1011 Shodex at 50.0 ºC with a flow of 0.8 mL/min, using a solution of 0.01N of sulphuric acid and water Milli-Q as mobile phase. A Waters IR detector 2414 and Waters dual λ absorbance detector 2487 (210 nm and 254 nm) was used to identify the sugars and their derivatives. The calibration reagents used for HPLC analysis were: cellobiose (+98%), glucose (+99%), fructose (+99%), glyceraldehyde (95%), pyruvaldehyde (40%), arabinose (+99%), glycolaldehyde (+98%), 5-hydroxymethylfurfural (99%), lactic acid (85%), formic acid (98%), glucuronic acid (99%), mannose (+99%), xylose (+99%), galactose (+99%), rhamnose (+99%), galacturonic acid (+99%), furfural (+99%), acetic acid (+99%), all of them purchased from Sigma-Aldrich and used without further modification.

For the analysis of sugars, sulphuric acid (96%) and calcium carbonate (+ 99 %), purchased from Panreac were used.

2.2.2 Analysis of molecular weights

Molecular weight of the hemicelluloses in the liquid extract was determined by Size Exclusion Chromatography (HPLC-SEC). The column used was a GPC column (SB-804 HQ; Shodex) protected by a guard column (SB-G; Shodex) at 35 ◦C with a flow rate of the mobile phase (NaNO3 0.1 M + NaN3 0.02% in Milli-Q water) set at 0.5 mL min−1. A Waters IR detector 2414 was used for the determination of the molecular weight of the extracted hemicelluloses. Calibration curve was obtained with a set of eight pullulan standards (STANDARD P-82; Shodex) ranged between 6.1 and 642 kDa of average molecular weight, dissolved in milli-Q water.

2.2.3 Determination of pH

The pH of the extracted solution (hydrolysate) was measured online, with intervals of 1 minute in the liquid outlet. An electronic pH-meter (Crison CRI10123.99) was used.

2.3 Raw Material Composition

Humidity, determined after drying a sample of wood in a convection oven at 105 ºC, was calculated to be 14% wt. Dry raw material was composed by: 20.8% of extractives, 16.2 % of lignin, 0.3% of ashes, 32.3% of cellulose (glucose), 23.7% of hemicelluloses (xylose, arabinose and acetic acid) and 6.8% of pectins (galacturonic acid). Amounts of single compounds are represented in Table S1 in the Supporting Information.

Along the manuscript, xylose will be chosen as the monosaccharide representing the hemicelluloses, as xylans constitutes the main hemicelluloses proceeding from hardwoods like *Catalpa bignonioides* [36].

Wood proceeding from branches and bark is known to have a great amount of extractives respect to stem wood [37, 38] and these results were confirmed by our composition analysis.

46.3% of extractives was water soluble, while the remaining 53.7% was soluble in ethanol. This proportion is similar to that found from other authors in other species like *Eucalyptus globulus* [39].

12.9% of the water soluble extractives were monosaccharides and sugar acids (32.0% glucuronic acid, 28.0% glucose, 14.0% xylose and 26.0% arabinose).

2.4. Experimental set-up and operation

This section is explained later within section 3 (lab and pilot scale for 1 reactor) and section 4 (dedicated to the pilot 5-reactor system manifold), as the scale-up was one of the objectives of the work.

3. Reactors scale-up: from lab scale to pilot scale

The set-up and the operational tips of both the laboratory scale and pilot scale reactors are described in this section; the criteria for the scale up is explained in detail. A discussion on the characterization of the hydrolysate effluents produced with these systems is included at the end of the section, comparing the results and assessing the effectiveness of the scale-up.

3.1 Laboratory scale flow-through reactor

Laboratory-scale reactor is schematized in Figure 2. A PU-2080 HPLC pump (P-01) took water from a deposit (D-01) and propelled it through a concentric tube heat exchanger working in counter-current mode (E-01, 2m length, 1/8”-1/4”). Then water passed through a pre-heater (H-01, 200 cm of 1/8” SS 316 pipe, electrically heated by two electric resistors of 300 W) which ensured a uniform temperature at the reactor inlet. Water entered from the top of the reactor (R-01), consisting of a SS316 pipe (R-01, 38 cm length,

½" O.D., 0.38" I.D.), charged with wood chips. Reactor was covered on the top and on the bottom by two metallic filters, in order to avoid the loss of solid particles during the experiments. The reactor was heated by three electric clamp resistors of 300 W/each, placed axially along a machined aluminum bar with 5.08 cm O.D. Outlet flow passed through a concentric tube heat exchanger E-01 (preheating the inner flow).

Pressure was controlled by a Go-back pressure valve (BPV-01) installed at the liquid outlet. The outer flow pH was measured online using an electronic pH-meter (Crison PH 29).

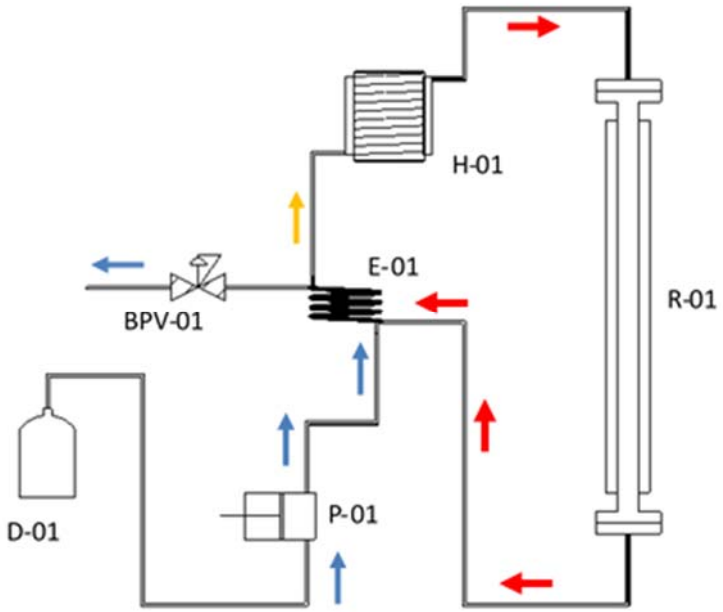


Figure 2. Schematic flow diagram of the lab-scale experimental system. Equipment: D-01 water deposit, P-01 pump, E-01 concentric tube heat exchanger, H-01 electric heater, R-01 flow-through reactor, BPV-01 Go-back pressure valve.

3.1.1 Operation of laboratory reactor

Wood from *Catalpa bignonioides* was used as raw material; 6 g of chips with a medium particle size of about 0.6 cm were loaded in the reactor R-01. A cold liquid pressure test was made before each experiment: cold water was circulated through the system for 5 min in order to check the presence of leaks and to ensure the wetting of the wood. After that, the pump was switched off, while electric heater and clamp resistances placed along the reactor were set at a temperature of 20 ºC above the operating temperature. When the temperature was reached, water was pumped through the system, starting the experiment (time 0). Experiments carried out with other raw materials in the laboratory scale reactor [16], indicated that the optimal flow to get a good mass transfer with the minimum amount of water, corresponded to 3.5 mL/min; the same flow rate was used in this work.

The first sample (time 0) was collected as soon as the first drop of liquid came out from the system. Water reached the operating temperature in about 7 min from the taking of the first sample. Samples were collected after 5, 10, 20, 30, 40, 60 and 90 minutes from the beginning of the operation. At the end of the process, the heating was turned off and fresh water was passed through the system to cool down the reactor. When a temperature of 50 ºC was reached, the pump was switched off, the system was depressurized and water was let to flow out. The reactor was finally dismounted, placed in a mechanical grip and, using a steel punch and a hammer, the wet solid was removed.

3.2 Scale-up to single stage pilot reactor

The scale-up of the laboratory reactor was made by following the criteria indicated in Table 1. The volume of the pilot reactor was 2000 mL, 73 times bigger than the laboratory-scale reactor. Geometrical similarity between the two systems was maintained,

by keeping constant the ratio between length and internal diameter (L/ID). An optimal flowrate of 250 mL/min was set in the pilot system to have the same superficial velocity of water as in the lab-system. The porosity of the bed was also preserved in both reactors, to have the same residence time of the liquid (aprox. 6.0 min).

Table 1. Scale up criteria from lab-scale to pilot-scale reactor.

Parameter	Unit	LAB SCALE	PILOT SCALE
Flow rate	mL/min	3.5	250
Internal diameter	cm	0.96	4
Length	cm	38	159
Volume	cm^3	27.5	2000
L/ID		40	40
Liquid Residence time	min	6.0	6.0
Porosity		0.71	0.71

One of the objectives of this work was to minimize the difficulties of replacing the biomass in the extractor in order to ease the operation and maintenance of the pilot reactor. At laboratory scale, there are many methods that can be used to remove the spent solid (some of them are 'brute-force' methods, e.g. drilling, pushing out with compressed air, etc.) that are difficult to use at pilot or industrial systems.

To facilitate the replacement of biomass in the flow-through reactor, a cartridge mode has been implemented.

The reactor unit consisted of 3 main parts (Figure 3a):

- An open cylinder, constructed with a wire mesh (7) that could be opened longitudinally. Internal diameter of the cylinder was 4 cm and length was 159 cm.
- Two stainless steel cylinders with the same diameter (4 and 5), one of which (5) had several orifices (with a diameter of 1 mm) at the bottom (6), working as a

filter. A glass wool layer could be placed over the holes to decrease the dimension of the voids. The wire mesh was inserted between the two cylinders, forming a cartridge, which was filled with biomass. The inner diameter of the two cylinders was 4.3 cm; so that the mesh adhered perfectly to the walls. The cylinders thickness was 2 mm.

- An outer stainless-steel cylinder (2) with two opening (A and B) closed at the upper end with a mechanized flange (1) with an opening (C), and with a ball valve (3) screwed on the lower end. Internal diameter of the valve and of the cylinder was 5.1 cm, so that the cartridge could be introduced from the bottom and inserted completely into the system.

This system greatly facilitated the replacement of biomass, which could be removed from the system by simply opening the valve and pulling out the cartridge. The longitudinal opening of the wire mesh, moreover, reduced the effort required to remove the wet biomass from the cartridge.

A flow diagram of the single-stage pilot system is represented in Figure 3b. A constant flow of water was drawn from a vessel (D-01) and propelled with a Tuthill DGS.68 pump (P-01) through the external tubes of a system composed by three concentric tube heat exchangers E-01 to E-03 (18 m total length, 1/4” internal tube-3/8” external tube) and then through an electric heater H-01 with a maximum power of 5 kW.

Given the high power of the heater, a special procedure was designed to avoid the overheating of this unit. Unlike in the laboratory scale system, where the heater was left over for a time heating with no contact with water, in the case of the pilot plant, water flowing through a coil was in constant contact with the heater wall.

A three way valve (3V-01) was placed between the heater and the reactor, which could direct the liquid flow to the inlet of the reactor (placed on the top) or out of the system. A ball valve (V-01) was placed just after the outlet of the reactor.

During the preheating phase, the 3-way valve was turned so that water did not enter the reactor, while valve V-01 was closed to prevent water from returning in the reactor through its output.

Water stream entered subsequently in the inner part of the concentric tube heat exchangers (E-01 to E-03), where it was cooled down by the feeding water flowing countercurrent in the external part, before leaving the system through a Go-back pressure valve (BPV-01). In this way it was possible to pre-heat the water to the desired temperature before introducing it into the reactor.

Reactor R-01 was homogeneously coated with four clamp resistors, which power was 250 W/each (total nominal power was 1 kW).

The entire system was thermally insulated with a layer of 2 cm glass wool, protected with aluminum foil.

Temperature of water entering and leaving the reactor was measured with two thermocouples placed at the inlet and at the outlet of R-01.

A flow-meter was placed after the pump to measure the liquid flow rate. An online pH-meter was placed after the Go-back pressure valve to measure the pH of the solution produced by the process.

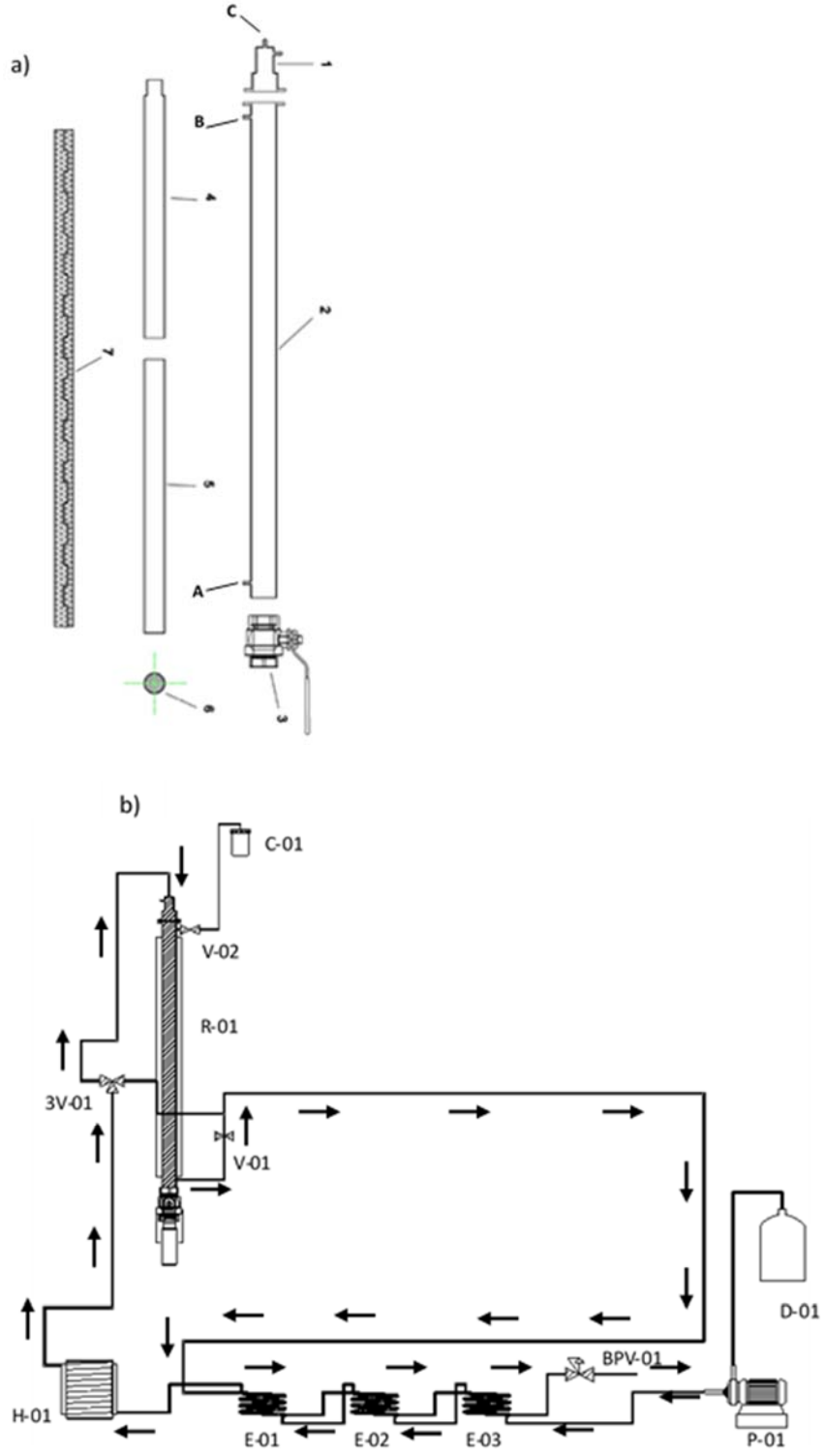


Figure 3. a) Section of reactor composition: 1. Cap of the outer SS cylinder; A,B,C. Openings; 2. External SS cylinder; 3. Ball valve; 4. Superior internal cylinder; 5. Inferior internal cylinder; 6. Bottom with orifices of the inferior cylinder, 7. Metallic mesh.

b) Schematic flow diagram of the pilot-scale experimental system. Equipment: D-01 water deposit, P-01 centrifugal pump, E-01, E-02, E-03 concentric tube heat exchangers, H-01 electric heater, R-01 flow-through reactor, 3V-01 three way valve, V-01, V-02 ball valves; BPV-01 Go-backpressure valve, C-01 plastic container.

3.2.1 Operation of single stage pilot reactor

The cartridge was assembled by introducing the wire mesh between the two half cylinders then. 250 g of chips with a medium particle size of about 2.0 cm were placed inside. The quantity and size of the particles was selected to maintain the same porosity as in the laboratory system.

At the beginning of the operation, the cartridge was introduced into the reactor through the bottom, and the ball valve (3) was closed.

A flow of cold water was pumped inside the reactor from the top, until it was completely filled. During this procedure, ball valve V-01 was closed to keep the water inside the reactor.

Another ball valve (V-02), was connected to an outlet (B) of the reactor placed on the top. This valve was opened during the filling procedure, and was connected to a plastic container (C-01). When water wet the container the reactor could be considered completely filled. At this moment, the ball valve V-02 was closed and the valve (V-01) was opened, letting water exiting from the reactor.

A cold liquid pressure test was made, by increasing the pressure of the system to 17 bar. Subsequently, the three-way valve was switched and the valve (V-01), so that the flow of water by-passed the reactor.

Heater was turned on to heat-up the liquid flow 20 ºC above the operating temperature; water contained in the reactor was preheated to 95 ºC through the clamp resistances which wrapped it around. Temperature of water inside the reactor was set to a value minor than 100 ºC to avoid the extraction of structural carbohydrates.

When the water reached the desired temperature, the three-way valve was switched, and the flow was directed into the reactor; at the same time, the ball valve (V-01) was opened, letting water exiting the reactor.

Time 0 (zero) was set at this moment and sample 0 (zero) was collected from the system outlet. Other samples were collected after 5, 10, 20, 30, 40, 60 and 90 minutes from the starting of the operation.

At the end of the process, the heating system was turned off and fresh water was passed through the system to cool it down. When a temperature of 50 ºC was reached, the pump was switched off, the system was depressurized and water was let to flow out. The ball valve (3) on the bottom of the reactor was opened and the cartridge containing biomass was extracted and dismounted to remove the solid.

3.3 Comparison between results obtained with laboratory and pilot reactor

Hydrothermal extraction was carried out with the laboratory scale and with the pilot reactor at 160 and 170 ºC. Figure 4a represents the cumulative yields of total xylose, produced with the two systems after the extraction process and the acid hydrolysis of the solution obtained. Yield was calculated as the ratio between the total mass of the xylose extracted and the total mass of xylose in the wood contained in the reactor at the beginning of the experiments.

A cumulated yield of 33.9% was obtained after 90 minutes of operation, using the laboratory scale reactor at 160 ºC; a yield of 35.7% was reached with the pilot reactor at the same temperature and extraction time.

In experiments conducted at 170 ºC, the yields increased in both cases, reaching final values of 38.8% and 41.7% when using the lab-scale and the pilot reactors respectively. Yields of all the compounds extracted are represented in Table S2 in the Supporting Information.

The positive effect of temperature in increasing the extraction yield is well known and widely documented [40-42]. However, in this work, the main concern was to identify the differences that occurred when the process was scaled-up.

Figure 4b shows the percent deviation between the yields obtained with laboratory scale and pilot scale reactors, at 160 and 170 ºC.

At both temperatures, the deviations followed a decreasing trend: it was 100% at time 0, after 5 min it decreased to 64.0 % in the experiment at 170 ºC and 44.0 % at 160 ºC. After 20 min the deviations reached negative values and then tended to 0.

The “100% deviation”, found at time 0 was associated to the fact that while with the laboratory system no xylose was produced, small amounts were detected in the solutions proceeding from the pilot plant at the very beginning of the extraction. The presence of this monosaccharide, rather than to hemicelluloses breakdown, was almost certainly due to the free sugars contained in the biomass, which can be extracted at temperatures below 100 ºC [43]. Considering the composition of water soluble extractives, determined as explained in the section 2.3, it was calculated that xylose extracted at time 0 with the pilot reactor, corresponded to the 25.8% of the monomeric xylose in the raw material. This initial yield of monomeric xylose is the same in the experiments carried out at 160 and

170 ºC, as temperature of water inside the reaction chambers was always set at 95 ºC (as explained in section 3.2.1) at the beginning of the operations.

The deviations between the yields obtained with pilot scale and laboratory scale plants were higher during the first 10 minutes of extraction. This behaviour could be due to: (1) the different temperature profile followed during the process, as represented in Figure S2 in the Supporting Information and (2) the differences in the time scale due to the piping, which length change have more influence at the beginning of the process, when the slope in the extraction curve is high.

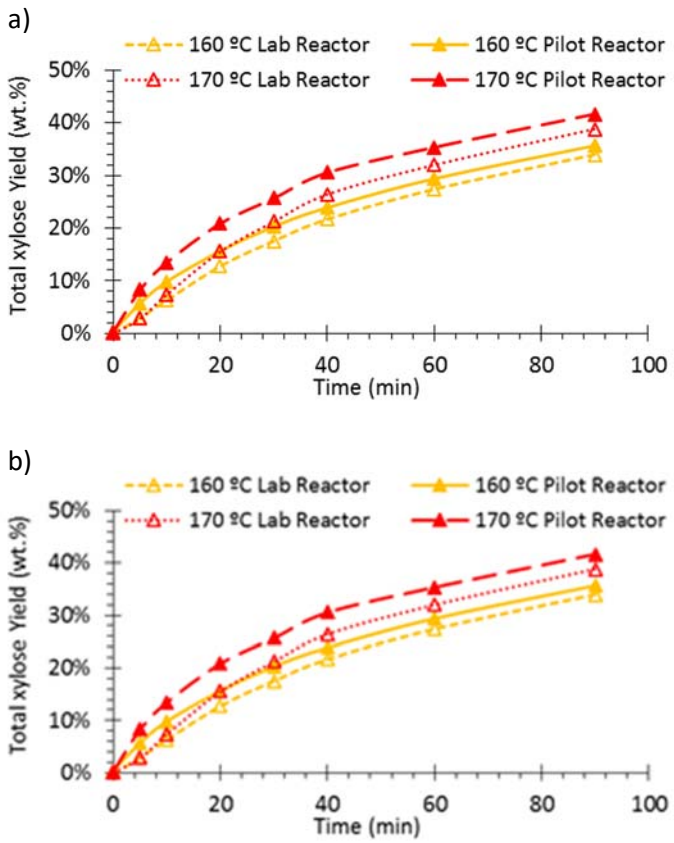
The warming up to the operating temperature occurred faster in the pilot plant (3 minutes less) in comparison with the laboratory scale system. This delay, due to the different method employed in the heating, is responsible for the initial deviation between the yields obtained in the two systems. In the pilot plant, the wood particles were completely submerged in water before starting the process. Then, they had undergone to a preheating to 95 ºC, during which their structure weakened and released part of the water-soluble compounds.

On the other hand, in the laboratory scale plant, part of the water that was injected during the pressure control step left the system during the preheating phase, when the pump was switched off. Thus, chips impregnation was less effective than in the pilot reactor, leading to a less breakdown of the wood particles [44, 45]. The lower amount of water inside the reactor also resulted in a greater inertia to reach the operating temperature, as it took about 5 min to completely fill the system and then warm it up.

Figure 4c represents the cumulative yield of monomeric xylose obtained at different times of extraction in the pilot and laboratory scale plant. As it is known, temperature favours the extraction of hemicellulose oligomers and increases hydrolysis and formation of

monosaccharides [46], which is in according with the results shown in Figure 4c. An increasing in temperature from 160 to 170 ºC meant an increasing in the yield of around 2%.

Comparing subplots in Figure 4 it is possible to deduce that the yield was slightly higher in the pilot reactor than in the lab scale in general. The higher differences appeared during the first 20 minutes of extraction, attributable mainly to the different preheating procedure. No degradation product, such as furfural (from xylose cyclodehydration) or 5-hydroxymethylfurfural (5-HMF from glucose dehydration), were detected in the experiments, suggesting that the temperatures and residence times tested were not strong enough to decompose the monosaccharides.



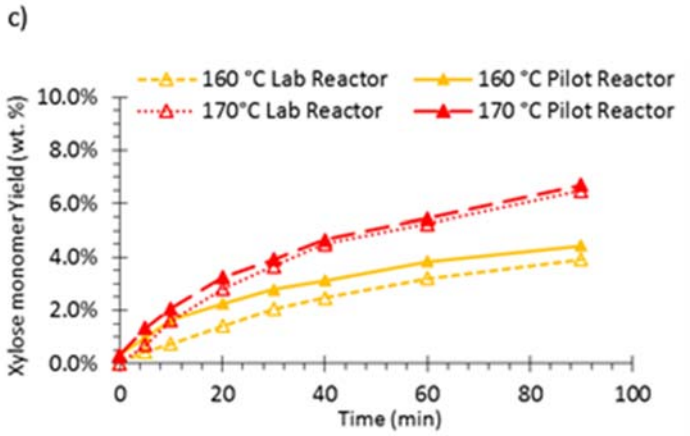


Figure 4 a) Yield of xylose extracted at 160 and 170 ºC with a lab-scale and a pilot-scale reactor; b) Percentage error between yields of xylose obtained with lab-scale and pilot-scale reactor at 160 ºC and 170 ºC. c) Cumulative yields of xylose monomers extracted with a lab-scale and a pilot-scale reactor at 160 and 170 ºC.

4. Multistage pilot flow-through reactor

After establishing the effectiveness of the scale-up, several modifications were made in the pilot plant, aimed at conducting the hydrothermal extraction in a continuous and rapid way: the single stage flow-through pilot plant was implemented to become a multistage flow-through pilot plant.

This section will explain the setup of the manifold-system and the standard operating mode for which it was designed. Finally, a study was conducted to verify the possibility of a continuous production of hemicellulose.

4.1 Set-up of multistage reactor

The complete layout of the plant is shown in Figure 5. Five reactors with the same geometry and operating principle as the one shown in Figure 3a, were connected in series (R-01 to R-05). A three-way valve (3V-01 to 3V-05) was placed before each reactor

(manifold). Each valve could divert the flow to the reactor inlet or to the next 3-way valve, by-passing the reactor. Ball valves (V-01, V-03, V-05, V-07 and V-09) placed after the reactors could be closed to prevent water return.

Right after the outlet of each reactor, needle valves (NV-01 to NV-05) connected to concentric tube heat exchangers (ES-01 to ES-05) allowed for the withdrawn of liquid samples from the reactors. Liquid solutions flowed through the internal pipe of heat exchangers (1 m length, 1/8" internal tube- 1/4" external tube) when needle valves were open, and were cool-down by tap water flowing through the external pipe.

A centrifugal pump Marathon Electric 5KH36 (P-02) took fresh water from a vessel (V-02) and transferred it through a pipe, ball valves (V-11 to V-15) could be opened to let water enter into the reactors. Valves V-02, V-04, V-06, V-08 and V-10, connected to plastic containers (C-01 to C-05) worked as level control system: reactors were filled when first drops wetted the containers.

Pump P-01 (Tuthill DGS.68) transferred water from a vessel (D-01), to the external section of three concentric tube heat exchangers E-01 to E-03 (18 m total length, 1/4" internal tube-3/8" external tube) and then through an electric heater H-01 with a maximum power of 5 kW.

Each reactor was coated with four clamp resistors with a total power of 1 kW/reactor. The water left the system after being cooled down in heat exchangers E-01 to E-03, and depressurized through a Go back-pressure valve (BPV-01). A pH meter placed after the valve measured online the pH of the final solution every minute. The whole system was thermally insulated with a layer of glass wool (about 2 cm) covered with aluminum foil.

4.2 Standard operation of multistage reactor

In a standard operation, three reactors (R-01, R-02 and R-03) were charged with biomass by inserting the cartridge, as explained in section 3.2.

System preparation

Valves (V-01, V-03, and V-05) placed after the outlet of the reactors were closed.

Centrifugal pump P-02 was turned on, valves V-11, V-12, and V-13 were opened to let water enter into the reactors (dotted arrows), and valves V-02, V-04 and V-06 were also opened to let water flow-out to plastic containers (C-01 to C-03) when reactors were completely filled.

When reactors were filled, the pump P-02 was switched off, while valves V-11, V-12, V-13 and V-02, V-04, V-06 were closed.

At this time, pump P-01 was turned on and set to the desired flow rate, feeding the system with fresh water.

3-way valves 3V-01, 3V-02 and 3V-03 were set in order to let water enter into the reactors, valves V-01, V-03, and V-05 were opened to let water flow out.

The direction of the flow is represented by the continuous arrows in Figure 5: water flowed through the external pipes of heat exchangers (E-01 to E-03), through the spirally wound pipes around the heater H-01, entered reactor R-01 from the top and left it from the bottom, passing through the biomass contained.

3-way valve 3V-02 and 3V-03 connected reactors R-02 and R-03 in series with R-01.

Water by-passed reactors R-04 and R-05, entered into the internal pipes of heat exchangers (E-01 to E-03) and left the system.

Pressurization and warming up

Pressure of the system was increased to 17 bar and a cold liquid pressure test was made, stabilizing at the same time the liquid old-up of the system.

When the liquid flow-rate (measured through a flow-meter placed after pump P-01) was constant, 3-way valves (3V-01 to 3V-03) were switched to make water by-pass the reactors R-01 to R-03, while valves V-01, V-03 and V-05 were closed to avoid water return.

Circulating water flow was pre-heated to 20 ºC above the operating temperature (by turning on the heater H-01), while water contained in reactors R-01 to R-03 was heated to 95 ºC by turning on the clamp resistors which covered the reactors.

Extraction

After reaching the desired temperature, 3-way valves 3V-01 to 3V-03 were switched and hot water flowed through the three reactors, extracting soluble compounds from biomass. Liquid samples could be withdrawn from each reactor at regular times, by opening the needle valves (NV-01 to NV-03).

When it was desired to interrupt the extraction in one of the reactors, i.e. R-01, the unit was isolated from the system, by switching the 3-way valve that preceded it (3V-01) and closing the ball valve V-01 simultaneously. Needle valve (NV-01) was opened to remove the water contained in the reactor and to depressurize it. Ball valve on the bottom of the reactor was opened and the cartridge containing the biomass was discharged.

Meanwhile, a new cartridge could be loaded in another reactor (i.e. R-04), which was filled with fresh water through pump P-02 and warmed up to 95 ºC. When extraction process ended in reactor R-01, circulating solution was let to enter in reactor R-04, by switching the 3-way valve 3V-04 and extracting compounds from the new biomass.

This operation could be repeated for each unit in the system, in this way each reactor could be integrated into the extraction process or could be by-passed. The system allowed continuous operation: each time that a reactor was stopped to replace the feedstock, another reactor could operate. Removal of the raw material was easy and fast, and it could be done without the necessity of disassembling the extraction unit. Moreover, all the solid could be recollected at the end of the extraction.

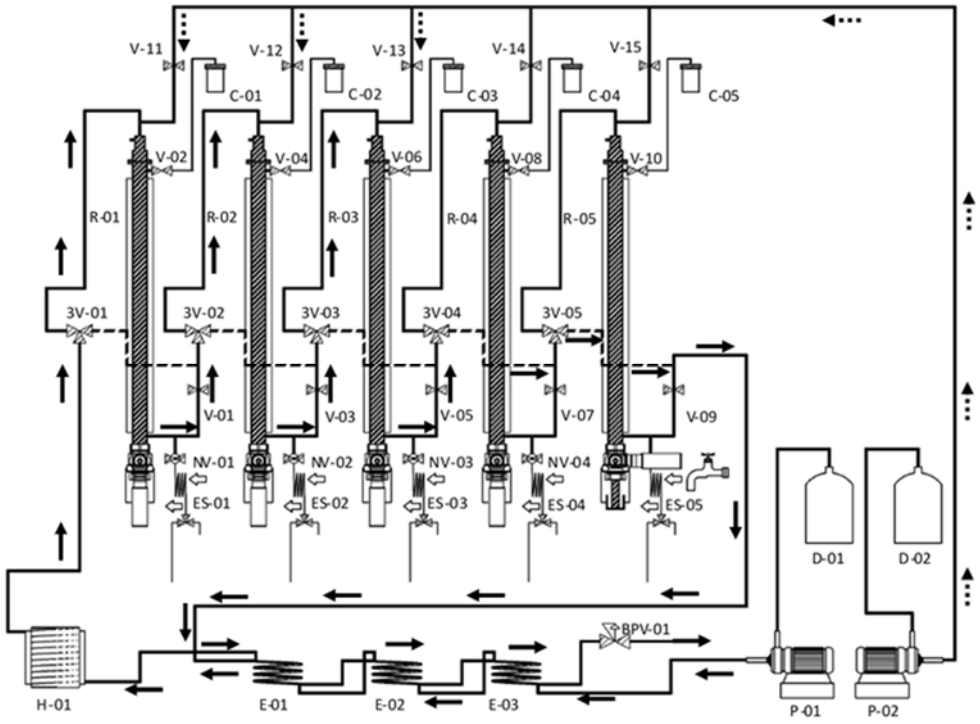


Figure 5. Schematic flow diagram of the multistage pilot flow-through reactor where three units are operating in series. Equipment: D-X water deposits, P-X pumps, E-X concentric tube heat exchanger, ES-X concentric tube heat exchangers for sample withdrawn, H-X electric heaters, R-X flow-through reactors, 3V-X three way valves, BPV-X Go-back pressure valves, V-X ball valves, NV-X needle valves, C-X plastic containers. Liquid flow entering in reactors R-01, R-02 and R-03 connected in series and by-passing reactors R-04 and R-05.

4.3 Extraction in series in the multistage pilot reactor

To assess the ability of the plant to work in a continuous way, with the possibility to quickly replace the biomass, as described in the paragraph 4.2, it was necessary to verify that the reactors worked in a homogeneous manner and that there were no alterations in the composition of the effluent produced by the individual reactors.

Another important matter to study was the evolution in the composition of the extracted products, varying their residence time within the system, to understand if there was degradation or depolymerization of the hemicelluloses extracted when flowing from one unit to the other.

Experiments were carried out at four different temperatures (140, 150, 160 and 170 ºC), using three reactors connected in series, with a constant water flow of 15 L/h. Temperature profiles inside the reactors are depicted in Figure S3a in the Supporting Information. In Figure S3b, the temperature of the water flow over time is plotted: before the heater (after preheating in the heat exchangers), after the heater (before reactor R1), after the reactors (before cooling through the heat exchangers) and at the system outlet (after cooling). In the system, 78% of the heat was recovered in the pre-heating, while 100% of the heat was dissipated during cooling.

The reactors were loaded with their respective cartridges, each one filled with 250 g of catalpa wood-chips with an average particle size of 2 cm. Reactors R1 to R3 were then pre-filled with distilled water and pre-heated to 95 ºC.

A water stream was also preheated to about 20 ºC above the operating temperature (thus 160, 170, 180 and 190ºC) and, at time 0, it was injected inside the first reactor; which outlet flow was entering into the second unit, connected in turn with the third unit in series.

In about 3 minutes, the three reactors reached the same operating temperature with a constant water stream flowing through them and preheating the feeding water before exiting the system.

The whole operation lasted 90 minutes. Liquid samples were collected at regular intervals of time (0, 5, 10, 20, 30, 40, 60 and 90 minutes) from each of the three reactors. After 90 min, the three units were isolated from the system. Then, they were emptied and the cartridges containing the biomass were removed. Pretreated wood proceeding from each unit was entirely collected, dried in an oven at 105 °C and finally weighted. No replacement of biomass was made (although the system is ready for a pseudo-continuous operation).

The whole liquid extract proceeding from each experiment was collected in a tank; six vials were filled with 2 mL of every solution and lyophilized to determine the average concentration of all the compounds extracted; the difference between this value and the concentration of hemicellulose in the final solution allowed to calculate the amount of lignin and extractives also resulting from the process.

Liquid samples withdrawn from each unit at various residence times were analyzed to determine the composition, the molecular weight and the ratio between monomers and oligomers contained in the products.

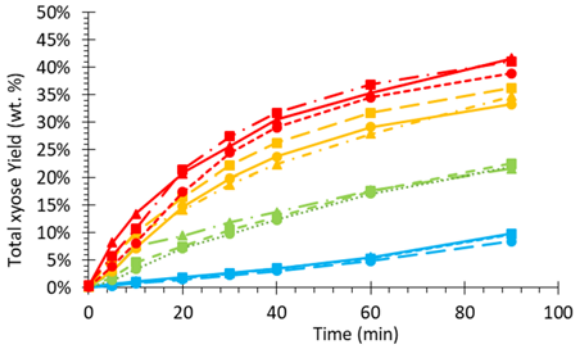
The mass balance was verified by adding the weight of the processed wood remaining at the end of the experiments and the mass of the total solid extracted during the experiments. The mass of the total solid extracted was calculated as the concentration of solid in the final solution multiplied by the total volume of liquid leaving the system during the experiments.

4.3.1 Temperature study in multistage pilot reactor

Figure 6a shows the cumulative yields of total xylose obtained in the multistage pilot plant at four different operating temperatures. Table S3 in the Supporting Information shows the results for all the compounds.

The residence time of liquid in the system was 6 min after crossing the unit R1, 12 min after R2, and 18 min after crossing R3.

a)



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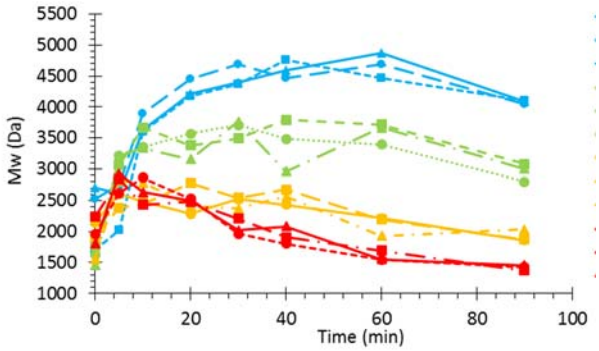

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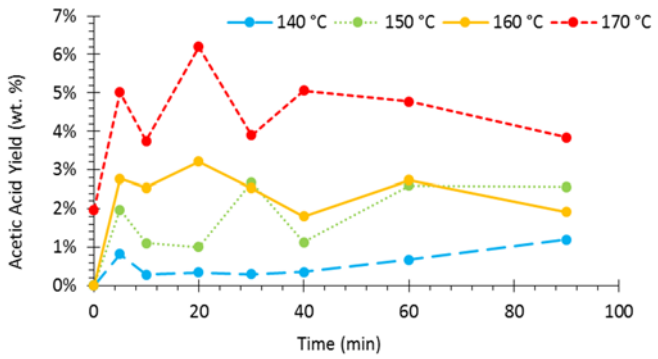

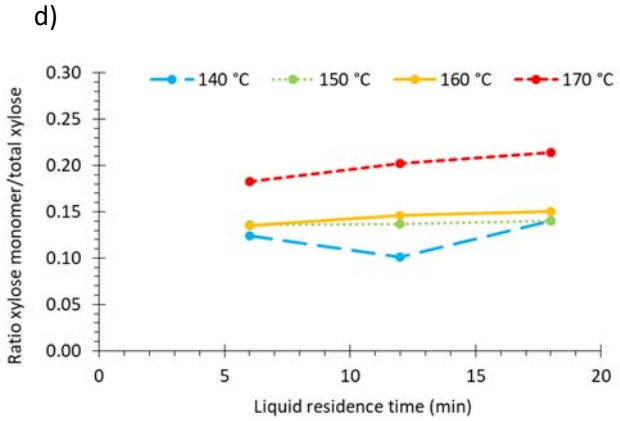

Figure 6 a) Cumulative yield of total xylose extracted, obtained from units 1, 2 and 3 during 90 min of operation. b) Molecular weight of hemicelluloses extracted obtained from units 1, 2 and 3 of the system during 90 min of operation. c) Instantaneous values of acetic acid yield obtained from unit 3 of the system during 90 min of operation. d) Ratio between monomeric xylose obtained after the hydrothermal treatment and total xylose extracted after 90 min of operation, as a function of the residence time of the extracted solutions within the system.

4.3.1.1 Yields of extracted hemicelluloses

Yields were calculated as the ratios between the mass of total xylose detected after acid hydrolysis of the liquid samples collected from each reactor and the total xylose contained in the biomass processed respectively in R1, R2 and R3. The figure shows that, as expected, the highest temperatures leaded to the highest yields. Furthermore, the reaction rates increased, since temperature influenced the mass transfer by increasing the diffusion coefficient inside the wood particles and opening the pore structure [30].

In addition, at constant temperature, the yields were very similar and the variation was minimal at increasing the liquid residence time in the system, as the values were very similar in the samples obtained from the three reactors.

Average values of cumulative yield after 90 min of operation were: 9.3±0.7%, 22.0 ±0.5%, 35.1±1.6% and 40.6±1.4%, respectively, at 140, 150, 160 and 170 ºC. The values indicate the average yields and the standard deviations between R1, R2 and R3. Thus, the experimental error was very low (between 2.2 and 7.9%).

During the process, the extracted products accumulated in the effluent flowing from one reactor to the nextr. The system operated as a long reactor divided into three equivalent sections where the extraction took place homogeneously. Moreover, there was no perturbation in the composition of the liquid effluent flowing from one unit to another.

4.3.1.2 Molecular weight of extracted hemicelluloses

Since the aim of this work was to verify the possibility of using the plant set-up for an industrial process, it was necessary to check if the effluent was homogeneous not only in terms of product yield, but also in terms of composition and length of the oligomers.

The molecular weight of the outputs from the three units during the process at different extractions times was then analysed. Values obtained at different temperatures from units R1, R2 and R3, are depicted in figure 6b. Results are represented in Table S4 in the Supporting Information. In all cases, the molecular weight exhibited an upward trend during the first 10 minutes of extraction, and then it remained constant or decreased with the extraction time. An exception occurred in the experiment carried out at 140 °C, wherein the higher molecular weights were obtained after 30 minutes of extraction.

In general, the molecular weight decreased with an increasing in the operating temperature. After 90 minutes of extraction, the molecular weight of hemicellulose oligomers extracted presented values of 4078±23, 2963±142, 1922±95 and 1417±41 Da at 140, 150, 160 and 170 ºC, respectively. Experimental errors were between 0.6 and 5.0%. This behavior was consistent with the results of other authors who worked with

other tree species [47, 48]. An increase in temperature is responsible for a higher cleavage of the hemicelluloses, thus reducing the size of the oligomers that detach from the matrix. A similar consideration can be made also by observing the polydispersity index (Mw/Mn) shown in Figure S4 in the Supporting Information, which decreased when increasing the temperature.

Hemicelluloses break more intensely and faster at high temperatures, for this reason, smaller and more homogeneous molecules were solubilized in experiments at 160 and 170 °C.

In the experiment conducted at 140 °C, polydispersity index increased from 1.2-1.7 during the first part of the reaction, reaching a maximum value of 2.5-3.2 after around 30-40 minutes, and then decreasing down to 2.2-2.4 till the end of the experiment. It seems that the extraction started with removing small molecules; as reaction time increased, larger molecules were broken, until their molecular weight was small enough to make them soluble.

4.3.1.2 Acidity of extracted hemicelluloses

As explained so far, the rupture and hydrolysis of the hemicelluloses occurred initially inside the wood chips, because of the kinetics enhanced temperature and catalyzed by the acetyl groups integrated the matrix. Subsequently, the oligomers solubilized in the liquid phase, experience a further hydrolysis catalyzed by the dissolved acetyl groups in the bulk liquid. Higher temperatures favored a greater release and solubilization of acetyl groups, and hence, a stronger and faster hydrolysis of hemicellulose oligomers. Figure 6c shows the instantaneous yield of acetic acid produced in the system during the experiments at different temperatures. The amount of acetic acid increased along with temperature,

enhancing the hydrolysis of the oligomers in the liquid phase. Moreover, while the formation of acetic acid at 140 and 150 ° C was almost linear over time, at 160 and 170 ° C there was a maximum production within 5 to 20 minutes from the beginning of the process. Therefore, the molecular weight of the extracted oligomers decreased from the first minutes of reaction at highest temperatures.

The variation of acetic acid extracted is reflected also in the pH of the solution leaving the reactor, measured online every minute. Values of pH are depicted in Figure S5 in the Supporting Information; more acidic solutions were obtained when increasing the operating temperature. At 140 and 150 ºC, pH decreased during the whole reaction, while at 160 and 170 ºC it reached a minimum value at around 20 minutes and then increased slowly.

At constant temperature, the molecular weights of the hydrolysate solutions extracted from the three units had similar values over time. This behaviour can suggest two hypotheses: 1) oligosaccharides extracted from biomass contained in a unit are further hydrolysed in the next unit, in which extraction of new oligosaccharides restored the average molecular weight value, 2) there was a simple accumulation of oligosaccharides and monosaccharides between one unit and the next one, and the oligosaccharides were not subsequently hydrolysed (when their residence time in the system increased passing through the next unit).

To solve this question, the ratio between the monomeric xylose extracted during the process and the total extracted xylose in each unit was analysed. In this way, it was possible to determine whether the extracted oligomers were hydrolysed when flowing from one unit to the next one, as shown next.

4.3.1.3 Monomers content in the liquid product

Figure 6d shows that the ratio between monomeric xylose and total xylose increased slightly with the residence time of the liquid within the system, with an increasing slope when temperature increased. Values are referred to the cumulative amount of sugars extracted from each unit at the end of the experiments (after 90 min of operation). The highest difference between the maximum and the minimum value resulted at 170 ºC, where the ratio of monomeric xylose to total xylose was 0.18 after 6 min and 0.21 after 18 min of liquid residence time. Considering the average values of the ratios at the various temperatures, it can be seen that between 140 and 160 ºC, the values were very similar. However, at 170 ºC, there was a general increasing in monomeric xylose compared to the total xylose extracted. Other authors have found only a slight formation of monomeric xylose from xylans hydrolysis at temperatures lower than 190 ºC and residence time below 20 minutes [49-51]. The phenomena may be due to the temperature itself, the main responsible of the cleavage of hemicelluloses, and to the presence of acetic acid, which at temperatures above 160 ºC increased its concentration in the aqueous medium catalysing the breakdown of the solubilized oligomers.

Degradation products as furfural or 5-HMF were under the detection limit at the conditions used in these experiments. Therefore, it can be stated that there was an accumulation of the compounds extracted from one unit to the next, while a weak hydrolysis of the oligosaccharides occurred with increasing the liquid residence time, more accentuated at high temperatures.

The final mass balance of the four experiments is shown in Figure 7. The errors (the highest error was below 10.5%) which is in the order of other authors in the field. As the

temperature rose, an increasing in the solubilized material was noticed, both of the hemicelluloses and of the other compounds, mostly made up of lignin and extractives.

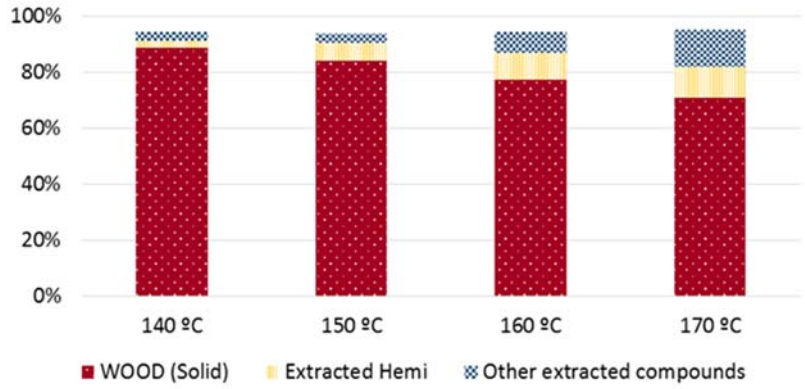

Figure 7 Mass balance calculated at the end of extractions at 140, 150, 150, 160 and 170 ºC.

4. Conclusions

Hemicellulose oligomers can be extracted from biomass using hot temperature water hydrolysis. Handling solids is always tricky, and a semicontinuous operation with batch solid and continuous liquid is a reliable option for both lab and pilot plant scale. In this work, we have established the basic criteria for the scale-up of a process from a 27.5 mL lab extractor (5 g wood particles) to a 2000 mL pilot extractor (400 g wood chips). A shape parameter of L/D=40, with a bed porosity of 0.71 and a liquid residence time of 6.0 min have demonstrated to be efficient for such purpose. The system operated with heat recovery of 85% of the heat.

The two scale systems were investigated extracting *Catalpa bignonioides* wood at 160 and 170 ºC using distilled water without any mineral acid or based addition. From the results obtained with 1 reactor we constructed a manifold of 5-reactors that can operate in series. In this work we demonstrated the operation with 3 reactors. It was verified that

the reactors worked homogeneously and that, at the operating conditions adopted, there was no degradation or substantial modification of the liquid product when increasing its residence time within the system. Therefore, it is possible to work continuously, replacing the biomass as indicated, without changings in the composition of the product. The highest yield in hemicelluloses, with a homogeneous distribution of molecular weight, was around 40% obtained when operating at 170 ºC.

Although the raw material had no ideal characteristics for extracting hemicellulose in a laboratory-level study, as the extractives and lignin content was high and the particle size was large. However, the material could be well used in an industrial context where only a minimal number of treatments is necessary, in order to reduce the costs. The disadvantages represented by this choice are reflected in the low purity of the extract of hemicellulose due to the considerable amount of extractives in the raw material, and in a lower yield respect to other experiments in which the wood particles were smaller and diffusion limitations played a minor role [24, 30].

Although some improvements are needed in the operations, to obtain products more pure in hemicellulose, the plant described in this document has proved to be versatile and suitable for its intended purpose and can be considered in industrial technology.

A possible solution to improve the process without excessively increasing the operational expenditure (OPEX) could be to perform a first hydrothermal pre-treatment at a temperature between 120 and 140 ºC, during which part of the undesirable soluble compounds would be eliminated without extracting the hemicellulose. Post-treatments to concentrate and fractionate the hydrolysate would be also necessary. Furthermore, a study to optimize the costs of grinding the wood (or any other biomass under examination) to obtain the best hemicellulose yield and target molecular weight is also recommended.

Acknowledgements

The authors acknowledge the Spanish Economy and Competitiveness Ministry, Project CTQ2015-64892-R (MINECO/FEDER). MEng. Gianluca Gallina wish to acknowledge the Spanish Economy and Competitiveness Ministry for the scholarship/predoctoral contract BES-2013-063556.

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CHAPTER 5

PROCESO Y PLANTA PILOTO MULTILECHO PARA FRACCIONAMIENTO DE BIOMASA.

PROCESO Y PLANTA PILOTO MULTILECHO PARA FRACCIONAMIENTO DE BIOMASA.

Abstract

The multi-stage pilot plant is composed by vertical reactors having liquid inlet above cartridges containing biomass, and a liquid outlet located in a lower part of the reactors. The lower ends of the reactors are connected to opening and closing devices of, located below the cartridges. The cartridges are independently introduced into the reactors and removed from the reactors , through the opening and closing of the devices. The operation is done without stopping the operation of the pilot plant, by isolating the reactors for exchanging the cartridges.

Resumen

La planta piloto multilecho comprende unos reactores en disposición vertical que tienen la entrada de fluido líquido por encima de unos cartuchos contenedores de la biomasa, y una salida de fluido líquido que está situada en una parte inferior de los reactores. En los extremos inferiores de los reactores conectan unos dispositivos de apertura y cierre de dichos reactores ubicados por debajo de los cartuchos; donde los cartuchos se introducen dentro de los reactores y se extraen del interior de dichos reactores, de forma independiente, a través de los dispositivos de apertura y cierre cuando se sitúan en una posición abierta; todo ello manteniendo el funcionamiento de la planta; donde es posible aislar de forma independiente los reactores para el intercambio de cartuchos cuando se precise, sin detener el funcionamiento de la planta piloto.

Objeto de la invención

La presente invención se refiere a un proceso y planta piloto multilecho para fraccionamiento de biomasa, donde se extraen y fraccionan en continuo compuestos solubles de la biomasa residual y no residual, utilizando como solvente agua o agua con una baja concentración de ácido o base; u otros fluidos. La planta de la invención permite facilidad y velocidad en la operación mediante un intercambio rápido del sólido exhausto sin detener el proceso.

Permite además operar con rapidez mediante un proceso en serie sin desmontar unos reactores de la planta piloto al final de los experimentos. Se destaca también que la planta de la invención permite operar con diferentes tipos de biomasa, sin limitaciones debidas al tamaño de las partículas de la biomasa, permitiendo también recolectar enteramente el sólido exhausto. Por último se destaca que las innovaciones de la invención consiguen reducir en gran medida el tiempo del proceso de extracción y también consiguen eliminar los tiempos de parada.

Problema técnico a resolver y antecedentes de la invención

En la actualidad, los materiales lignocelulósicos pertenecen a las materias primas de segunda generación, y se pueden obtener de varias fuentes, tales como residuos de madera, residuos agrícolas o municipales; que no interfieren con los cultivos para el consumo humano directo. Se componen principalmente de lignina, celulosa y hemicelulosa, asociadas en una estructura resistente, cuya ruptura requiere una cantidad considerable de energía. Sin embargo, gracias a su composición diferenciada, permiten obtener combustibles y múltiples productos químicos de alto valor.

Una forma prometedora, limpia y barata para despolimerizar la hemicelulosa en monosacáridos es el proceso llamado hidrólisis hidrotermal, que simplemente consiste en el tratamiento de la biomasa con agua con diferentes temperaturas, o con agua con una baja concentración de ácido.

La forma más eficiente para extraer la hemicelulosa de la biomasa es el uso de sistemas en el flujo, en el que unos reactores se cargan con pellets o polvo de biomasa, mientras que un flujo continuo de líquido (agua o agua con ácido) se introduce continuamente en los reactores, extrayendo e hidrolizando la hemicelulosa. Para asegurarse de que el sistema sea eficiente, es necesario que el ratio entre la biomasa y el agua sea alto, a fin de obtener un producto concentrado.

Uno de los principales problemas de las instalaciones actualmente existentes para la finalidad descrita, es cargar y descargar el material sólido contenido en cada reactor una vez que se haya completado el proceso de extracción. De hecho, la biomasa húmeda se hincha y se compacta, lo que hace imposible su remoción sin parar el sistema, y sin abrirlo. Esto implica tiempos de inactividad largos. En muchos casos se utilizan partículas con dimensiones del orden de micras, que presuponen la trituración de la biomasa, con el consiguiente consumo de energía, y el bombeo de la suspensión constituida de agua y biomasa, lo que también supone un consumo de energía añadido.

La patente con nº de publicación WO 2015009986 A2 describe un método para la obtención de hemicelulosa utilizando agua o agua con ácido, a través de un reactor en flujo.

La patente con nº de publicación US 6022419 A se refiere a un reactor en el que el volumen ocupado por el sólido (serrín), disminuye continuamente por efecto de un muelle, que lo empuja hacia una extremidad.

La patente con nº de publicación WO 2011091044 A1 describe un procedimiento para extraer celulosa y hemicelulosa de la biomasa, con agua subcrítica y supercrítica, a través de un reactor continuo alimentado con una suspensión de agua y biomasa.

La patente con nº de publicación US 6228177 B1 describe un sistema en serie, para la extracción de material lignocelulósico, en el que los reactores se enfrían mediante inmersión en agua fría.

También es conocida la publicación de King SD referida a "The future of industrial biorefineries". In: Forum WE, editor; 2010.

Descripción de la invención

Con el fin de alcanzar los objetivos y evitar los inconvenientes mencionados en los apartados anteriores la invención propone una planta piloto multilecho para fraccionamiento de biomasa que tiene por objeto extraer y fraccionar en continuo bioproductos como polifenoles, betaglucanos, cafeína y biopolímeros como la hemicelulosa, desde biomasas lignocelulósica residual y no residual de diferentes procedencias.

Las biomasas vegetales contienen muchos productos interesantes. Algunos de estos, como los polifenoles contenidos en las semillas y hollejos de uvas, los beta-glucanos contenidos en la avena, en la cebada y en las setas, y especialmente la hemicelulosa, componente esencial de las plantas, y que figuran en todos los productos de origen vegetal, se pueden extraer sólo con agua con diferentes temperaturas.

Los primeros dos compuestos mencionados tienen gran importancia en la salud y el bienestar humano: los polifenoles son compuestos con capacidad antioxidante que han despertado un gran interés en la salud y en la prevención de enfermedades asociadas a un

aumento de los procesos de oxidación celular (cáncer, enfermedades cardiovasculares y enfermedades neurodegenerativas); los betaglucanos mejoran el control de la glucosa en sangre, así como los niveles de lípidos tales como el colesterol o triglicéridos.

La hemicelulosa, cuando se aísla a partir de biomasa en masas moleculares por encima de los 3-5 kDa, tiene propiedades únicas. Se puede utilizar para producir películas para el envasado de aplicaciones en sustitución a los plásticos sintéticos, funciona como barreras contra la penetración de oxígeno; otra aplicación importante es la producción de aerogeles para aislar productos alimentarios.

La xilosa a partir de la hemicelulosa, por ejemplo, se puede convertir en furfural, que es un precursor utilizado en diferentes campos, tales como el refino de petróleo, plásticos, farmacéutica y agroquímica. La xilosa puede ser también hidrogenada o enzimáticamente transformada en xilitol, que es un agente edulcorante y también se utiliza para la prevención de las caries dental.

La idea de la transformación de biomasa en energía, materiales y productos químicos, define el concepto de biorrefinería, tema particularmente interesante hoy en día, teniendo en cuenta las cuestiones relacionadas con los combustibles fósiles y derivados.

Dos categorías de materia prima dominan la investigación: primera y segunda generación. Productos de primera generación se fabrican a partir de biomasa comestible tales como plantas ricas en almidón o aceitosas; productos de segunda generación utilizan biomasa que consiste en las partes no comestibles residuales de los actuales cultivos u otras fuentes no alimentarias, como las hierbas perennes o algas. Estos son ampliamente reconocidos como poseedores de un potencial significativamente mayor para reemplazar productos de origen fósil (King, 2010).

Los materiales lignocelulósicos pertenecen a las materias primas de segunda generación, y se pueden obtener de varias fuentes, tales como residuos de madera, residuos agrícolas o municipales, que no interfieren con los cultivos para el consumo humano directo. Se componen principalmente de lignina, celulosa y hemicelulosa, asociadas en una estructura resistente, cuya ruptura requiere una cantidad considerable de energía. Sin embargo, gracias a su composición diferenciada, permiten obtener combustibles y múltiples productos químicos de alto valor.

La biomasa lignocelulósica se puede fraccionar en celulosa, hemicelulosa y lignina, a partir de las cuales se pueden producir azúcares, combustibles, materiales y productos químicos. Desde los residuos de elaboración se puede producir energía. La planta de la invención ofrece grandes mejoras en la extracción de hemicelulosa, una parte integral del proceso de biorrefinería.

Además la planta de la invención incluye numerosas innovaciones para la extracción de bioproductos a partir de biomasa, y permite:

- Extracción continua de biopolímeros y biocompuestos a partir de biomasa, con agua subcrítica (hasta 16 bar y 200 ºC).
- Intercambio rápido del sólido exhausto sin parar el proceso.
- Ahorro energético entre el 85% y el 95% mediante un sistema de intercambio de calor con aprovechamiento en producto y en las tomas de muestra.
- Posibilidad de operar con diferentes tipos de biomasa, y sin limitaciones debidas al tamaño de partículas.
- Facilidad y velocidad en la operación, contacto físico mínimo con el aparato.

La planta piloto multilecho es aplicable a las industrias de procesado de café soluble; sin embargo en dichas industrias se utiliza simplemente el extractor vacío, ya que unas

estructuras tubulares que incluyen dichas industrias son suficientemente grandes como para desalojar el sólido exhausto. En cambio, a nivel de planta piloto el material sólido se atasca y crea graves problemas para hacer posible una operación de extracción en continuo. Con la planta de la invención se logra cargar la biomasa en unos cartuchos que se insertan en caliente en cada reactor, de forma que una serie de válvulas logran aislar los lechos de modo que se pueda intercambiar la biomasa exhausta sin parar el proceso de fraccionamiento y extracción conseguido en la planta de la invención.

La planta piloto multilecho de la invención comprende unos reactores intercomunicados entre sí mediante un circuito de tuberías por donde fluye un fluido líquido impulsado por al menos una moto-bomba; donde los reactores incluyen unos cartuchos contenedores de la biomasa, una entrada de fluido líquido que recorre la biomasa contenida dentro de los cartuchos y una salida de fluido líquido que contiene diversas sustancias extraídas de la biomasa alojada dentro de los cartuchos.

La planta piloto de la invención comprende además unos reactores en disposición vertical que tienen la entrada de fluido líquido por encima del cartucho, y la salida de fluido líquido está situada en una parte inferior de los reactores.

En los extremos inferiores de los reactores conectan unos dispositivos de apertura y cierre de dichos reactores ubicados por debajo de los cartuchos; donde los cartuchos se introducen dentro de los reactores y se extraen del interior de dichos reactores a través de los dispositivos de apertura y cierre cuando se sitúan en una posición abierta.

El circuito de tuberías comprende unas partes de ida por las que discurre el fluido líquido dirigido hacia las entradas de fluido líquido dentro de los reactores, y unas partes de retorno por las que discurre el fluido liquido cuando sale de los reactores; donde en dichas partes del circuito de tuberías se intercalan unas válvulas que permiten aislar de forma

independiente los reactores para el intercambio de cartuchos sin detener el funcionamiento de la planta piloto.

Cada una de las partes de ida del circuito de tuberías incluye una primera válvula de tres vías y una segunda válvula de dos vías intercalada en una derivación que arranca de la entrada al reactor.

Cada una de las partes de retorno del circuito de tuberías incluye al menos una primera válvula de dos vías.

Una salida de la primera válvula de tres vías conecta con un tramo de tubería que alimenta al reactor; mientras que otra salida de la primera válvula de tres vías alimenta a un tramo intermedio que conecta con otra primera válvula de tres vías intercalada en otra parte de ida del circuito de tuberías.

La primera válvula de dos vías está intercalada en un tramo de tubería que arranca de la salida del reactor y conecta con dicho tramo intermedio.

La planta de la invención incluye una válvula de aguja de dos posiciones intercalada en un tramo extremo de tubería que conecta con el tramo de tubería de la salida del reactor y con un intercambiador de calor que conecta con una segunda válvula de tres vías que tiene una salida que conecta con un primer tubo para tomar muestras del fluido líquido, y una segunda salida que conecta con un segundo tubo.

Un tramo inicial del circuito de tuberías incluye unos intercambiadores de calor iniciales en combinación con un calentador principal ubicado a continuación de dichos intercambiadores de calor iniciales.

La planta piloto multilecho comprende una válvula de contrapresión para regular la presión dentro de los reactores; donde dicha válvula de contrapresión está ubicada en una derivación inicial de la parte de ida del circuito de tuberías.

La parte de retorno de un último reactor correspondiente con la salida de fluido líquido de dicho último reactor, conecta con una derivación de retroalimentación que desemboca en una zona inicial de la parte de ida del circuito de tuberías.

Cada uno de los reactores tiene una salida adicional superior donde conecta una derivación de tubería que desemboca en un contenedor; donde en dicha derivación de tubería está intercalada una tercera válvula de dos vías.

En una realización, cada uno de los dispositivos de apertura y cierre de los reactores comprende una válvula de esfera.

La planta piloto comprende unos dispositivos de paracaídas fijados a los dispositivos de apertura y cierre ubicados en los extremos inferiores de los reactores; donde dichos dispositivos de paracaíadas amortiguan la caída de los cartuchos cuando se extraen del interior de los reactores.

Los reactores están revestidos homogéneamente mediante unas resistencias envolventes para calentar dichos reactores y mantener la temperatura. Cada uno de los reactores de la planta comprende:

- una malla tubular que se llena con biomasa.
- un carcasa tubular interior que tiene una base inferior perforada y una base superior abierta; donde dentro de dicha carcasa tubular interior se ubica la malla tubular.
- una carcasa tubular exterior donde se aloja el cartucho constituido por la carcasa tubular interior y la malla tubular.

La malla tubular comprende dos medias partes desmontables que se acoplan entre sí a través de dos generatrices opuestas que siguen una trayectoria quebrada.

La carcasa tubular interior comprende dos partes separadas: una primera parte que incluye la base inferior perforada, y una segunda parte que incluye un estrechamiento donde se ubica la base superior abierta.

La carcasa tubular exterior de cada reactor comprende un cuerpo principal y una tapa que cierra el cuerpo principal por su extremo superior, mientras que su extremo inferior conecta con el dispositivo de apertura y cierre; donde la tapa incluye una boca de entrada de fluido líquido dentro del reactor; y donde el cuerpo principal incluye una boca de salida de fluido líquido y la salida adicional que conecta con la derivación de tubería que desagüa parte del fluido líquido en el contenedor.

En una realización de la invención, la malla tubular, carcasa tubular inferior y carcasa tubular exterior tienen una configuración cilíndrica y están constituidas por un material metálico.

Cada intercambiador de calor ubicado en la parte de retorno del circuito de tuberías, comprende dos tubos concéntricos: exterior e interior; donde desde el tubo interior se descarga el fluido líquido contenido en el reactor, enfriado por agua que fluye por el tubo exterior.

Cada uno de los dispositivos de paracaídas comprende una placa frontal que pende de al menos dos elementos de suspensión conectados a una parte del dispositivo de apertura y cierre; donde dicha placa frontal está ubicada por debajo del dispositivo de apertura y cierre para recibir el cartucho cuando se abre dicho dispositivo de apertura y cierre.

La planta piloto comprende una primera moto-bomba que se usa al principio del proceso para llenar los reactores antes de que el circuito de tuberías se abra para llevar a cabo el proceso de extracción y fraccionamiento en continuo de los compuestos solubles de la

biomasa residual y no residual. Dicha primera moto-bomba se alimenta de un fluido líquido limpio o no, contenido dentro de un primer depósito.

La planta piloto comprende además una segunda moto-bomba que impulsa el fluido líquido a través del circuito de tuberías para llevar a cabo el proceso de extracción y fraccionamiento en continuo de los compuestos solubles de la biomasa residual y no residual. Dicha segunda moto-bomba se alimenta de un fluido líquido contenido dentro de un segundo depósito.

El proceso para el fraccionamiento de biomasa llevado a cabo por la planta piloto multilecho descrita comprende las siguientes fases:

- calentar el fluido líquido mediante los intercambiadores de calor iniciales.
- calentar el fluido líquido mediante el calentador principal.
- introducir el fluido líquido dentro de los reactores por encima de los cartuchos;
- calentar los reactores mediante las resistencias envolventes ubicadas alrededor de los reactores.
- extraer el fluido líquido del interior de los reactores.
- variar la temperatura del fluido extraído de los reactores a través de los intercambiadores de calor.
- cambiar los cartuchos de los reactores por otros nuevos sin detener el proceso.
- tomar muestras del fluido extraído del interior de los reactores después de pasar por los intercambiadores de calor a través de los primeros tubos.
- recoger el fluido extraído del interior de los reactores después de pasar por los intercambiadores de calor, a través de los segundos tubos.
- regular la presión dentro de los reactores 1 mediante la válvula de contrapresión.

- canalizar parte del fluido líquido que se introduce dentro de los reactores hasta los contenedores; donde cuando se llenan dichos contendores se interrumpe el flujo de líquido hacia los contenedores.

En una realización el proceso comprende una fase adicional en la que se retorna el fluido líquido extraído del último reactor hacia la parte inicial del circuito de tuberías.

A continuación para facilitar una mejor comprensión de esta memoria descriptiva y formando parte integrante de la misma, se acompaña una serie de figuras en las que con carácter ilustrativo y no limitativo se ha representado el objeto de la invención.

Breve descripción de las figuras

Figura 1. Muestra una vista de la planta piloto multilecho para fraccionamiento de biomasa, objeto de la invención. Comprende un conjunto de reactores en combinación con otros elementos para llevar a cabo la extracción y el fraccionamiento en continuo de compuestos solubles. También es objeto de la invención el proceso para llevar a cabo el fraccionamiento de la biomasa.

Figura 2. Muestra una vista de una parte de la planta de la invención.

Figura 3. Muestra una vista en explosión de uno de los reactores junto con una válvula de esfera, que forma parte de la planta de la invención.

Figura 4. Muestra una vista en sección del reactor.

Figura 5. Muestra una vista en planta de una de las piezas del reactor.

Descripción de un ejemplo de realización de la invención

Considerando la numeración adoptada en las figuras, la planta piloto multilecho para fraccionamiento de biomasa comprende varios reactores 1 en disposición vertical intercomunicados entre sí mediante unas partes de ida por las que discurre un fluido líquido dirigido hacia unas entradas de fluido líquido de los reactores 1, y unas partes de retorno por las que discurre el fluido liquido cuando sale de los reactores 1; donde cada uno de los reactores trabaja en serie con los demás, con la posibilidad de poder ser excluido un reactor 1 de los demás; y donde dichas partes de ida y de retorno forman parte de un circuito de tuberías por el que fluye el fluido líquido.

Cada uno de los reactores 1 comprende:

- una malla tubular 2 de metal formada por dos medias partes 2a, 2b desmontables que se acoplan entre sí a través de dos generatrices opuestas que siguen una trayectoria quebrada; donde dicha malla tubular 2 se llena con biomasa.
- una carcasa tubular interior 3 de acero inoxidable con una base inferior perforada 4 y una base superior abierta 5; donde dentro de dicha carcasa tubular interior 3 se ubica la malla tubular 2; y donde dicha carcasa tubular interior 3 comprende dos partes separadas: una primera parte 3a que incluye la base inferior perforada 4 y una segunda parte 3b que incluye un estrechamiento 6 donde se ubica la base superior abierta 5.
- una carcasa tubular exterior 7 de acero inoxidable, donde se aloja el cartucho constituido por la carcasa tubular interior 3 y la malla tubular 2. Dicha carcasa tubular exterior 7 comprende un cuerpo principal 7a y una tapa 7b que cierre el cuerpo principal 7a por su extremo superior, mientras que su extremo inferior conecta con una válvula de esfera 9.

En la realización que se muestra en las figuras, la malla tubular 2, carcasa tubular inferior 3 y carcasa tubular exterior 7 tienen una configuración cilíndrica. Con esta disposición descrita, el conjunto del cartucho se inserta desde abajo dentro de la carcasa tubular exterior 7 a través de la válvula de esfera 9 cuando está en posición abierta hasta que se introduce completamente dentro de dicha carcasa tubular exterior 7.

En este momento la válvula de esfera 9 se cierra y al terminar la extracción haciendo pasar un fluido líquido por el interior del reactor 1, se procede a la apertura de la válvula de esfera 9 cayendo hacia abajo por gravedad el cartucho que se extrae hacia afuera, de manera que la salida del cartucho se amortigua mediante un dispositivo de paracaídas 10 ubicado en la zona de salida de la válvula de esfera 9.

Un flujo constante de agua (u otro fluido líquido adecuado) entra por una boca de entrada 11 ubicada en la parte superior de cada reactor 1 realizando un recorrido hacia abajo por el interior de dicho reactor 1 hasta que sale por una boca de salida 12 situada en la parte inferior del reactor 1; donde la boca de entrada 11 está situada en la tapa 7b de la carcasa tubular exterior 7 y la boca de salida 12 está situada en una parte inferior del cuerpo principal 7a de dicha carcasa tubular exterior 7.

El flujo líquido de salida puede ir al siguiente reactor 1, o puede ser desviado con el fin de excluir el siguiente reactor 1, y pasar al próximo reactor 1. De esta manera la biomasa puede ser descargada (a través de la apertura de la válvula de esfera 9 y la remoción rápida del cartucho) del reactor 1 en el que se completa el proceso de extracción, y otro reactor 1 se puede cargar con otro cartucho, luego desviar en ese reactor 1 el flujo líquido para continuar con el proceso de extracción. La planta de la invención permite un funcionamiento rápido y continuo sin paradas.

Otra característica importante de la planta de la invención, es el ajuste de la temperatura del agua y el control de energía. Para ello, antes de entrar en los reactores 1, el agua pasa a través de un calentador principal 13 formado por un serpentín en espiral enrollado alrededor de un cuerpo macizo metálico; donde dicho serpentín en espiral está cubierto por una resistencia eléctrica de abrazadera.

El agua entra a continuación en los reactores 1, que están revestidos homogéneamente con unas resistencias envolventes 14 de abrazadera. Después de dejar el reactor 1, el fluido líquido entra en un intercambiador de calor 15 de tubos concéntricos. En la parte exterior fluye el suministro de agua de la planta, que se calienta previamente a través de unos intercambiadores de calor iniciales 16 y luego pasa al calentador principal 13 descrito anteriormente. Todo el sistema está aislado térmicamente con una capa de lana de vidrio cubierta con papel de aluminio. El ahorro total de calor es del 85% y el ahorro de enfriamiento está cercano al 100%.

Cada reactor 1 puede ser vaciado del fluido líquido caliente presurizado antes de extraer el cartucho constituido por la malla tubular 2 y carcasa tubular interior 3. Entre cada par de reactores 1 adyacentes se sitúa el intercambiador de calor 15 con tubos concéntricos: exterior e interior; donde desde el tubo interior se descarga el líquido contenido en el reactor 1, enfriado por agua que fluye por el tubo exterior. Estos intercambiadores de calor 15 también permiten la toma de muestras de líquido de cada reactor durante la reacción del fluido líquido con la biomasa.

Dependiendo del compuesto que se desea extraer de los reactores, diferentes temperaturas son necesarias.

La extracción de los polifenoles requiere una temperatura entre 60 y 80 °C; la extracción de beta-glucanos alrededor de los 100 °C; mientras que la extracción de la hemicelulosa

requiere temperaturas entre 110 y 210 °C. Por tanto, es necesario un control de la temperatura eficaz, que se realiza por medio de unos dispositivos controladores PID.

Para alcanzar temperaturas por encima de 100 °C, es necesario aumentar también la presión dentro de los reactores 1, que se regula a través de una válvula de contrapresión 17 ubicada en una derivación inicial 18 del circuito de tuberías de la planta.

La planta incluye además válvulas de seguridad en cada reactor 1 que evitan cualquier sobrepresión. También se incluye un termostato que evita el sobrecalentamiento del cuerpo macizo metálico del calentador principal 13.

Para que la operación de extracción sea continúa, es necesario descargar rápidamente los reactores 1. Dado que el sistema de la planta de la invención está bajo presión, una apertura repentina de la válvula de esfera 9 podría causar una fuga de vapor de agua sobrecalentado. Por esta razón, entre un reactor y otro adyacente se ha colocado el correspondiente intercambiador de calor 15 con los tubos concéntricos: exterior e interior. Según se ha descrito anteriormente, desde el tubo interior se descarga el líquido contenido en el reactor 1 después de la operación de extracción, y por el tubo exterior fluye agua fría que luego alimenta a la planta de la invención.

Al principio del proceso, la biomasa se introduce dentro de la malla tubular 2, que se puede abrir longitudinalmente, y que se inserta entre las dos partes 3a, 3b de la carcasa tubular interior 3. La primera parte 3a está abierta por el extremo superior y por el extremo inferior tiene la base inferior perforada 4 con orificios de diámetro entre 5 mm y 0.5 mm. Según se ha referido anteriormente, el conjunto de la malla tubular 2 y de la carcasa tubular interior 3 se inserta, a través de la válvula de esfera 9, dentro de la carcasa tubular exterior 7 cerrada por la extremidad superior mediante la tapa 8 que se puede remover en el caso de que se atasque el reactor 1.

El tamaño de las partículas de biomasa cargadas en el reactor 1, tiene que ser superior al tamaño del diámetro de los orificios de la base inferior perforada 4 de la carcasa tubular interior 3, que funciona como un filtro impidiendo que la biomasa solida sea removida del lecho y arrastrada a través de los distintos elementos de la planta de la invención.

En una realización como la mostrada en las figuras 2 y 3, la planta de la invención comprende una unidad operativa de reactor 1 donde se lleva a cabo el proceso para el fraccionamiento de la biomasa.

En cambio, en la figura 1, se muestra una planta que incluye varias unidades de reactores conectados, que pueden operar en serie uno con otro, o por separado, y donde también se lleva a cabo el proceso para el fraccionamiento de la biomasa.

Una vez que un reactor 1 se ha cargado con la biomasa, como se ha descrito anteriormente, se empuja el cartucho hasta que entra completamente, y posteriormente se cierra la válvula de esfera 9 situada por debajo del cartucho. En este momento, se llena el reactor 1 con agua, impulsada mediante una primera moto-bomba 19, asegurándose de que el líquido entra en el reactor 1 y no se escapa a través del circuito de tuberías.

Por esta razón, se aisla cada reactor 1 del resto del sistema de la planta piloto mediante el cierre de una primera válvula 20 de dos vías y una segunda válvula de aguja 21 situadas a la salida de cada reactor 1. También se abre una segunda válvula 22 de dos vías y se mueve una primera válvula 23 de tres vías, de manera tal que el agua entre sólo en un reactor 1, y en el segmento de tubo entre el reactor y dicha primera válvula 23 de tres vías.

La primera válvula 23 de tres vías puede desviar el flujo hacia el siguiente reactor 1 o hacia la siguiente primera válvula 23 de tres vías, evitando el reactor 1 anterior.

Una tercera válvula 24 de dos vías conectada a una salida adicional superior 25 de cada reactor 1, se mantiene abierta hasta que se comprueba que un contenedor 26 empieza a llenarse de fluido líquido a través de una derivación de tubería que arranca de dicha tercera válvula 24 y desemboca en el contenedor 26. En ese momento se cierran las válvulas de dos vías 22 y 24, y se desconecta la primera moto-bomba 19 interrumpiendo el flujo de fluido líquido hacia el reactor 1.

En esta situación, la biomasa contenida dentro del reactor 1 está completamente sumergida en el fluido líquido, y el reactor 1 está aislado del sistema de la planta piloto.

El reactor 1 se calienta homogéneamente mediante las resistencias envolventes 14 hasta una temperatura ligeramente inferior a la temperatura mínima que se necesita para empezar la extracción del compuesto que se quiere extraer.

La hemicelulosa empieza a extraerse a temperaturas superiores a 100 ºC; de manera que si se decide extraer dicho compuesto de hemicelulosa hay que precalentar el reactor 1 y esperar a que el reactor 1 alcance temperaturas superiores a 100 ºC.

Una segunda moto-bomba 27 se activa para impulsar el flujo de líquido que pasa previamente a través de los intercambiadores de calor iniciales 16, funcionando como un liquido de enfriamiento de otro líquido caliente que sale del sistema, y al mismo tiempo consiguiendo un primer precalentamiento. Consecutivamente consigue un ulterior precalentamiento, mediante el calentador principal 13, hacia una temperatura superior a la cual se requiere operar; siguiendo el fluido líquido después hacia la primera válvula 23 de tres vías.

Como se ha referido anteriormente, en una realización, dicha primera válvula 23 de tres vías sigue colocada en una posición en la que el flujo de líquido es desviado hacia la siguiente primera válvula 23 de tres vías, y no hacia el reactor 1.

Cuando el flujo alcanza la temperatura requerida, se sube la temperatura del reactor hacia las condiciones con las cuales se quiere operar; ajustándose la primera válvula 23 de tres vías, de manera que el flujo de líquido entre en el reactor 1, y al mismo tiempo se abre la primera válvula 20 de dos vías para que el líquido pueda salir del reactor 1. En este momento empieza la extracción de los compuestos solubles desde la biomasa en el reactor 1: el flujo de líquido entra en el reactor 1, pasa a través del lecho de biomasa, extrae los compuestos solubles, y sale del reactor.

El proceso descrito hasta ahora, se refiere básicamente al funcionamiento de una sola unidad de reactor 1.

El flujo de líquido que sale del reactor 1 llega a otra primera válvula 23 de tres vías, de manera que actuando sobre esta primera válvula 23 de tres vías se puede dirigir el flujo al siguiente reactor 1 o hacia la siguiente primera válvula 23 de tres vías. Esta operación se puede repetir para cada unidad de reactor en el sistema de la planta piloto. De esta manera, cada reactor 1 se puede integrar en el proceso de extracción, o se puede omitir.

Después de salir del último reactor 1 utilizado en el sistema de la planta piloto, la corriente de líquido caliente es devuelta y entra en la parte interior de los intercambiadores de calor iniciales 16 transfiriendo calor al líquido alimentado en el sistema. La salida de líquido, por lo tanto se enfría, y se despresuriza a través de la válvula de contrapresión 17, y finalmente sale del sistema.

La válvula de contrapresión 17 regula la presión de todo el sistema de extracción, de manera que cuando se desea interrumpir la reacción en un reactor 1, se desvía el flujo de líquido, bloqueando el suministro a la unidad de reactor 1 correspondiente para ser aislado del sistema.

Para ello, se gira la primera válvula 23 de tres vías que precede al respectivo reactor 1 y se cierra la primera válvula 20 de dos vías colocada a la salida de dicho reactor 1, excluyendo así el correspondiente reactor 1 del sistema.

El flujo de líquido puede ser dirigido a la unidad de reactor 1 subsiguiente (cargado con un cartucho de biomasa, como se ha explicado anteriormente), o puede ser dirigido a la primera válvula 23 de tres vías siguiente, que a su vez puede dirigir el flujo.

La válvula de aguja 21 tiene una salida que desemboca en el intercambiador de calor 15, de manera que cuando se abre dicha válvula de aguja 21, el líquido contenido en el reactor 1 pasa al intercambiador de calor 15 y se enfría mediante otro líquido que fluye en la parte exterior del intercambiador de calor 15. De esta manera, el líquido presurizado contenido dentro del reactor no se vaporiza durante la apertura de la válvula de aguja 15, y sale como un líquido normal sin presión.

Este sistema de la planta piloto permite vaciar completamente cada reactor del líquido, una vez que se termina el proceso de extracción.

A la salida del intercambiador de calor 15 se conecta una segunda válvula 28 de tres vías que permite dirigir el líquido que sale hacia un primer tubo 29 más corto para tomar una muestra del producto, o hacia un segundo tubo 30 más largo conectado a un sistema de desagüe.

Después de vaciar el reactor 1 del líquido, se abre la válvula de esfera 9 por debajo del reactor 1, haciendo que el cartucho que contiene la biomasa exhausta, caiga en el dispositivo de paracaídas 10. En esta situación, el cartucho puede ser retirado y abierto, de manera que la mala tubular 2 se extrae del interior de la carcasa tubular interior 3 y

posteriormente se abre la malla tubular 2 longitudinalmente, para que se pueda recoger toda la biomasa en estado sólido.

Cuando un reactor termina su funcionamiento, y se extrae el cartucho, otro cartucho contenedor de la biomasa fresca se puede volver a cargar rápidamente, reiniciando el proceso de extracción en el nuevo cartucho montado.

La parte de retorno del último reactor 1 correspondiente con la salida de fluido líquido de dicho último reactor 1, conecta con una derivación de retroalimentación 8 que desemboca en una zona inicial de la parte de ida del circuito de tuberías.

La primera moto-bomba 19 se usa al principio del proceso para llenar los reactores 1 antes de que el circuito de tuberías se abra para llevar a cabo el proceso de extracción y fraccionamiento en continuo de los compuestos solubles de la biomasa residual y no residual. Dicha primera moto-bomba 19 se alimenta de un fluido líquido limpio o no, contenido dentro de un primer depósito 31.

En cambio la segunda moto-bomba 27 impulsa el fluido líquido a través del circuito de tuberías para llevar a cabo el proceso de extracción y fraccionamiento en continuo de los compuestos solubles de la biomasa residual y no residual. Dicha segunda moto-bomba 27 se alimenta de un fluido líquido contenido dentro de un segundo depósito 32.

Por tanto las dos moto-bombas 19, 27 se alimentan de forma independiente de fluidos líquidos contenidos dentro de los depósitos 31, 32; que en una realización de la invención dichos fluidos líquidos son agua.

Cada uno de los dispositivos de paracaídas 10 comprende una placa frontal 10a de metal que pende de dos elementos de suspensión 10b conectados a una parte de la válvula de esfera 9; donde dicha placa frontal 10a está ubicada por debajo de dicha válvula de esfera

9. En una realización de la invención los elementos de suspensión 10b comprenden unas cadenas y en otra realización comprenden por ejemplo unos tirantes.

Con esta disposición descrita, la placa frontal 10a se puede mover hacia adelante o hacia atrás para permitir la entrada y salida del cartucho. En el momento de remoción del cartucho, la placa frontal 10a se posiciona justo por debajo de la válvula de esfera 9 para recibir el cartucho que cae hacia abajo por gravedad directamente sobre dicha placa frontal 10a.

Reivindicaciones

1. Planta piloto multilecho para fraccionamiento de biomasa, que comprende unos reactores intercomunicados entre sí mediante un circuito de tuberías por donde fluye un fluido líquido impulsado por al menos una moto-bomba; donde los reactores incluyen unos cartuchos contenedores de la biomasa, una entrada de fluido líquido que recorre la biomasa contenida dentro de los cartuchos y una salida de fluido líquido que contiene diversas sustancias extraídas de la biomasa alojada dentro de los cartuchos; caracterizada por qué:

- comprende unos reactores (1) en disposición vertical que tienen la entrada de fluido líquido por encima de los cartuchos, y la salida de fluido líquido está situada en una parte inferior de los reactores (1);
- en los extremos inferiores de los reactores (1) conectan unos dispositivos de apertura y cierre de dichos reactores (1) ubicados por debajo de los cartuchos; donde los cartuchos se introducen dentro de los reactores (1) y se extraen del interior de dichos reactores a través de los dispositivos de apertura y cierre cuando se sitúan en una posición abierta;

- el circuito de tuberías comprende unas partes de ida por las que discurre el fluido líquido dirigido hacia las entradas de fluido líquido dentro de los reactores (1), y unas partes de retorno por las que discurre el fluido liquido cuando sale de los reactores 1; donde en dichas partes del circuito de tuberías se intercalan unas válvulas que permiten aislar de forma independiente los reactores (1) para el intercambio de cartuchos sin detener el funcionamiento de la planta piloto.

2. Planta piloto multilecho para fraccionamiento de biomasa, según la reivindicación 1, caracterizada por qué:

- cada una de las partes de ida del circuito de tuberías incluye una primera válvula (23) de tres vías y una segunda válvula (22) de dos vías intercalada en una derivación que arranca de la entrada al reactor;
- cada una de las partes de retorno del circuito de tuberías incluye al menos una primera válvula (20) de dos vías;

donde,

- una salida de la primera válvula (23) de tres vías conecta con un tramo de tubería que alimenta al reactor (1); mientras que otra salida de la primera válvula (23) de tres vías alimenta a un tramo intermedio que conecta con otra primera válvula (23) de tres vías intercalada en otra parte de ida del circuito de tuberías;
- la primera válvula (20) de dos vías está intercalada en un tramo de tubería que arranca de la salida del reactor (1) y conecta con dicho tramo intermedio.

3. Planta piloto multilecho para fraccionamiento de biomasa, según la reivindicación 1, caracterizada por que incluye una válvula de aguja (21) de dos posiciones intercalada en un tramo extremo de tubería que conecta con el tramo de tubería de la salida del reactor (1) y con un intercambiador de calor (15) que conecta con una

segunda válvula (28) de tres vías que tiene una salida que conecta con un primer tubo (29) para tomar muestras del fluido líquido, y una segunda salida que conecta con un segundo tubo (30).

4. Planta piloto multilecho para fraccionamiento de biomasa, según la reivindicación 2, caracterizada por que un tramo inicial del circuito de tuberías incluye unos intercambiadores de calor iniciales (16) en combinación con un calentador principal (13) ubicado a continuación de dichos intercambiadores de calor iniciales (16).
5. Planta piloto multilecho para fraccionamiento de biomasa, según la reivindicación 1, caracterizada por que comprende una válvula de contrapresión (17) para regular la presión dentro de los reactores (1); donde dicha válvula de contrapresión (17) está ubicada en una derivación inicial (18) de la parte de ida del circuito de tuberías.
6. Planta piloto multilecho para fraccionamiento de biomasa, según la reivindicación 1, caracterizada por que la parte de retorno de un último reactor (1) correspondiente con la salida de fluido líquido de dicho último reactor (1), conecta con una derivación de retroalimentación (8) que desemboca en una zona inicial de la parte de ida del circuito de tuberías.
7. Planta piloto multilecho para fraccionamiento de biomasa, según la reivindicación 1, caracterizada por que cada uno de los reactores (1) tiene una salida adicional superior (25) donde conecta una derivación de tubería que desemboca en un contenedor (26); donde en dicha derivación de tubería está intercalada una tercera válvula (24) de dos vías.

8. Planta piloto multilecho para fraccionamiento de biomasa, según la reivindicación 1, caracterizada por que cada uno de los dispositivos de apertura y cierre de los reactores (1) comprende una válvula de esfera (9).
9. Planta piloto multilecho para fraccionamiento de biomasa, según la reivindicación 1, caracterizada por que comprende unos dispositivos de paracaídas (10) fijados a los dispositivos de apertura y cierre ubicados en los extremos inferiores de los reactores (1); donde dichos dispositivos de paracaíadas (10) amortiguan la caída de los cartuchos cuando se extraen del interior de los reactores (1).
10. Planta piloto multilecho para fraccionamiento de biomasa, según la reivindicación 1, caracterizado por que los reactores (1) están revestidos homogéneamente mediante unas resistencias envolventes (14) para calentar dichos reactores (1) y mantener la temperatura.
11. Planta piloto multilecho para fraccionamiento de biomasa, según la reivindicación 1, caracterizado por que cada uno de los reactores (1) comprende:

- una malla tubular (2) que se llena con biomasa;
- un carcasa tubular interior (3) que tiene una base inferior perforada (4) y una base superior abierta (5); donde dentro de dicha carcasa tubular interior (3) se ubica la malla tubular (2);
- una carcasa tubular exterior (7) donde se aloja el cartucho constituido por la carcasa tubular interior (3) y la malla tubular (2).

12. Planta piloto multilecho para fraccionamiento de biomasa, según la reivindicación 11, caracterizada por que la malla tubular (2) comprende dos medias partes (2a, 2b) desmontables que se acoplan entre sí a través de dos generatrices opuestas que siguen una trayectoria quebrada.

13. Planta piloto multilecho para fraccionamiento de biomasa, según la reivindicación 11, caracterizada por que la carcasa tubular interior (3) comprende dos partes separadas: una primera parte (3a) que incluye la base inferior perforada (4), y una segunda parte (3b) que incluye un estrechamiento (6) donde se ubica la base superior abierta (5).
14. Planta piloto multilecho para fraccionamiento de biomasa, según las reivindicaciones 7 y 11, caracterizada por que la carcasa tubular exterior (7) de cada reactor (1) comprende un cuerpo principal (7a) y una tapa (7b) que cierra el cuerpo principal (7a) por su extremo superior, mientras que su extremo inferior conecta con el dispositivo de apertura y cierre; donde la tapa (7b) incluye una boca de entrada (11) de fluido líquido dentro del reactor (1); y donde el cuerpo principal (7a) incluye una boca de salida (12) de fluido líquido y la salida adicional (25) que conecta con la derivación de tubería que desagüa parte del fluido líquido en el contenedor (26).
15. Planta piloto multilecho para fraccionamiento de biomasa, según la reivindicación 11, caracterizada por que la malla tubular (2), carcasa tubular inferior (3) y carcasa tubular exterior (7) tienen una configuración cilíndrica y están constituidas por un material metálico.
16. Planta piloto multilecho para fraccionamiento de biomasa, según la reivindicación 3, caracterizada por que cada intercambiador de calor (15), ubicado en la parte de retorno del circuito de tuberías, comprende dos tubos concéntricos: exterior e interior; donde desde el tubo interior se descarga el fluido líquido contenido en el reactor (1), enfriado por agua que fluye por el tubo exterior.

17. Planta piloto multilecho para fraccionamiento de biomasa, según la reivindicación 9, caracterizada por que cada uno de los dispositivos de paracaídas (10) comprende una placa frontal (10a) que pende de al menos dos elementos de suspensión (10b) conectados a una parte del dispositivo de apertura y cierre; donde dicha placa frontal (10a) está ubicada por debajo del dispositivo de apertura y cierre.
18. Planta piloto multilecho para fraccionamiento de biomasa, según la reivindicación 1, caracterizada por que comprende:

- una primera moto-bomba (19) para llenar los reactores (1); donde dicha primera motobomba (19) se alimenta de un fluido líquido contenido dentro de un primer depósito (31);
- una segunda moto-bomba (27) que impulsa el fluido líquido a través del circuito de tuberías; donde dicha segunda moto-bomba (27) se alimenta de un fluido líquido contenido dentro de un segundo depósito (32).

19. Proceso para el fraccionamiento de biomasa, llevado a cabo por la planta piloto multilecho descrita en una las reivindicaciones anteriores 1 a 18, caracterizado por que comprende las fases:

- calentar el fluido líquido mediante los intercambiadores de calor iniciales (16);
- calentar el fluido líquido mediante el calentador principal (13);
- introducir el fluido líquido dentro de los reactores (1) por encima de los cartuchos;
- calentar los reactores (1) mediante las resistencias envolventes (14) dispuestas alrededor de los reactores (1);
- extraer el fluido líquido del interior de los reactores (1);

- variar la temperatura del fluido extraído de los reactores (1) a través de los intercambiadores de calor (15);
- cambiar los cartuchos de los reactores (1) por otros nuevos sin detener el proceso;
- tomar muestras del fluido extraído del interior de los reactores después de pasar por los intercambiadores de calor (15) a través de los primeros tubos (29);
- recoger el fluido extraído del interior de los reactores (1) después de pasar por los intercambiadores de calor (15), a través de los segundos tubos (30);
- regular la presión dentro de los reactores (1) mediante la válvula de contrapresión;
- canalizar parte del fluido líquido que se introduce dentro de los reactores (1) hasta los contenedores (26); donde cuando se llenan dichos contendores (26) se interrumpe el flujo de líquido hacia los contenedores (26).

20. Proceso para el fraccionamiento de biomasa, según la reivindicación 19, caracterizado por que comprende una fase adicional de retornar del fluido líquido extraído del último reactor (1) hacia la parte inicial del circuito de tuberías.

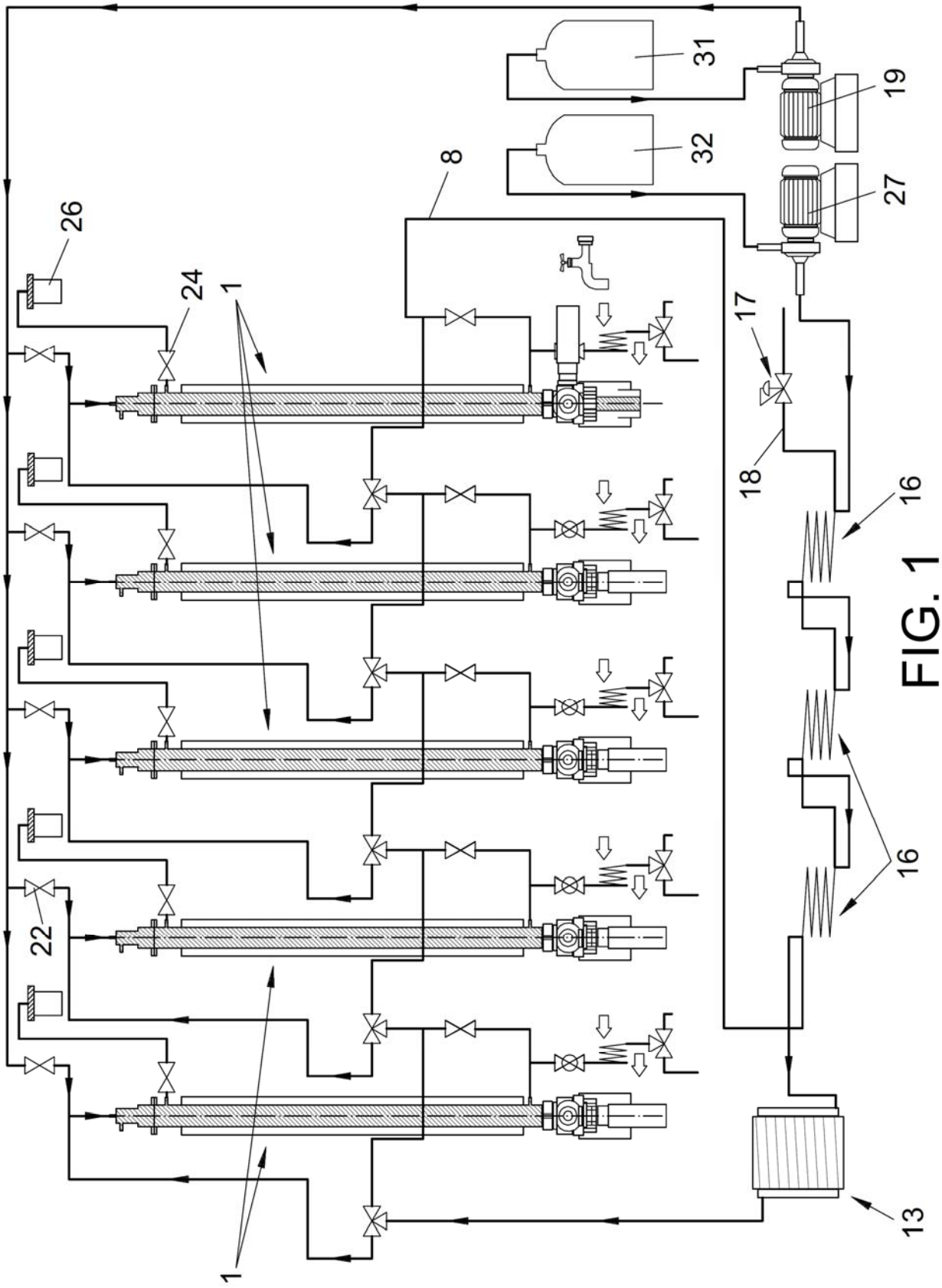

FIG. 1

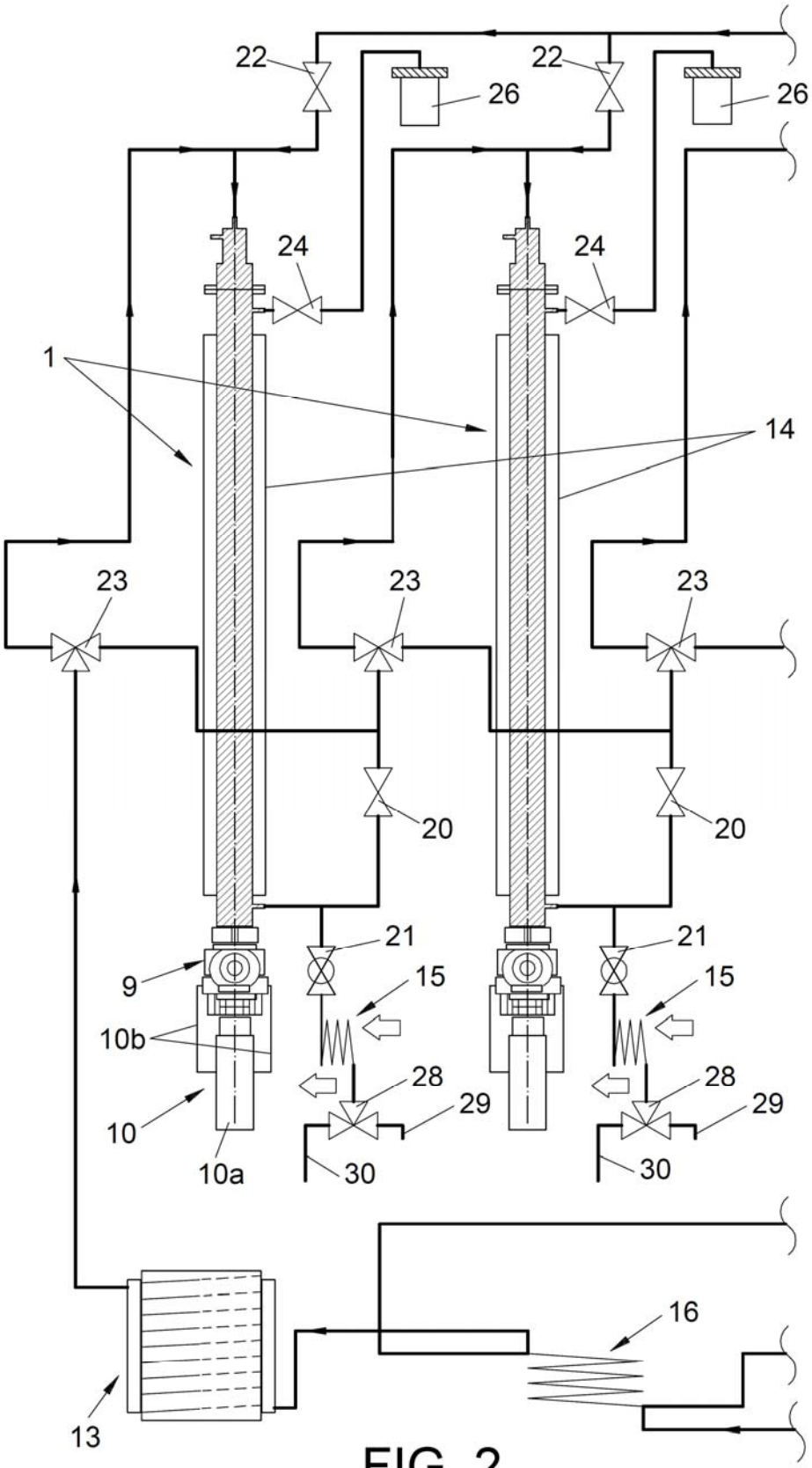
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FIG. 2

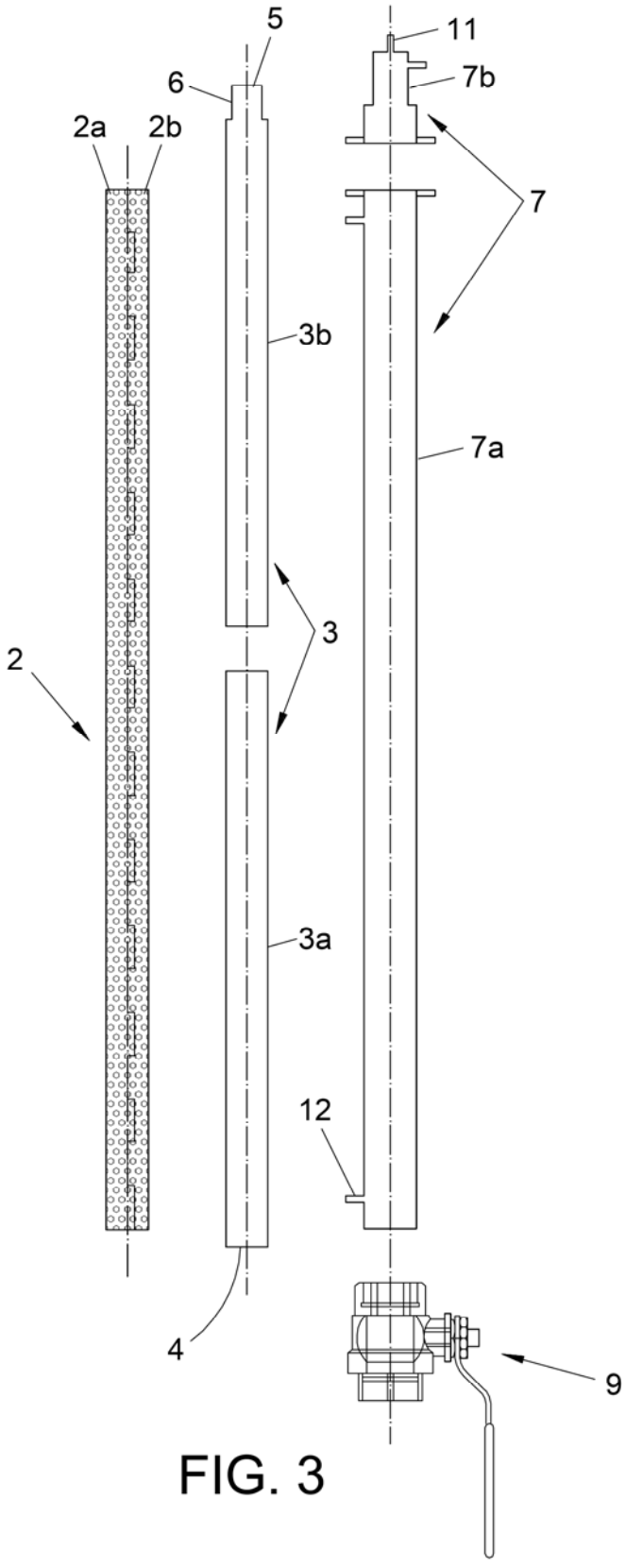


FIG. 3

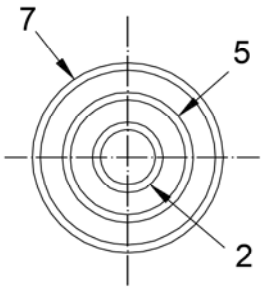


FIG. 4

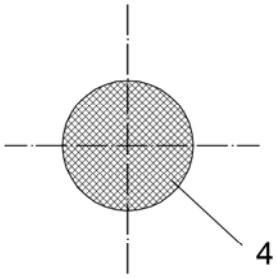


FIG. 5

CHAPTER 6

BUISNESS PLAN.

SWEETGREEN, EL EDULCORANTE PERFECTO.

CHAPTER VI

SWEETGREEN, EL EDULCORANTE PERFECTO.

Abstract

With Sweet Green we intend to introduce a natural xylitol sweetener to the market. Xylitol is a product with the same flavor as sugar, with fewer calories, with beneficial effects for oral hygiene, which helps reduce problems of obesity and diabetes without the drawbacks of artificial sweeteners known so far.

Sweet Green-xylitol is manufactured with a new technology, patented by us, that uses only natural resources (water and biomass), making the product greener than existing sweeteners.

1. IDEA Y PROPUESTA DE VALOR

Con Sweet Green pretendemos introducir en el mercado un edulcorante natural a base de xilitol. El xilitol es producto con el mismo sabor que el azúcar, con menos calorías, con efectos benéficos para la higiene bucodental, que ayuda a reducir problemas de obesidad y diabetes sin los inconvenientes de los edulcorantes artificiales conocidos hasta el momento.

Sweet Green-xilitol se fabrica con una nueva tecnología, patentada por nosotros, que emplea solo recursos naturales, haciendo el producto más ecológico y más económico respecto a los edulcorantes existentes.

2. DESCRIPCIÓN DEL MODELO DE NEGOCIO

Actualmente, el azúcar de siempre (de remolacha o de caña) no está muy de moda. Esto queda demostrado, por ejemplo, en el libro de recetas de Sarah Wilson, The I Quit Sugar

Cookbook [1], que salió en 2015 y se convirtió en best-seller del New York Times. Y se confirma también por el hecho que el porcentaje de consumidores que buscan alternativas al azúcar está aumentando bruscamente: ahora es la era de "otros" edulcorantes naturales. No sólo ricos en sabor, o con sabores distintivos, sino que se busca una dulzura nutritiva, en línea con el deseo de calidad y excelencia también en términos de salud.

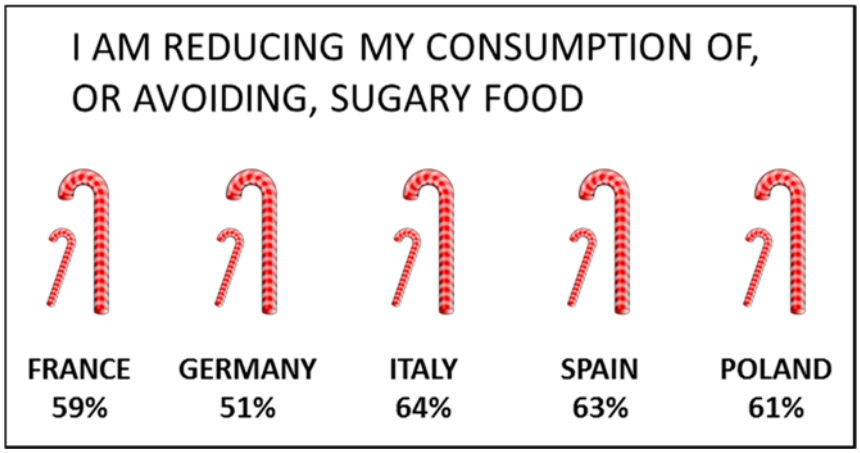


Figura 1: Tendencia hacia la reducción de la utilización de azúcar en Europa [2].

De hecho, la principal razón del éxito del mercado de edulcorantes alternativos es el crecimiento de la conciencia de salud entre los consumidores. La potencia y eficacia de los sucedáneos del azúcar para mantener el peso corporal y para controlar el nivel de azúcar en la sangre ayuda a aumentar la demanda de edulcorantes.

El aumento de la tasa de obesidad y otros problemas de salud como la diabetes, el sobrepeso olas enfermedades cardiovasculares, están impulsando al consumo de edulcorantes alternativos a nivel mundial.

El crecimiento se atribuye principalmente a la creciente demanda de alimentos y bebidas saludables, bajas en calorías y sin azúcar.

Uno de los edulcorantes naturales más interesante se llama **xilitol**: un carbohidrato natural que tiene el mismo sabor y apariencia que el azúcar de mesa regular. Está disponible extensamente en la naturaleza, ya que se puede obtener de cualquier materia vegetal.

La diferencia del xilitol en comparación con el azúcar regular y todos los demás edulcorantes existentes en el mercado, se encuentra en sus propiedades únicas.

- Es un azúcar con poder dulcificante muy parecido al de la sacarosa (azúcar de mesa), mismo sabor, pero con un 40% de calorías menos.

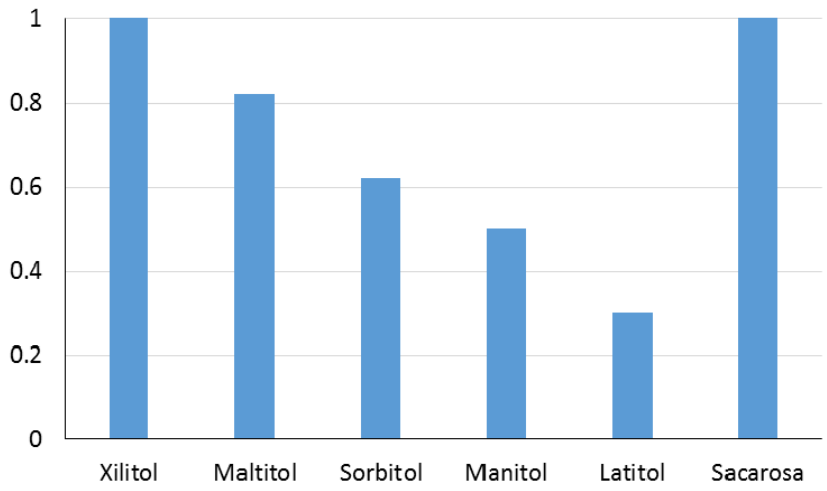


Figura 2: Poder edulcorante de diferentes productos dulcificantes

- En comparación con otros edulcorantes como el azúcar de mesa, el xilitol tiene una acción muy débil sobre los niveles de glucosa en la sangre y la insulina. El índice glucémico del xilitol es 7, mientras que el del azúcar refinado es 60-70. El xilitol, desde el punto de vista del índice glucémico, representa una alternativa al azúcar

refinado adecuado para aquellos en riesgo de diabetes y aquellos que sufren de obesidad y problemas metabólicos.

- El xilitol tiene beneficios para la salud de los dientes y la boca. Entre las bacterias responsables de la placa encontramos *Streptococcus mutans*. Esta bacteria se alimenta de glucosa en la boca gracias a los alimentos, pero no puede usar xilitol. Así que reemplazar el azúcar con xilitol podría ayudar a mantener esta bacteria fuera de juego. El xilitol, sustituyendo al azúcar e introducido en la dieta, puede reducir las caries dentales y disminuir la caída de dientes de un 80 a un85%.

SWEET GREEN, el producto fabricado por nosotros, no es solamente un edulcorante a base de xilitol; su producción se lleva a cabo a través de un proceso real de biorefinería, una instalación que integra procesos de conversión de biomasa para producir combustibles, energía y productos químicos.

Al producir múltiples productos, una biorefinería aprovecha los varios componentes de biomasa y sus intermediarios, maximizando así el valor derivado de la biomasa utilizada como materia prima.

Desde 2014, en el grupo de Procesos a Alta Presión de la Universidad de Valladolid, dirigido por la Catedrática Mª José Cocero Alonso, estamos trabajando en la creación de una biorefinería en la cual se utilicen residuos agrícolas y forestales para crear productos con valor añadido.

En la tesis doctoral de Gianluca Gallina, en particular, se ha diseñado y construido un sistema a escala piloto que permite procesar biomasa procedente de madera de poda, pulpa de remolacha, residuos del vino y otros deshechos agrícolas y forestales, para producir compuestos de alto valor añadido.

El producto más valioso existente en el mercado, que se puede fabricar con esta tecnología, es el xilitol.

Los sistemas industriales existentes para la finalidad descrita, utilizan productos químicos como ácidos o bases, con la necesidad de purificar el producto y de eliminar los residuos tóxicos. Otro punto crítico viene de la materia prima que se utiliza normalmente para la producción de xilitol; las biomasas más utilizadas para su producción son:

- salvado de trigo cultivado en China, derivado de plantaciones GM (genéticamente modificadas) que ocupan millones de hectáreas y consumen enormes cantidades de agua, contribuyendo masivamente a la desertificación;

- abedules procedentes de países escandinavos, plantados sólo para ser cortados y procesados para producir xilitol.

Nuestro sistema se diferencia de los demás en cuanto a que:

- utiliza solamente agua como disolvente, reduciendo el impacto medioambiental y minimizando los costes asociados con la eliminación de residuos tóxicos;
- utiliza residuos agrícolas y forestales proporcionados por agricultores conocidos y localizados en el territorio;
- es capaz de trabajar de manera continua, rápida y económica.

La tecnología pudo ser patentada gracias al programa Prometeo 2016 convocado por la Fundación General de la Universidad de Valladolid.

El programa tiene entre sus objetivos fomentar la colaboración entre agentes generadores de conocimiento y especialistas en la transferencia de conocimiento. Como valor añadido, persigue generar una inercia en el proceso de registro como

propiedad industrial de productos tecnológicos desarrollados por los participantes, dotándolos así de una protección jurídica cara a su posible explotación comercial.

Posteriormente el proyecto fue premiado con el Primer Premio VIVERO 2016 organizado por la Fuescyl.

El concurso tiene por objeto formar emprendedores y nutrir una bolsa de promotores empresariales universitarios (PEU) que ayuden a crear nuevas empresas de base tecnológica a partir de los resultados de los proyectos de investigación y tecnologías de las universidades de Castilla y León.

Al primer y al segundo clasificado se entregaron dos premios con las siguientes características:

- Ayuda, valorada en 6.000 euros, para desarrollar una prueba de concepto, un prototipo o un producto piloto que permita chequear la viabilidad práctica de la nueva idea empresarial y/o realizar un primer test de mercado.
- Dotación económica de hasta 6.000 euros para la constitución del capital social de la nueva empresa.

2.1 Nuestros clientes

Los *clientes potenciales* a los cuales nos dirigimos son los mayores consumidores de azúcar en España y fabricantes de repostería, bebidas, golosinas y chicles. Promoviendo las propiedades únicas de nuestro producto, esperamos empujar a nuestros clientes a utilizar nuestro edulcorante en sustitución de los demás.

Habiendo analizado el mercado existente, a continuación detallamos algunas de las empresas a las que nuestro producto les podría interesar, y por lo tanto, convertirse en potenciales clientes:

FINI 

http://www.fini.es/es/empresa

Fini Golosinas es la mayor empresa española en la fabricación y distribución de golosinas. Marca líder en España en caramelo de gelatina, marshmallow y regaliz. Es la compañía confitera que más crece, hasta un 49,1%, en 2015. Fini es líder del sector gracias a sus productos únicos y transgresores, reconocibles por los consumidores de todas las edades.

Helios 

http://www.heliosesvida.es

El grupo Helios, con sede en Valladolid, se ha situado como líder de mermeladas en España y desarrolla continuamente nuevos productos. Está formado por diferentes empresas, ocho centros productivos, oficinas comerciales, en países como España, Alemania, Francia y Reino Unido.

AMC Group 

http://www.amcgrupo.eu/

AMC es un grupo empresarial dedicado al sector de la alimentación en el mercado global, enfocado a la Marca de la Distribución, con actividad en zumos, smoothies y bebidas naturales de fruta. El Grupo AMC alcanzó una cifra de negocios consolidada de 1.131 millones de euros en el ejercicio 2016, mostrando un crecimiento del 14% en el volumen

de sus operaciones respecto al ejercicio anterior. En su política tienen un compromiso con la salud y con el medioambiente.

Reina

https://postresreina.com/

Industria de chocolates, turrones, productos de pastelería, dulces de navidad y postres lácteos. La empresa ha conseguido ser líder en su sector, incrementando su producción día a día, realizando, en la actualidad, más de 1.500.000 de postres diarios. Dicen de ellos: “El respeto por el medio ambiente forma parte de nuestro concepto empresarial”.

Juver

http://www.juver.com/

Productora de zumos, la marca Juver proviene del nombre del propio fundador (Juan Valverde), y desde sus inicios ha estado relacionada con un estilo de vida saludable. Consolidarse como una empresa ambientalmente responsable, ha sido desde siempre uno de los objetivos estratégicos de Juver.

Sin embargo, siendo conscientes de la limitada producción que inicialmente podremos desarrollar, durante el primer año te actividad los *clientes reales* hacia los que nos dirigiremos serán pequeñas pastelerías, herboristerías y tiendas ecológicas cuyas necesidades de este producto son más limitadas y por lo tanto, nos permitirían atender sus demandas con la producción actual.

Durante el segundo año se construirá una planta industrial capaz de satisfacer la demanda de las grandes industrias nombradas antecedentemente.

3. DESCRIPCIÓN DEL EQUIPO PROMOTOR

SWEET GREEN va a estar constituida en un primer momento por dos socios: Gianluca Gallina y Juan García Serna. Los dos son autores de la patente que van a explotar y tienen un perfil científico e ingenieril adapto al alto nivel tecnológico de la empresa.

Sweet Green es una empresa que nace del Grupo de Alta Presión que forma parte del Departamento de Ingeniería Química de la Universidad de Valladolid en el que María José Cocero Alonso, catedrática de la Universidad de Valladolid, ha liderado el grupo de Alta Presión en los últimos 19 años.

A continuación, se va a detallar el cv de los fundadores de la empresa.

Juan Garcia Serna

Asesor i+D

Gianluca Gallina

Desarrollo Producto

Juan Garcia Serna (Palencia-España, 1977). Ingeniero Químico (2000), doctor en Ingeniería Química (2005) por la Universidad de Valladolid y Profesor Titular de Universidad del Departamento de Ingeniería Química y Tecnología del Medio Ambiente (2010).

Realizó la formación postdoctoral en la universidad de Abo Akademi en Turku (2013), con beca Salvador de Madariaga. Ha trabajado como Ingeniero de Procesos en la empresa Técnicas Reunidas (2000-2002) en diseño de procesos para industria petroquímica.

Tiene experiencia investigadora en desarrollo de procesos sostenibles con reacción, modelado cinético, fraccionamiento de biomasa y procesos de repolimerización con CO_2.

Ha dirigido 5 tesis doctorales y está dirigiendo a 4 estudiantes de doctorado. Presenta índice Hirsch = 17, habiendo publicado 55 artículos en revistas indexadas y participado con más de 70 comunicaciones a congresos.

Ha sido IP en 3 proyectos nacionales, 1 regional y contratos artículos 83 por un valor cercano a 0.3 millones €, destacando la colaboración con REPSOL para mejora del proceso de oxidación húmeda de la planta de REPSOL química en Tarragona.

Ha participado en varios proyectos europeos SHYMAN y WINESENSE entre otros. Es coordinador de Prácticas en Empresa (2015) y coordinador del Máster en Ingeniería Química (2016). Es experto en Ingeniería de Procesos y de Proyectos.

Gianluca Gallina, (Montebelluna-Italia, 1987) inició los estudios de Ingeniería Química en la Universidad de Padua en 2006 y se graduó en 2011.

En junio de 2013 trabajó en la Universidad Åbo Akademi en Turku (Finlandia). La experiencia ha sido muy interesante y educativa: aprendió mucho sobre química verde, ingeniería, catálisis, procesos químicos y cinética.

Su interés por los procesos verdes y la ingeniería ambiental lo han empujado a iniciar su doctorado en el Grupo de Procesos de Alta Presión del Departamento de Ingeniería Química (Universidad de Valladolid) en febrero de 2014.

Su doctorado se enfocó en la extracción de hemicelulosa de diferentes biomasas residuales como por ejemplo madera de poda o pulpa de remolacha.

Diseñó y construyó una planta piloto para la extracción de hemicelulosa que permite operar de forma continua y rápida gracias a un sistema innovador de carga y descarga de la biomasa. Con este sistema ganó el premio Prometeo y pudo patentar la tecnología.

Su interés en la transferencia de conocimiento desde el ámbito académico a la industria le hizo participar en el concurso Vivero (2016), cuyo objetivo era formar a empresarios y ayudar a crear nuevas empresas basadas en la tecnología basadas en los resultados de proyectos de investigación y tecnologías de las universidades de Castilla y León, consiguiendo el primer premio.

4. MISIÓN, VISIÓN Y VALORES DE SWEET GREEN

4.1 Misión

Nuestra misión es ser proveedores de xilitol producido con nuestra tecnología verde para las principales industrias de alimentación de España, principalmente del sector de repostería, refrescos y bebidas.

4.2 Visión

Queremos ser marca de referencia de edulcorantes naturales para el sector de alimentación bajo en calorías del mercado nacional e internacional.

4.3 Valores:

Orientación al cliente: queremos que el cliente esté satisfecho con la calidad y el precio del producto que ofrecemos, contactaremos con ellos y responderemos a sus peticiones.

Respeto del medioambiente: nuestra tecnología respeta plenamente el medio ambiente, nuestro objetivo futuro es demostrarlo con certificaciones medioambientales.

Fiabilidad: nuestra experiencia técnica nos permite garantizar la fiabilidad y la calidad de nuestros productos.

Salud: nuestro producto es saludable y proviene de materias primas con origen conocida y controlada.

5. PROCESO DE VALIDACIÓN

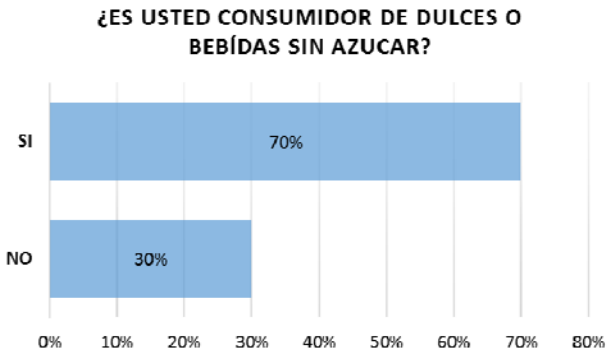
El proceso de validación de la idea comenzó con una encuesta, tras un ejercicio de identificación de cuáles eran para nosotros las principales hipótesis.

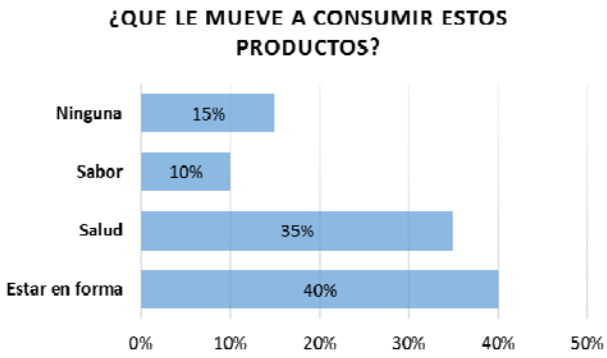
HIPÓTESIS:

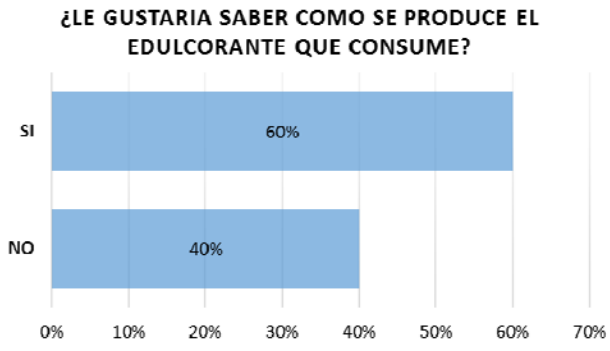
1. Creemos que el mayor problema de nuestros clientes será encontrar un edulcorante con el sabor del azúcar, a bajo precio.
2. Creemos que los productos naturales son más aceptados públicamente que los productos formulados químicamente.
3. Creemos que nuestros clientes puedan ser industrias de producción de dulces.
4. Creemos que nuestros usuarios quieren comer dulces sin preocuparse por la forma física
5. Creemos que nuestros usuarios quieren comer dulces con una preocupación menor por la higiene dental.
6. Creemos que los dulcificantes existentes que sustituyen al azúcar son muy caros.

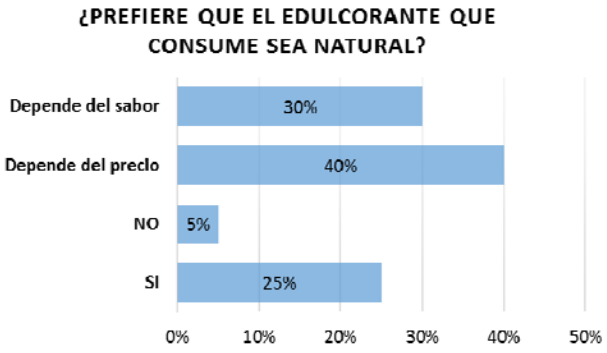
7. Creemos que nuestro principal competidor en el mercado serán otras industrias que producen dulcificantes, aunque con características diferentes.

Se entrevistó a una muestra de 20 sujetos con diferente procedencia social y diferentes edades. Los principales resultados se muestran en las gráficas que siguen.









Evaluando estas entrevistas y considerando un análisis del mercado hecha con referencias bibliográficas, se ha considerado que la producción de un edulcorante con las características de SWEET GREEN sería competitivo en el mercado.

El proceso ha sido totalmente validado por los usuarios, y está en proceso de validación para los clientes.

Industrias de repostería y de bebidas “sin azúcar” se han mostrado interesadas en el producto, pero requieren elevadas cantidades que en este momento no pueden ser obtenidas a causa de la pequeña dimensión de nuestra maquinaria.

Tiendas de productos ecológicos han demostrado sus intereses hacia el producto con las cantidades limitadas que se pueden fabricar en este momento.

Se ha realizado un producto mínimo viable para enseñarlo a clientes y consumidores. El vídeo se presenta en este enlace:

https://www.dropbox.com/s/dn9ish2vxeap0gh/PMV%20Yuzz.mpg?dl=0

Gracias a la tecnología que tenemos fue posible diseñar y construir un sistema para la producción del producto.

El sistema se ha patentado para poder proteger nuestro proceso de la competencia.

Figura 3: Imagen del prototipo realizado para la producción de SWEET GREEN.

6. DESCRIPCIÓN DEL PRODUCTO

SWEET GREEN xilitol es un producto de alta calidad que se extrae sólo de materias primas vegetales. Es dulce y sabroso al igual que el azúcar, con un 40% de calorías menos; ayuda a mantener los dientes sanos y es adecuado para los diabéticos.

Las materias primas de procedencia son: madera, pulpa de remolacha, salvado de trigo y residuos del vino de origen localizados en territorio español.

A diferencia del xilitol presente en el mercado, que se obtiene a partir de maíz chino o de abedules del norte de Europa, SWEET GREEN xilitol se produce respetando el medioambiente y sin ningún peligro para la salud del consumidor.

El maíz de procedencia china, sin embargo, no tiene que seguir ningún protocolo y los agricultores son libres de usar herbicidas y otros compuestos químicos. Por lo tanto, el producto final podría estar contaminado y no ser saludable. En España, los cultivos deben cumplir con varias directivas y reglamentos, lo cual significa que el producto final es controlado y seguro.

La extracción de SWEET GREEN xilitol además no emplea disolventes químicos como en los procedimientos convencionales; de hecho, se utiliza agua como único disolvente.

SWEET GREEN xilitol es ideal para endulzar bebidas, mermeladas, postres y caramelos; nuestros clientes serán por eso “industrias del dulce”.

7. ANÁLISIS DEL ENTORNO Y SITUACIÓN DEL MERCADO

7.1 Edulcorantes alternativos: un mercado creciente

En España, según Asemac , Asociación Española de la Industria de Panadería, Bollería y Pastelería, la industria de la alimentación y bebidas es la primera rama industrial dentro de la economía española. Este sector se ha ido adaptando a los nuevos hábitos de vida y a las preferencias actuales del consumidor con nuevas tecnologías y nuevos productos, contribuyendo al auge de los productos alimenticios que ofertan beneficios para la salud a través de productos con menor contenido en azúcares simples, en grasa total o en sal, ricos en fibra y minerales, elaborados con grasas saludables, etc.; con tal intensidad que en algunos casos pueden llegar a presentar una penetración superior a la del producto homólogo.

De acuerdo con el estudio de Hábitos Alimentarios en la Comunidad de Madrid [3], la mayoría de los consumidores se fija en el etiquetado de los productos, sobre todo en la información nutricional, en los ingredientes y en las fechas de caducidad. Los agentes de la distribución resaltan que, en el caso concreto de la bollería y la pastelería industrial, no es frecuente encontrar a un comprador analizando una etiqueta o preguntando por las características nutricionales y los ingredientes de los productos. Lo que más impacta son los mensajes nutricionales y de salud como por ejemplo “light” o “sin azúcar”.

El mercado de los edulcorantes alternativos está en crecimiento rápido. Se espera que el mercado global de edulcorantes alternativos aumente de manera constante durante el período 2015-2021. El informe elaborado por Transparency Market Research evidencia las tendencias actuales del mercado y proporciona previsiones para el período 2015-2021.

Actualmente, el mercado de los edulcorantes mueve 40 millones de euros al año en España y es un sector en continuo crecimiento, ya que según el estudio AC Nielsen de junio de 2015, este mercado aumentó un 4,4% su valor en 2015 respecto el 2014. Uno de cada tres españoles (37,5%) se declara consumidor de edulcorantes y el 45% los toma habitualmente y más de una vez al día, según datos Nielsen y del estudio Gfk de marzo de 2015.

El mercado mundial de edulcorantes alternativos se estimaba en 12.101,9 millones de dólares en 2015 y se espera que alcance 15.466,7 millones de dólares en 2021 (un 3% más).

Los edulcorantes alimentarios, se pueden clasificar según su origen y naturaleza química [4]:

- Edulcorantes Naturales: monosacáridos (glucosa, fructosa, galactosa), disacáridos (sacarosa, lactosa, maltosa), polioles de primera generación (sorbitol, xilitol, manitol), estevia.

- Edulcorantes Sintéticos: sacarina, ciclamato, aspartamo, sucralosa, etc.

En general hemos de valorar que aunque la mayoría de edulcorantes naturales contienen más calorías que los edulcorantes artificiales, también es verdad que tienen beneficios nutricionales o propiedades medicinales interesantes.

7.2 Mercado del xilitol

El mercado de xilitol está creciendo más rápidamente que el mercado de los otros edulcorantes. Las pocas calorías, excelente sabor, versatilidad y dulzor equivalente al azúcar, un efecto mínimo sobre los niveles de azúcar en la sangre y la insulina, y los beneficios para los dientes favorecen la demanda del producto.

El mercado mundial de xilitol se estima en 190,9 mil toneladas, valorado en 725,9 millones de dólares en 2016. Según un nuevo informe de investigación de Global Market Insights, Inc. se espera que el tamaño del mercado de xilitol llegue a 1,12 mil millones de dólares en 2023 (un 4% más en 7 años).

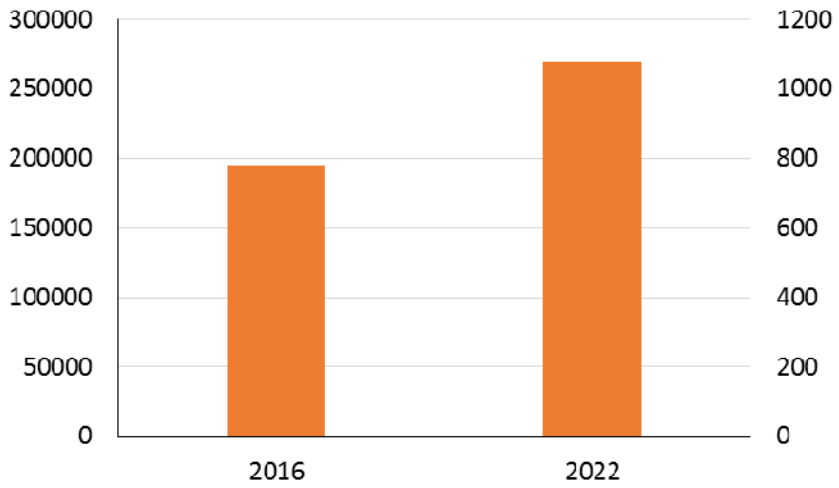


Figura 4: Gráfica de la demanda creciente de xilitol.

En 1996, el Comité Mixto FAO/OMS de Expertos en Aditivos Alimentarios (JECFA), organismo que asesora a la Organización Mundial de la Salud y a la Organización de Agricultura de las Naciones Unidas, confirmó la seguridad del xilitol para el consumo humano y le asignó una Ingesta Diaria Admisible (ADI) “no especificada”. La Ingesta

Diaria Admisible es la cantidad de un aditivo que puede incluirse diariamente en la dieta, durante toda la vida, sin riesgo. Una ADI “no especificada” es la categoría más segura para un aditivo. El Comité Científico sobre la Alimentación de la Unión Europea también determinó que el xilitol es admisible para usos dietéticos. Productos con xilitol incluyen chocolate, pasta de dientes y enjuague bucal, productos horneados, postres y dulces congelados.

La utilización de xilitol en la producción de chicle domina la mayor parte del mercado total del xilitol, generando 450 millones de dólares de negocio en el 2015. Se estima un aumento de producción del 6% en 2023. Los chicles de xilitol evitan la sequedad de la boca, los riesgos de caries dentales y aumentan la salud oral en niños y adultos. El xilitol además actúa como edulcorante y proporciona sabor y efecto de enfriamiento.

En el 2023 se estima un aumento del empleo de xilitol del 5,4% en alimentos como bebidas y productos de panadería (galletas, pasteles, tartas y bollos), con la generación de más de 45 millones de dólares. La tendencia se debe a la creciente preferencia del consumidor por edulcorantes naturales en la dieta para la prevención de enfermedades como diabetes y obesidad.

Si a todas las características que se enumeran, se añade también que nuestra tecnología es capaz de producir xilitol de una manera ecológica y a bajo coste, será fácil entender por qué hemos visto una oportunidad de mercado en la producción y venta de este compuesto.

7.3 Competencia

En España, los productores de edulcorantes alternativos son Nutrisweet SL y Zukán; las dos no producen xilitol. La mayoría de las industrias funcionan como distribuidores de edulcorantes.

A nivel global se encuentran industrias productores de edulcorantes como: Tate & Lyle Plc, Cargill, Incorporated, Ajinomoto Co., Inc., Archer-Daniels-Midland Company, Ingredion Incorporated, Roquette Frères SA, NutraSweet Company, BENEO-Palatinit GmbH, Hermes Sweeteners Ltd. y EI du Pont de Nemours entre otros.

El mayor fabricante de xilitol en el mundo es la compañía danesa Danisco (Du Pont), junto con otros proveedores de China (Shoji Foodtech Co, Shandong Futaste Co Ltd., ZuChem Inc., Zhejiang Huakang Pharmaceutical Co.).

En estos últimos años se ha puesto de moda el uso de estevia, una planta que, refinada es 200 veces más dulce que el azúcar, de acuerdo con el Centro Médico Langone. Estevia refinado es un edulcorante de mesa, que se encuentra en algunos refrescos, postres y chicles. El problema principal de este edulcorante es que deja un sabor amargo y metálico debido al esteviosido, su principal componente [5].

En el mercado no existe un producto que además de contener xilitol, siga un proceso totalmente ecológico como el nuestro.

7.4 Biorefinería, una industria en expansión

No podemos hablar de SWEET GREEN sin explicar el concepto de biorefinería, la clave fundamental para la realización de nuestra idea de negocio.

Como hemos mencionado antes, las biorefinerías son instalaciones que de un modo sostenible transforman biomasa en un amplio espectro de productos energéticos, alimentos y bioproductos.

El rápido crecimiento de la población humana y la consiguiente demanda creciente de alimentos, energía y agua son los más graves desafíos en los que el mundo se está enfrentando. A lo largo de muchas discusiones impulsadas por la Comunidad de la Industria Química en el Foro Económico Mundial en 2008 y 2009, las biorefinerías industriales fueron identificadas como una posible solución que puede ayudar a mitigar la amenaza del cambio climático y la demanda aparentemente ilimitada de energía, combustibles, productos químicos y materiales (Fernández, 2008).

Dos de los principales impulsores de la industria, además de la seguridad energética y las preocupaciones ambientales, son los mandatos y las políticas (Tabla 1). El 31 de enero de 2007, la Comisión Europea (CE) ha propuesto nuevas normas sobre las energías renovables y obliga a que, en 2020, un 10 % de la energía utilizada en el sector del transporte sea renovable.

Además de las motivaciones políticas, una serie de tendencias han puesto los bioproductos en la agenda estratégica de muchas industrias.

- Dada la limitada disponibilidad de reservas de combustibles fósiles, fuentes alternativas y sostenibles para satisfacer las necesidades de la humanidad podría ser la única alternativa viable.
- Muchos países se esfuerzan por reducir la exposición económica y geopolítica asociada a su necesidad de petróleo, mediante la sustitución de al menos parte de su combustible y materia prima con la producción nacional de alternativas de base biológica.

- La creciente demanda y presiones reglamentarias para la biomasa aumentará el tamaño del mercado global de los productos agrícolas y forestales.

United States	Brazil	European Union	China	India
Mandate of 36 billion gallons of biofuels by 2022. Volumetric tax credit: USD 0.51/gal ethanol + USD 1.00/gal biodiesel. Cellulosic biofuel producer tax credit: USD 1.01/gal. Small producer tax credit: USD 0.1/gal. USD 1 billion in support for 2nd generation technology.	30+ year commitment to 'alcohol program'. Annual blending target for ethanol (25%). Biodiesel target of 5% by 2013. Lower taxes for ethanol (E100) than gasoline. FFV sales tax of 14% compared to 16% for gasoline-only vehicles.	5.75% blending target by 2010 and 10% by 2020. Discussion on target waiver triggered by food crisis, but no change of policy so far. Country-level subsidies average USD 1.90/gal for ethanol and USD 1.50/gal for biodiesel. Penalty fee in 5 countries for noncompliance with biofuel target.	Plan to substitute 20% of crude imports by 2020. Target of 1.7 billion gallons of ethanol by 2010. Investments in feedstock-rich countries. Commitment to develop non-food based biofuels - COFCO (Nat. Food Corp.) with PetroChina and Sinopec - 2nd generation multiple projects.	Blending targets in current drafts are 5% by 2012, 10% by 2017, 20% for long term. Target of 20% biofuels by 2020. Duty-free imports of jatropha to support biodie-sel. Individual states may set additional measures to promote biofuels or restrict transport of molasses over state boundries.
***Corn/ Lignocellulose**	***Sugarcane**	***Rapeseed/ Lignocellulose**	***Lignocellulose/ Various**	***Various**

Tabla 1: Estado de la política actual, mandatos y subsidios en cinco grandes regiones del mundo.

Se estima que la posesión de plantas de conversión de biomasa y la venta de los productos finales podrá producir para el año 2020 potenciales de ingresos de 80 mil millones de dólares para los biocombustibles, 10-15 mil millones de dólares para productos químicos y bioplásticos, 65 millones para energía y calor.

Dos categorías de materia prima dominan la investigación: primera y segunda generación. Productos de primera generación se fabrican a partir de biomasas comestibles tales como plantas ricas en almidón o aceitosas; productos de segunda generación utilizan biomasa que consiste en las partes no comestibles residuales de los actuales cultivos u otras fuentes no alimentarias, como las hierbas perennes o algas. Estos son ampliamente reconocidos

como poseedores de un potencial significativamente mayor para reemplazar productos de origen fósil [6].

Los materiales lignocelulósicos pertenecen a las materias primas de segunda generación, y se pueden obtener de varias fuentes, tales como residuos de madera, residuos agrícolas o forestales, que no interfieren con los cultivos para el consumo humano directo.

Un esquema de biorefinería se presenta en la figura 5. La biomasa lignocelulósica se puede fraccionar en tres componentes principales: celulosa, hemicelulosa y lignina, a partir de las cuales se pueden producir azúcares, combustibles, materiales y productos químicos. Desde los residuos de elaboración se puede producir energía.

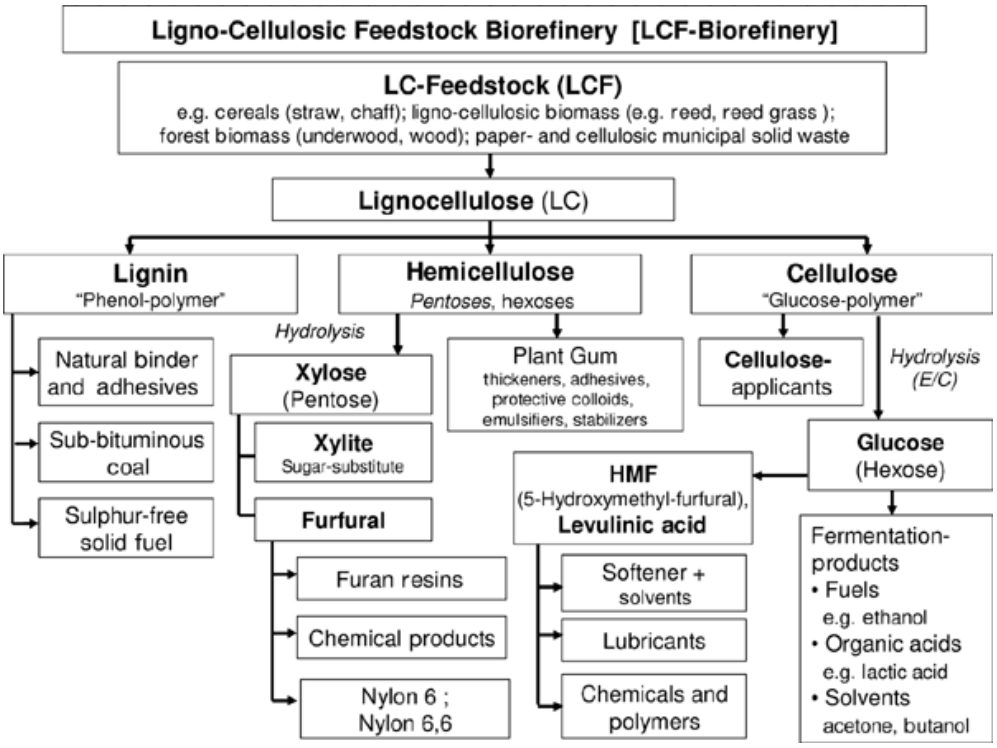


Figura 5: Esquema del concepto de biorrefinería lignocelulósica [7].

CHAPTER VI

Nuestro producto se origina a partir de hemicelulosa, un compuesto que además de para la producción de xilitol, puede tener varias aplicaciones:

- producción de biocombustibles;
- producción de películas para el envasado;
- producción de geles para el suministro de fármacos
- producción de furfural, que se usa en la producción de plásticos y también en el campo farmacéutico.

Los sistemas industriales existentes para extraer hemicelulosa utilizan productos químicos, con la necesidad de purificar el producto y de eliminar los residuos tóxicos.

Sin embargo, la hemicelulosa se puede extraer con agua a temperatura superior a 100 ºC. Esto tiene numerosas ventajas económicas y ambientales.

Actualmente no existe ninguna tecnología a nivel industrial adaptada para extraer hemicelulosa utilizando solamente agua como único disolvente; especialmente, no existen plantas que sean capaces de trabajar con cantidades representativas de biomasa (del orden de kilogramos) a nivel de laboratorio y piloto, y que permitan el trabajo cuasi-continuo. SWEET GREEN ha conseguido alcanzar este objetivo.

7.5 Biomasa en España

Según un estudio del Observatorio Industrial del Sector de Fabricantes de Bienes de Equipo, el potencial de biomasa disponible en España, bajo hipótesis conservadoras, se sitúa en torno a 87 millones de toneladas de biomasa primaria en verde, incluyendo restos de masas forestales existentes, restos agrícolas, masas existentes sin explotar y cultivos energéticos a implantar.

Actualmente, la contribución de la biomasa a la necesidad de energía primaria está muy por debajo del potencial disponible, y se produce fundamentalmente por la utilización de leña para quemar en chimeneas y estufas. No obstante, las tecnologías para la utilización de combustibles vegetales en sistemas de calefacción doméstica han experimentado un gran desarrollo en los últimos años y han alcanzado niveles de eficiencia, fiabilidad y confort muy parecidos a los de los sistemas tradicionales de gas y de gasóleo.

En noviembre de 2009 la Asociación Española de Valorización Energética de la Biomasa (AVEBIOM), creó el Observatorio Nacional de Calderas de Biomasa (ONCB), que pretende analizar la situación del sector y su evolución para realizar un planteamiento de futuro y reducir incertidumbres.

Los primeros datos recogidos por el ONCB muestran que aunque hay un mayor número de instalaciones en el ámbito doméstico, es en la industria donde encontramos más kW instalados debido al uso de calderas de mayor potencia.

En el sector industrial son las fábricas relacionadas con el mundo agrario y de la madera las que mayor número de instalaciones y potencia reúnen, debido, sobre todo, a la mayor facilidad de acceso a la materia prima.

A pesar de ello, el precio de la biomasa todavía es bastante bajo, como se puede ver en la siguiente tabla:

Tabla 2: Costes indicativos de la biomasa. Fuente: CECU, Calderas de biomasa para sistemas de calefacción doméstica, Proyecto RES & RUE Dissemination.

Biomasas	Coste *€/kg*
Leña para quemar 25% humedad (*)	0,103
Leña para quemar 35% humedad	0,093
Leña para quemar 45% humedad	0,077
Astillas de haya/encina 25% humedad	0,067
Astillas de haya/encina 35% humedad	0,062
Astillas de haya/encina 50% humedad (**)	0,057
Astillas de álamo 25% humedad	0,052
Astillas de álamo 35% humedad	0,044
Astillas de álamo 50% humedad	0,036
Pellet de madera humedad máx. 10%	0,180
Pulpa de remolacha (pellets)	0.150
Pulpa de remolacha (prensada ensilada)	0.070

(*) Leña seca de dos años

(**) madera recién cortada

El gráfico que sigue muestra la oscilación del precio de astillas de madera desde 2014 hasta 2017. Son precios medios a cliente final calculados trimestralmente e incluyen el

21% de IVA y un transporte medio de 100 km en formato a granel. Como se puede ver, el precio no ha sufrido grandes cambios en este período de tiempo.

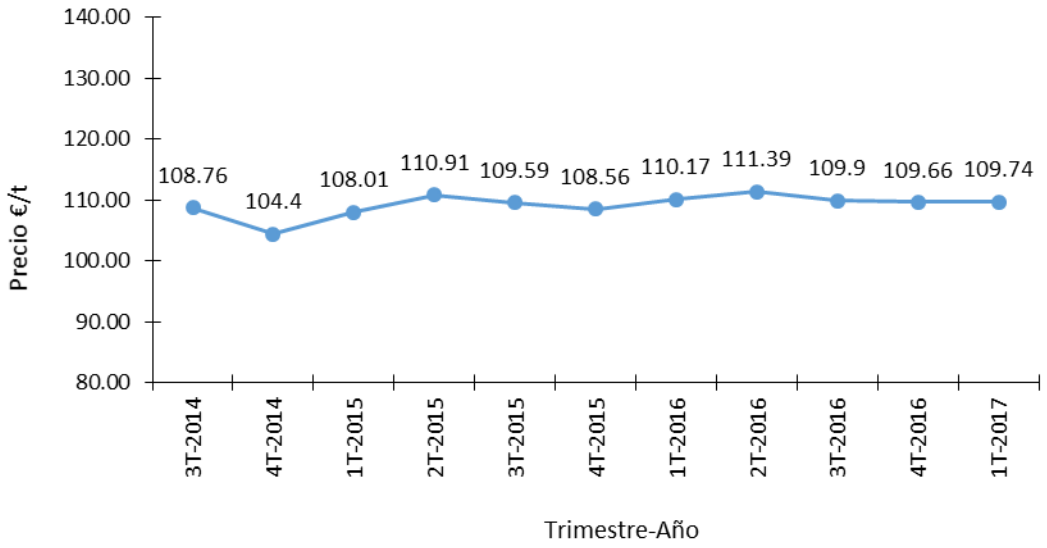


Figura 6: Precios de astillas a granel en los últimos 4 años. Fuente: Asociación Española de Valorización Energética de la Biomasa (AVEBIOM)

8 NUESTRA MATERIA PRIMA Y NUESTROS PROVEEDORES

Hasta ahora, se ha hablado de biomasa de origen forestal, pero tenemos que añadir un elemento muy importante. España es el país europeo con mayor potencial de biomasa de podas y replantaciones de vid, olivo y frutal. Según el periódico El Economista, se generan más de ocho millones de toneladas al año de restos de madera, correspondientes a podas agrícolas y renovación de plantaciones (PARP) de vid, olivo y frutal.

Todo este potencial, sin embargo, no se está aprovechando. La mayoría de las ocho millones de toneladas anuales no suelen utilizarse; se quema o bien se procede a su vertido. Entre las principales razones por las que no se emplea, se encuentra el hecho

de que esta agro-biomasa tiene más dificultades en la cadena de suministro y en la conversión energética en comparación con las astillas forestales, cuyas cadenas de suministro y mercado están establecidos de manera estable.

Este desaprovechamiento se produce a pesar de las ventajas que conlleva tanto para el agricultor como para los productores de frutas y las bodegas, porque permitiría optimizar estos recursos y obtener una mayor rentabilidad en su actividad al reducir costes y disminuir la huella de carbono.

Para la producción de SWEET GREEN xilitol, por lo tanto:

- utilizamos una materia prima económica, con una gran ventaja para nuestro proceso;
- contribuimos a la salud del medio ambiente aprovechando un producto que de otro modo se quemaría;
- impulsamos la economía del sector agrícola que encuentra una nueva oportunidad de negocio en un mercado totalmente nuevo.

9 ANÁLISIS DAFO

FORTALEZAS	**DÉBILIDADES**
Ser los primeros: en la actualidad no existe tecnología igual.	Falta de marca reconocida, el consumidor puede no valorar su diferencia.
Bajo coste de las materias primas y del proceso	Necesidad de una inversión inicial bastante alta.
Tecnología patentada	Necesidad de optimizar las características del producto.
Producción continua, sin necesidad de parar el proceso.	Actualmente el nivel de productividad es bajo; necesidad de contratar personal y trabajadores.
OPORTUNIDADES	**AMENAZAS**
Pocas empresas en España producen edulcorantes	Posible diminución del precio de edulcorantes importados.
Tendencia al consumo de productos naturales y saludables.	Poco conocimiento general de las propiedades del producto.
Incentivos a la constitución de empresas con estas características.	Hay varios grupos de investigación trabajando sobre este tema.

9.1 Soluciones

- La promoción del producto se llevará a cabo a través de publicidad y marketing en ferias y congresos.
- Aunque el producto sea nuevo, sus numerosas ventajas medioambientales y económicas hacen que sea muy atractivo desde un punto de vista comercial.
- La productividad se puede aumentar fácilmente, gracias a una mayor automatización del proceso.
- Una colaboración inicial con la universidad, donde actualmente se encuentra la instalación de producción, puede ayudar a reducir los costes de puesta en marcha inicial.
- Para construir una planta de este tipo y optimizar la producción se requiere una formación y conocimientos muy específicos.

10 NUESTROS OBJETIVOS

10.1 Objetivos cualitativos

Nuestro primer objetivo es la satisfacción del cliente. Queremos transmitir confianza y transparencia sobre nuestro producto y sobre nuestro proceso de producción; ofreciendo un producto natural, de alta calidad, ecológico y a precios competitivos.

Queremos ser la marca lider en España para la producción de edulcorantes.

10.2 Objetivos cuantitativos

Queremos acabar el primer año con un superavit mínimo de 20.000 euros para invertirlos en la construcción de la nueva maquinaria de producción durante el segundo año.

El crecimiento interanual esperado a partir del segundo año es del 4%.

11 ESTRATEGIAS

11.1 Estrategia de diferenciación

Con SWEET GREEN xilitol, ofrecemos un producto innovador que permite a nuestros clientes introducir una nueva línea de productos con buen sabor, más saludables, más naturales y con origen conocida; el consumidor final podrá aceptar fácilmente y voluntariamente las novedades.

Nos proponemos asegurar un producto de calidad, que pueda crear la percepción de unicidad en el mercado y crear una fidelización y una lealtad de los consumidores y de los clientes.

11.2 Estrategia económica

Ofrecemos un producto más económico gracias a las materias primas, el proceso y la tecnología que utilizamos.

11.3 Estrategia de desarrollo del producto

Queremos aumentar las ventas, desarrollando productos mejorados o nuevos en los mercados actuales. En particular, nuestro objetivo es de modificar los productos actuales:

- añadiendo valor social o emocional;
- mejorando la seguridad o percepción de salud;
- lanzando productos ecológicos;
- estableciendo un programa de control de calidad.

12 PLAN DE MARKETING OPERATIVO

12.1 Producto

SWEET GREEN xilitol es un edulcorante constituido por 99% de xilitol procedente de biomasa agrícola y forestal. Tiene un aspecto sólido, cristalino, incoloro; totalmente similar al azúcar de mesa, con el mismo sabor y consistencia.

Tiene efectos positivos sobre la salud oral y es adapto para los diabéticos.

Producido en España, con materias primas vegetales de procedencia española; realizado con un proceso 100% natural y ecológico utilizando solamente agua como disolvente.

Ideal para ser utilizado como dulcificante en bebidas, postres, bollos y golosinas.

En la primera fase, y con el fin de comprobar la viabilidad y afianzar la cartera de clientes, la producción será a granel, en envases de polietileno de 200 g, 500 g, 1 Kg y 5 Kg.

12.2 Precios:

En el mercado hay principalmente dos tipos de xilitol: uno producido en China, procedente de salvado de trigo, y otro producido en Escandinavia, procedente de madera de árboles. El precio del xilitol puro (a granel) es alrededor de 10 €/Kg.

El precio de envases de 0.5-1 Kg es alrededor de 25 €/Kg.

A los precios indicados tienen que ser añadidos los gastos de transporte y exportación.

El precio de nuestro producto se acercará a los precios existentes en el mercado, con la ventaja de tener nuestros clientes en el mismo país de la producción, permitiéndonos tener por ello un coste de distribución más bajo.

12.3 Promoción

Por ser un producto nuevo, es importante promocionar sus beneficios. Hay que estimular el conocimiento, interés, evaluación, prueba, aceptación y finalmente recompra de SWEET GREEN xilitol.

La empresa tendrá página web donde se describirán los productos y servicios que proporcionamos así como los contactos y direcciones para cubrir cualquier duda o pedido.

A mayores se expondrá el producto en congresos y ferias específicos de biomasa, alimentación, pastelería y alimentos naturales. Entre todos:

- EXPOBIOMASA 2017 (Valladolid, 26-29 septiembre 2017): “reunión de todos los profesionales y grupos de inversión que participan en generar ahorros a los consumidores de biomasa.”
- ALIMENTARIA BARCELONA 2018 (Barcelona, 16 -19 Abril 2018): “uno de los salones de Alimentación y Bebidas más importantes del mundo. 14 salones diferentes sectorizados por producto u origen.”

Además se acudirá personalmente a ofrecer nuestro producto y servicio a posibles futuros clientes.

13 BREVE DESCRIPCIÓN DE LA TECNOLOGÍA

Una forma limpia y barata para extraer compuestos solubles de la biomasa consiste en el tratamiento con agua caliente.

Las instalaciones actualmente existentes para la finalidad descrita, consisten en un reactor constituido de un tubo cargado con pellets de biomasa, atravesado por un flujo de agua. Uno de los principales problemas es cargar y descargar el material sólido una vez completada la extracción. La biomasa húmeda se hincha y se compacta, lo que hace imposible su remoción sin parar el sistema y abrirlo. Por esta razón se ha inventado un

sistema que hace posible la operación sin tener que parar la planta, esperar que se enfríe y desmontarla durante las fases de carga y descarga. El sistema permite además de recolectar enteramente el sólido exhausto, operación crítica por el proceso.

Se ha diseñado una planta piloto compuesta por 5 reactores, cada uno de los cuales trabaja en serie con los demás o puede ser excluido del sistema.

Cada reactor se compone de:

- Un cilindro abierto en los extremos, construido con una malla de metal que se puede abrir longitudinalmente.
- Dos cilindros de acero inoxidable con el mismo diámetro, uno de los cuales tiene una parte inferior con orificios de 1 mm de diámetro. La malla metálica se inserta entre los dos cilindros, formando un cartucho, que se llena con biomasa.
- Un cilindro exterior de acero inoxidable cerrado en el extremo superior y con una válvula de globo en el extremo inferior. El cartucho se inserta desde abajo en este sistema hasta que entra por completo. En ese momento la válvula se cierra. Al terminar la extracción, la válvula se abre y el cartucho cae en un sistema de paracaídas.

Un flujo constante de agua entra por la parte superior de cada reactor hacia abajo hasta que sale por un agujero situado en la parte inferior. El flujo de salida puede ir al siguiente reactor, o puede ser desviado con el fin de excluir la siguiente unidad, y pasar a la próxima. De esta manera la biomasa puede ser descargada de la unidad en la que se completa el proceso de extracción, y otra unidad se puede cargar con otro cartucho para continuar la extracción.

Antes de entrar en los reactores, el agua pasa a través de un pre-calentador donde se recupera hasta el 90% de energía invertida, y entra a continuación en los reactores, los cuales están a una temperatura constante regulable. Después de salir del reactor, el líquido

entra en un intercambiador de calor de tubos concéntricos. En la parte exterior fluye el suministro de agua de la planta, que se precalienta y luego pasa al pre-calentador.

El reactor puede ser vaciado del agua caliente presurizada antes de extraer el cartucho. Entre un reactor y el otro existe un intercambiador de calor con tubos concéntricos; desde el tubo interior se descarga el líquido contenido en el reactor, enfriado por agua que fluye en el tubo exterior. La tecnología ha sido presentada a la Oficina Española de Patentes y Marcas con el objetivo de ser patentada.

14 RECURSOS HUMANOS

Durante el primer año, Gianluca Gallina, asesorado por Juan García Serna, va a trabajar a tiempo completo para producir xilitol, y para perfeccionar y optimizar el prototipo y el proceso de producción y purificación.

Ambos van a desarrollar la función comercial, para mostrar el producto en ferias y congresos así como directamente en empresas de repostería, mermeladas, bebidas y golosinas para captar clientes.

A principio del segundo año, cuando es previsto el aumento de la producción, se va a contratar un trabajador, contratado por 30 horas semanales, que se dedicará a seguir el proceso de producción. No se requiere particular formación.

En este periodo Gianluca Gallina se ocupará de la actividad comercial y de las relaciones con los clientes.

Juan García Serna se ocupará principalmente del trabajo de I+D y de asesoría tecnológica.

15 PROCESOS – PRODUCCIÓN

PROCESOS

1. Captación de la biomasa
2. Transformación de la biomasa en xilitol: a través de nuestra tecnología.
3. Venta directa del producto resultante.

Hay otros procesos paralelos, como puede ser un trabajo de investigación en perfeccionar y mejorar la tecnología, además de procesos de promoción y marketing de la empresa. El trabajo de I+D se realizará en la universidad de Valladolid, y será financiado a través de un articulo 83.

Año 1

PRODUCCIÓN

El producto a fabricar se hará durante el primer año con maquinaria de la UVa que está ubicada en el Departamento de Ingeniería Química y Tecnología del Medio Ambiente con quien se ha llegado a un acuerdo de explotación de uso.

El coste de alquiler se ha fijado a 1.500 €/mes e incluye los gastos de electricidad y luz de la producción.

Los clientes, durante el primer año, se han identificado en tiendas ecológicas, herboristerías y pequeñas pastelerías localizadas en la provincia de Valladolid.

Se estima una producción de 30 Kg de SWEET GREEN xilitol/semana a 22,5 €/Kg.

El consumo de materias primas (biomasa) será de 150 Kg/semana, con un coste de 0,05 €/Kg.

En este primer punto de la empresa, la producción resultante se trasladará a una oficina alquilada que actuará de almacén donde se guardará para su distribución a los clientes; oficina que también sirve como depósito de las materias primas. La estructura estará

localizada en el Parque Científico UVa, tendrá una superficie de 23 m^2, con un coste de alquiler de 276 €/mes (con gastos incluidos, menos internet), más 2 meses de fianza.

FINANCIACIONES

- 30.000 €: capital social (2/3 aportados por Gianluca Gallina y 1/3 por Juan García Serna)
- 4.860 €: procedentes del premio Vivero 2016 con lo cual fue premiada la tecnología.
- 1.000 €: financiación de Michelin para estar en el Parque Científico UVa.
- 1.500 €: beca *Crea* del Ayuntamiento de Valladolid.

OTROS GASTOS

- 450 €: coste de establecimiento
- 300 €: ordenador, amortizables en 4 años.
- 500 €: material de oficina (sillas, mesa, estanterías) amortizables en 10 años.
- 5.000 €: materiales y productos de laboratorio.
- 80 €: licencias software amortizable en 2 años.
- 400 €: primas de seguro.
- 1700 €: publicidad, promoción y viajes.
- 6000 euros: artículo 83 con universidad.
- 50 €: tributos.
- 60 €/mes: asesoría legal.
- 50 €/mes: internet.

SALARIOS

- 600 €/mes + 30 % de seguridad social (salario emprendedor)

Año 2

PRODUCCIÓN

Durante el segundo año, se prevé la construcción de una planta de producción más grande, capaz de satisfacer a la demanda de producto de clientes como grandes industrias de repostería, bebidas, golosinas y chicles.

En este momento, la producción se trasladará a una nave con una superficie de 200 m^2 localizada en el Parque Tecnológico de Boecillo (Valladolid). El precio del alquiler es de 1.000 €/mes + 2 meses de fianza. La nave actuará de almacén, centro de producción y oficina.

Se requiere una inversión inicial de 150.000 €, amortizables en 10 años, para construir la nueva maquinaria.

Se estima una producción de 220 Kg de SWEET GREEN xilitol/semana a 9 €/Kg.

El consumo de materias primas (biomasa) será de 1.000 Kg/semana, con un coste de 0,05 €/Kg.

FINANCIACIONES

- 22.000 €: tesorería del año anterior
- 45.000 €: proyecto de inversión en PYMES para empresas en Castilla y Leon (30% de la inversión)
- 90.000 €: préstamo bancario a largo plazo con interés del 5% en 10 años.

OTROS INGRESOS

- 552 €: fianza año anterior

OTROS GASTOS

- 1.000 € + 5% facturación: contrato de transferencia de la patente.
- 1.700 €: publicidad, promoción y viajes.
- 12.000 €: materiales y productos de laboratorio.
- 250 €: tributos.
- 8.400 euros: artículo 83 con universidad
- 60 €/mes: asesoría legal.
- 500 €/mes: electricidad.
- 200 €/mes: agua.
- 50 €/mes: internet.

SALARIOS

- 1.000 €/mes + 30 % de seguridad social (salario emprendedor).
- 600 €/mes + 30 % de seguridad social (salario trabajador).

Año 3

El lugar de producción será el mismo que en el año 2. Se prevé un aumento de producción y venta gracias a nuevos clientes.

Se aumentarán también los salarios de los socios y trabajadores.

Los números explicados en este apartado se introducirán en el plan económico financiero presentado en el apartado siguiente.

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16 PLAN ECONOMICO FINANCIERO

A continuación, se presenta el plan económico-financiero que se ha estimado para los 3 primeros años de SWEET GREEN.

Se presentan primero los presupuestos de tesorería del año 1 y del año 2. El presupuesto del año 2 se ha añadido porqué al principio de este periodo hay un cambio sustancial en la estructura de los costes y de los beneficios, como explicado antecedentemente.

Presupuesto de Tesorería AÑO 1

		Nº CASILLA		MES 1	MES 2	MES 3	MES 4	MES 5	MES 6	MES 7	MES 8	MES 9	MES 10	MES 11	MES 12	TOTAL AÑO
INGRESOS	De explotación	1	2+3	3,037.50	3,037.50	3,037.50	3,037.50	3,037.50	3,037.50	3,037.50	3,037.50	3,037.50	3,037.50	3,037.50	3,037.50	36,450.00
	Ventas	2		3,037.50	3,037.50	3,037.50	3,037.50	3,037.50	3,037.50	3,037.50	3,037.50	3,037.50	3,037.50	3,037.50	3,037.50	36,450.00
	Otros ingresos	3		0.00												0.00
	De financiación	4	5+6	34,860.00	0.00	1,000.00	0.00	0.00	0.00	1,500.00	0.00	0.00	0.00	0.00	0.00	37,360.00
	Propia	5		30,000.00	0.00	0.00										30,000.00
	Ajena	6		4,860.00	0.00	1,000.00				1,500.00						7,360.00
	TOTAL INGRESOS PERIODO	7	1+4	37,897.50	3,037.50	4,037.50	3,037.50	3,037.50	3,037.50	4,537.50	3,037.50	3,037.50	3,037.50	3,037.50	3,037.50	73,810.00
GASTOS	Variables	8	9+10+11+12+13	7,257.00	825.00	1,225.00	825.00	1,175.00	1,325.00	825.00	825.00	1,325.00	1,175.00	975.00	990.00	18,747.00
	Materiales Consumidos/Compras	9		45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	540.00
	Mano de obra directa. Personal	10		780.00	780.00	780.00	780.00	780.00	780.00	780.00	780.00	780.00	780.00	780.00	780.00	9,360.00
	Comisiones	11														0.00
	Amortizaciones	12													165.00	165.00
	Otros	13		6,432.00		400.00		350.00	500.00			500.00	350.00	150.00		8,682.00
	Fijos	14	15+16	2,836.00	2,386.00	2,386.00	2,386.00	2,386.00	2,386.00	2,386.00	2,386.00	2,386.00	2,386.00	2,386.00	2,386.00	29,082.00
	Alquileres	15		276.00	276.00	276.00	276.00	276.00	276.00	276.00	276.00	276.00	276.00	276.00	276.00	3,312.00
	Suministros	16	17+18+19+20+21	2,560.00	2,110.00	2,110.00	2,110.00	2,110.00	2,110.00	2,110.00	2,110.00	2,110.00	2,110.00	2,110.00	2,110.00	25,770.00
	Electricidad	17														0.00
	Agua	18														0.00
	Teléfono	19		50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	600.00
	Mano de obra indirecta	20														0.00
	Otros suministros	21		2,510.00	2,060.00	2,060.00	2,060.00	2,060.00	2,060.00	2,060.00	2,060.00	2,060.00	2,060.00	2,060.00	2,060.00	25,170.00
	Financieros	22		0.00	0.00	0.00										0.00
	Impuesto sociedades	23		0.00	0.00	0.00										0.00
	TOTAL GASTOS DEL PERIODO	24	8+14+22+23	10,093.00	3,211.00	3,611.00	3,211.00	3,561.00	3,711.00	3,211.00	3,211.00	3,711.00	3,561.00	3,361.00	3,376.00	47,829.00
SUPERÁVIT/DÉFICIT DE TESORERÍA		25	7-24	27,804.50	-173.50	426.50	-173.50	-523.50	-673.50	1,326.50	-173.50	-673.50	-523.50	-323.50	-338.50	25,981.00
+/- SUPERÁVIT-DÉFICIT MES ANTERIOR		26		0.00	27,804.50	27,631.00	28,057.50	27,884.00	27,360.50	26,687.00	28,013.50	27,840.00	27,166.50	26,643.00	26,319.50	25,981.00
RESULTADO NETO		27		27,804.50	27,631.00	28,057.50	27,884.00	27,360.50	26,687.00	28,013.50	27,840.00	27,166.50	26,643.00	26,319.50	25,981.00	51,962.00

Presupuesto de Tesorería AÑO 2														
		Nº CASILLA		MES 1	MES 2	MES 3	MES 4	MES 5	MES 6	MES 7	MES 8	MES 9	MES 10	MES 11	MES 12	TOTAL AÑO
INGRESOS	De explotación	1	2+3	9,462.00	8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	107,472.00
	Ventas	2		8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	106,920.00
	Otros ingresos	3		552.00												552.00
	De financiación	4	5+6	135,000.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	135,000.00
	Propia	5		45,000.00	0.00	0.00										45,000.00
	Ajena	6		90,000.00	0.00											90,000.00
	TOTAL INGRESOS PERIODO	7	1+4	144,462.00	8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	8,910.00	242,472.00
GASTOS	Variables	8	9+10+11+12+13	153,305.00	11,305.00	2,705.00	2,305.00	2,655.00	2,805.00	2,305.00	5,305.00	2,805.00	2,655.00	2,555.00	17,470.00	208,175.00
	Materiales Consumidos/Compras	9		225.00	225.00	225.00	225.00	225.00	225.00	225.00	225.00	225.00	225.00	225.00	225.00	2,700.00
	Mano de obra directa. Personal	10		2,080.00	2,080.00	2,080.00	2,080.00	2,080.00	2,080.00	2,080.00	2,080.00	2,080.00	2,080.00	2,080.00	2,080.00	24,960.00
	Comisiones	11														0.00
	Amortizaciones	12													15,165.00	15,165.00
	Otros	13		151,000.00	9,000.00	400.00		350.00	500.00		3,000.00	500.00	350.00	250.00		165,350.00
	Fijos	14	15+16	2,988.00	2,988.00	2,988.00	2,988.00	2,988.00	2,988.00	2,988.00	2,988.00	2,988.00	2,988.00	2,988.00	2,988.00	35,856.00
	Alquileres	15		1,000.00	1,000.00	1,000.00	1,000.00	1,000.00	1,000.00	1,000.00	1,000.00	1,000.00	1,000.00	1,000.00	1,000.00	12,000.00
	Suministros	16	17+18+19+20+21	1,988.00	1,988.00	1,988.00	1,988.00	1,988.00	1,988.00	1,988.00	1,988.00	1,988.00	1,988.00	1,988.00	1,988.00	23,856.00
	Electricidad	17		500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00	6,000.00
	Agua	18		200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00	2,400.00
	Teléfono	19		50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	600.00
	Mano de obra indirecta	20														0.00
	Otros suministros	21		1,238.00	1,238.00	1,238.00	1,238.00	1,238.00	1,238.00	1,238.00	1,238.00	1,238.00	1,238.00	1,238.00	1,238.00	14,856.00
	Financieros	22		375.00	375.00	375.00	375.00	375.00	375.00	375.00	375.00	375.00	375.00	375.00	375.00	4,500.00
	Impuesto sociedades	23		0.00	0.00	0.00										0.00
	TOTAL GASTOS DEL PERIODO	24	8+14+22+23	156,668.00	14,668.00	6,068.00	5,668.00	6,018.00	6,168.00	5,668.00	8,668.00	6,168.00	6,018.00	5,918.00	20,833.00	248,531.00
SUPERÁVIT/DÉFICIT DE TESORERÍA		25	7-24	-12,206.00	-5,758.00	2,842.00	3,242.00	2,892.00	2,742.00	3,242.00	242.00	2,742.00	2,892.00	2,992.00	-11,923.00	-6,059.00
+/- SUPERÁVIT-DÉFICIT MES ANTERIOR		26		25,000.00	12,794.00	7,036.00	9,878.00	13,120.00	16,012.00	18,754.00	21,996.00	22,238.00	24,980.00	27,872.00	30,864.00	18,941.00
RESULTADO NETO		27		12,794.00	7,036.00	9,878.00	13,120.00	16,012.00	18,754.00	21,996.00	22,238.00	24,980.00	27,872.00	30,864.00	18,941.00	12,882.00

Pérdidas y Ganancias

	AÑO 1	AÑO 2	AÑO 3
Importe cifra de negocio			
Importe cifra de ventas	36,450.00	106,920.00	138,996.00
Variación de existencias			
Variación de existencias	500.00	2,500.00	
Aprovisionamientos			
Consumo de mercaderías	-6,040.00	-17,200.00	-23,510.00
Otros ingresos de explotación			
Otros ingresos			
Subvenciones de explotación			
Gastos de personal			
Gastos de personal	-9,360.00	-24,960.00	-32,760.00
Otros gastos de explotación			
Arrendamientos	-3,312.00	-12,000.00	-12,000.00
Reparación y conservación			
Servicios profesionales	-24,720.00	-10,320.00	-12,720.00
Transportes			
Primas de seguros	-400.00	-400.00	-400.00
Servicios bancarios y similares			
Publicidad y promoción	-1,000.00	-1,000.00	-1,000.00
Suministros	-600.00	-9,720.00	-10,680.00
Gastos de viaje	-700.00	-700.00	-700.00
Tributos	-150.00	-250.00	-300.00
Gastos establecimiento	-450.00		
Licencias y altas suministros		-4,536.00	-5,443.20
Otros gastos			
Amortización del inmovilizado			
Amortización del inmovilizado	-165.00	-15,215.00	-15,215.00
Imputación de subvenciones de inmovilizado no financiero			
Subvenciones a la inversión	1,380.00	5,865.10	5,865.10
Otros resultados			
Ingresos Excepcionales		552.00	
Gastos excepcionales			
RESULTADO DE EXPLOTACIÓN	**-8,567.00**	**19,536.10**	**30,132.90**
Ingresos financieros			
Gastos financieros		-4,500.00	-4,142.23
RESULTADO FINANCIERO		**-4,500.00**	**-4,142.23**
RESULTADO ANTES DE IMPUESTOS	**-8,567.00**	**15,036.10**	**25,990.67**
Impuesto sobre beneficios		-3,759.02	-6,497.67
BENEFICIO O PERDIDA DESPUÉS DE IMPUESTOS	**-8,567.00**	**11,277.07**	**19,493.00**

Balance

ACTIVO	AÑO 1	AÑO 2	AÑO 3
Activo no corriente	**1,267.00**	**138,052.00**	**122,917.00**
Inmovilizado Intangible			
Investigación y Desarrollo			
Patentes, licencias y marcas		1,000.00	1,000.00
Aplicaciones informáticas	80.00	80.00	160.00
Derechos de traspaso			
Canon franquicia			
- Amortización acumulada Inmov. Intang.	-40.00	-130.00	-220.00
Inmovilizado Material			
Terrenos			
Construcciones			
Instalaciones y maquinaria		150,000.00	150,000.00
Elementos de transporte			
Equipo proceso de información	300.00	300.00	300.00
Otro inmovilizado material	500.00	500.00	500.00
- Amortización acumulada Inmov. Material	-125.00	-15,250.00	-30,375.00
Inversiones financieras			
Fianzas - depósitos L/P	552.00	1,552.00	1,552.00
Activo corriente	**26,866.00**	**28,296.59**	**52,884.95**
Existencias	500.00	3,000.00	3,000.00
Clientes			
Deudores y otros			
Hacienda deudora	378.42	21,305.76	6,151.57
Tesorería	22,000.00	30,000.00	45,000.00
Otros activos corrientes/Recursos libre di	3,987.58	-26,009.17	-1,266.62
Total Activo	**28,133.00**	**166,348.59**	**175,801.95**

PASIVO	AÑO 1	AÑO 2	AÑO 3
Patrimonio Neto	**27,413.00**	**77,824.98**	**91,452.88**
Capital Social	30,000.00	30,000.00	30,000.00
Reservas		-8,567.00	2,710.07
Subvenciones	5,980.00	45,114.90	39,249.80
Resultados después de impuestos	-8,567.00	11,277.07	19,493.00
Pasivo no corriente		**82,844.59**	**75,331.41**
Préstamos L/P		82,844.59	75,331.41
Acreedores L/P (Préstamos no Bancarios)			
Pasivo corriente	**720.00**	**5,679.02**	**9,017.67**
Préstamos y Créditos C/P			
Proveedores			
Acreedores C/P			
Seguridad Social	180.00	480.00	630.00
Hacienda pública IRPF	540.00	1,440.00	1,890.00
Hacienda pública IVA			
Hacienda pública Impuesto Soc.		3,759.02	6,497.67
Total pasivo	**28,133.00**	**166,348.59**	**175,801.95**

17 ESTRUCTURA LEGAL DE LA EMPRESA

La estructura legal que adoptará la empresa será de Sociedad Limitada (S.L.), compuesta por dos socios capitalistas.

Uno de los dos socios, Juan García Serna, siendo PDI (Profesor Docente Investigador), no podrá ser socio trabajador. Por eso SWEET GREEN firmará un Artículo 83 con la Universidad de Valladolid, que permitirá a Juan García Serna el cobro de las ventas.

El Capital inicial, aportado directamente por los dos socios será de 30.000 €, que se destinará a financiar inversiones y necesidades de liquidez.

La constitución se hará mediante estatutos y escritura pública firmados ante notario y presentados posteriormente en el Registro Mercantil.

Se ha optado por este tipo de estructura por las ventajas que ofrece:

- relativa sencillez en cuanto a trámites burocráticos;
- costes de constitución asequibles;
- el nº de socios es el mínimo posible;
- respecto a un autónomo, las sociedades tienen mayor facilidad de acceso al crédito bancario.

18 PRÓXIMOS HITOS

En Septiembre 2017 se comenzará a hablar con algunos potenciales clientes para determinar cuánta producción necesitarían, así como con proveedores para garantizar su suministro.

Paralelamente se procederá a la creación de la empresa, aprovechando el dinero de los premios recibidos y de las aportaciónes de los socios. En este momento se podrá comenzar a suministrar el producto a dichos clientes.

RESUMEN EJECUTIVO

LOGO:

Descripción de la empresa

SWEET GREEN (S.L.) es una empresa que fabrica un edulcorante a base de xilitol. Nuestra empresa usa proceso totalmente natural que utiliza biomasa y agua como únicas materias primas.

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sweetgreensl87@gmail.com

Problema que resuelve

- Ofrecemos un producto con el mismo sabor del azúcar, con menos calorías, con efectos benéficos para la higiene bucodental, que ayude a reducir los problemas de obesidad y diabetes.

- utilizamos solamente agua como disolvente, reduciendo el impacto medioambiental y minimizando los costes asociados con la eliminación de residuos tóxicos;
- utilizamos residuos agrícolas y forestales proporcionados por agricultores conocidos y localizados en el territorio.

SECTOR:
Industria de base/ de bienes de consumo

Solución

El proceso es posible gracias a una tecnología novedosa que hemos patentado.

EQUIPO:
El equipo fundador tiene una elevada experiencia científica e ingenieril adapta al alto nivel tecnológico de la empresa.

Gianluca Gallina: socio capitalista y trabajador. Ingeniero químico.
Juan García Serna: socio capitalista no trabajador. Ingeniero químico. Profesor Docente Investigador. Sus trabajos se contratarán vía artículos 83 LOU.

Mercado

Durante el primer año de actividad los clientes reales hacia los que nos dirigiremos serán pequeñas pastelerías, herboristerías y tiendas ecológicas cuyas necesidades de este producto son más limitadas y por lo tanto, nos permitirían atender sus demandas con la producción actual.

A partir del segundo año, gracias a un incremento de producción debido a la

	construcción de una maquinaria más grande, los clientes a los cuales nos dirigiremos serán los mayores consumidores de azúcar en España y fabricantes de repostería, bebidas, golosinas y chicles. Promoviendo las propiedades únicas de nuestro producto esperamos empujar nuestros clientes a utilizar nuestro edulcorante en sustitución de los demás.
ALIANZAS/ PARTNERS: Nuestros principales aliados, así como proveedores de materias primas, serán agricultores conocidos ubicados en la zona de Castilla y León, que nos proporcionarán residuos agrícolas y forestales, asegurándonos su origen.	**Competencia** En España, los productores de edulcorantes alternativos son Nutrisweet SL y Zukán; las dos no producen edulcorantes naturales (no formulados químicamente). La mayoría de las industrias funcionan como distribuidores de edulcorantes. El mayor fabricante de xilitol al mundo es la compañía danesa Danisco (Du Pont), junto con otros proveedores de China.
INVERSIONES **Inversión inicial:** 37.300 € durante el primer año: 30.000 € derivante de capital social y 7.300 € de subvenciones. 150.000 € durante el segundo año: 22.000 € de tesoreria del año 1, 45.000 € de subvenciones y 90.000 € de préstamo bancario a largo plazo.	**Ventaja Competitiva** En España no existen productores de edulcorantes naturales (no formulados químicamente) alternativos al azúcar. En el mercado internacional no existe un producto que además de contener xilitol, siga un proceso totalmente ecológico como el nuestro.
USO DE LOS FONDOS • Alquiler maquinaria **(**año 1) • Instalaciones y maquinaria (año 2) • Publicidad y marketing • Salarios • Artículo 43 • Arrendamientos • Materiales laboratorio	**Modelo de Negocio. Tipo de modelo** Nuestro modelo de negocio es de tipo multiplicativo, ya que ofrecemos un producto con valor agregado hacia el cliente. Nuestro objetivo es atender a un número cada vez mayor de clientes, transmitiendo confianza y transparencia sobre nuestro producto y sobre nuestro proceso de producción.

	Hitos conseguidos y futuros ✓ Se ha conseguido patentar la tecnología necesaria para producir nuestro producto. ✓ Se ha conseguido una primera financiación de 6.000 euros para crear el capital social de la empresa, gracias al primer premio Vivero 2016 ➢ En Septiembre 2017 se procederá con la creación de la empresa, aprovechando el dinero de los premios recibidos y de las aportaciones de los socios.

	Año 1	**Año 2**	**Año 3**
INGRESOS	36.950	109.420	138.996
GASTOS	46.732	81.086	99.513
EBITDA	-9.782	28.334	39.482

CONCLUSIONS

HEMICELLULOSE PRODUCTION USING HOT PRESSURIZED WATER: FROM LAB TO PILOT SCALE.

In this thesis, the fractionation of wood from different tree species was thoroughly studied and developed, using hot-pressurized water as solvent. The investigation begun with the performance of experiments in a laboratory-scale reactor and ended up with the design, construction and test of a multistage pilot reactor.

1. *Search for optimal operating conditions for hemicellulose extraction using a semi-continuous laboratory scale reactor.*

Optimal conditions to maximize hemicellulose yield, avoiding the degradation of monosaccharides, were identified through experiments carried out with a laboratory-scale flow-through reactor, using *Eucalyptus globulus* wood as raw material. A final yield of C_5 sugars (detected with HPLC after hydrothermal extraction and acid hydrolysis of the liquid extract) of 67 %wt. was obtained at these conditions, corresponding to a temperature of 185 °C and a residence time below 2 min. The yield of hexoses was very low, as the temperature was not sufficiently high to extract and depolymerize cellulose. Yield of C_5 and C_6 was maximum (respectively 82 %wt. and 65 %wt.) at the highest temperature tested (285 ºC). On the other hand, the amount of degradation products was also maximum at that conditions, resulting to be 9.8 %wt. with respect to the total initial weight of the raw material. A total of 85.7 wt% of the pulp was extracted at this temperature, leaving a residual solid containing mainly lignin, with a few fibres of cellulose and hemicellulose oligomers.

2. Processing of lignocellulosic biomass derived oligomers and monomers, with subcritical and supercritical water, to obtain high value products.

About 80 wt%. of the pulp (including C_5, C_6 and degradation products) was extracted at 260 ºC and a residence time below 2 min, using holm oak chips as raw material in a semi-continuous reactor. Yield of total C_5 was around 90 % at these conditions, mainly

represented by oligomers. A little amount of 5-HMF, pyruvaldehyde, glycolaldehyde and lactic acid, deriving by the degradation of monosaccharides was detected at this stage of the process.

Outlet flow from the semi-continuous reactor was then mixed with a stream of supercritical water to hydrolyze the extracted oligomers of cellulose and hemicellulose into monomers, and subsequently transform monomers into higher value products. This operation was conducted in a continuous reactor with reaction times between 0.23 s and 12.5 s.

The main products of this further hydrolysis were lactic acid, glycolaldehyde and pyruvaldehyde.

High residence times favored the production of glycolaldehyde + pyruvaldehyde and the degradation of lactic acid to acetic acid. A final yield of glycolaldehyde + pyruvaldehyde of 24 wt%. (calculated as mass of product/mass of soluble material in raw biomass) was obtained with a temperature of 350 ºC and a reaction time of 8.6 s. Shorter reaction times lead to a highest formation of lactic acid (a yield of 25 wt% was obtained at 400 ºC and 0.23 s) and to a minor degradation of the same to acetic acid.

3. Investigation on hemicellulose extraction from 10 different species of tree; detection of a correlation between the composition of the biomass, its structure and the yield of hemicellulose extracted.

After investigating the optimal operating conditions for obtaining high yields in hemicellulose extraction, a study was carried out to identify which tree species were best suited for obtaining hemicellulose through extraction with pressurized hot water. This work was carried on with the collaboration of “Reaction Engineering and Industrial

Chemistry Laboratory" at Åbo Akademi (Turku, Finland) with Dr. Henrik Grenmán and Ak. Prof. Tapio Salmi.

Experiments conducted in a batch-wise cascade reactor at 160 ºC, with wood proceeding by 10 different species of tree, indicated that the highest yield of hemicellulose extracted and dissolved in the liquid phase was reached using eucalyptus as raw material (40.3 wt%).

Hemicellulose extraction was demonstrated to be disfavored by a high amount of cellulose or lignin in the raw material. In addition to the composition, the biomass structure greatly influenced the capacity of hot pressurized water to extract the hemicellulose.

This structure was indirectly studied through a mathematical model based on TGA analysis of the raw materials. Results showed that woods with high volatilization kinetics for lignin during a TGA, enhance the hemicellulose extraction, if lignin char formation kinetics is not also promoted.

4. *Scale-up of semi-continuous reactor from laboratory-scale to pilot-scale for the extraction of hemicelluloses from lignocellulosic biomass with liquid hot pressurized water.*

Knowledge about the hydrothermal extraction process, obtained at the laboratory-scale, allowed us to scale-up the flow-through reactor with a volume of 27.5 mL and to design a pilot reactor with 2000 mL. Extraction of hemicelluloses from *Catalpa bignonioides* wood was carried out with the 2 scale systems at 160 ºC and 170 ºC, demonstrating that a geometrical parameter of L/D=40, with a bed porosity of 0.71 and a liquid residence time of 6.0 min were efficient for such purpose.

A manifold of 5-reactors was designed, based on the working mechanism of the 1 pilot reactor. It was verified that this multistage system worked homogeneously, with no degradation or substantial modification of the liquid product when increasing its residence time within the system. The plant proved to be versatile and suitable for a continuous extraction of hemicelluloses, and can be considered in industrial technology.

5. Detailed description of the pilot plant and its operation.

A patent was written, specifying the characteristics, the detailed operation and , the novelties of the multistage pilot plant developed in this thesis.

6. Design of a business model and evaluation of the viability of a company created around the production and sale of xylitol.

Finally, a business model based on the modification of the product obtained by this pilot plant, proved the viability of the project. The business plan has been rewarded with two prizes.

FUTURE WORK

From the studies developed in this PhD, it can be concluded that the fractionation process of lignocellulosic biomass with hot pressurized water can be brought to industrial level, and the pilot-plant designed and constructed during this thesis is a good option for obtaining a fast, versatile and wieldy set-up. Despite that, some improvements are needed in the operations, to obtain products more pure in hemicellulose. We would like to get a real product that can be used for the manufacture of materials such as plastics or hydrogels, or which can be converted into xylitol by catalytic hydrolysis and hydrogenation.

As anticipated in chapter four, a first solution to improve the process could be to perform a two-step hydrothermal pre-treatment; the first at a temperature between 120 and 140 ºC, during which part of the undesirable soluble compounds would be eliminated, and the second at a temperature between 160 and 180 ºC to extract hemicelluloses.

Moreover, different biomasses, proceeding from wheat straw, beet pulp and spent coffee should be tested to obtain hemicelluloses with different composition and molecular weight respect to hemicelluloses proceeding from wood.

Post-treatments to concentrate and fractionate the hydrolysate would be also necessary, through the use of membranes and ultrafiltration technologies.

Once a pure product is obtained, next step would be the creation of hydrogels based on hemicelluloses, and the production of sugar alcohols through catalytic hydrogenation.

All these goals will be realized during the thesis of a new PhD student belonging to our research group.

RESUMEN

PRODUCCIÓN DE HEMICELULOSA UTILIZANDO AGUA CALIENTE PRESURIZADA: DESDE ESCALA LABORATORIO HASTA ESCALA PILOTO.

RESUMEN

El rápido crecimiento de la población humana y la consiguiente creciente demanda de alimentos, energía y agua son los desafíos más grandes que el mundo está enfrentando.

El cambio climático es otra amenaza grave para la humanidad y es necesaria una reducción significativa de los gases de efecto invernadero para evitar consecuencias destructivas para el medio ambiente. A lo largo de muchas discusiones impulsadas por la Comunidad de la Industria Química en el Foro Económico Mundial en 2008 y 2009, las biorrefinerías industriales fueron identificadas como una posible solución que puede ayudar a mitigar la amenaza del cambio climático y de la demanda aparentemente ilimitada de energía, combustibles, productos químicos y materiales.

El concepto de biorrefinería es análogo al concepto de refinería de petróleo, y asume la producción de combustible, productos químicos y energía de diferentes tipos de biomasa. La elección de qué tipo de biomasa usar, puede estar influenciada por factores económicos, ambientales o geográficos; también existe una dependencia directa entre la materia prima, la tecnología utilizada para su conversión y la gama de productos que se pueden obtener.

Las tecnologías de primera generación se basan en fermentar y destilar la glucosa contenida en los cultivos como maíz o caña de azúcar para producir etanol. Las críticas hacia el uso de la biomasa se dirigen principalmente a este tipo de tecnología, culpada por proporcionar una seria competencia para la producción de alimentos.

La biomasa lignocelulósica se considera la fuente más prometedora para la producción de biocombustibles y productos químicos. Los materiales lignocelulósicos pertenecen a materias primas de segunda generación y pueden obtenerse de diversas fuentes, tales como residuos de madera, residuos agrícolas o municipales, sin interferir con los cultivos

directos para el consumo humano. En una biorefinería lignocelulósica, las materias primas pueden ser pretratadas y fraccionadas en celulosa, hemicelulosa y lignina.

Los compuestos fenólicos de lignina se pueden utilizar para producir materiales como plásticos o adhesivos, la glucosa de la celulosa puede convertirse en metanol o productos químicos, mientras que otros productos químicos, combustibles, polímeros y materiales pueden obtenerse a partir de hemicelulosa. Residuos de celulosa, hemicelulosa y lignina, pueden ser utilizados para la cogeneración de energía y calor.

El primer paso para la conversión de biomasa en productos de valor añadido es el fraccionamiento de su estructura en sus tres principales componentes: hemicelulosas, celulosa y lignina.

En comparación con otros métodos como los pretratamientos ácidos o alcalinos, los pretratamientos hidrotérmicos (que utilizan agua presurizada a alta temperatura como disolvente y medio de reacción) ofrecen varias ventajas: no se utilizan solventes tóxicos o corrosivos, no se requieren reactores especiales y no hay costes asociados a la recuperación o eliminación de productos químicos.

Dependiendo del compuesto a extraer, se necesitan diferentes temperaturas. La extracción selectiva de extractivos hidrosolubles requiere temperaturas alrededor de 100 ° C, la extracción de hemicelulosa entre 140 y 190 ° C, mientras que la extracción de celulosa requiere temperaturas superiores a 240 ºC.

En la fase líquida, los compuestos extraídos experimentan un proceso de hidrólisis y degradación en el que, dependiendo de la temperatura y el tiempo de residencia, los oligosacáridos se despolimerizan en monosacáridos y luego en productos de degradación. Por lo tanto, la temperatura y el tiempo de residencia son parámetros fundamentales para aumentar la selectividad hacia ciertos productos que otros.

En esta tesis se investigó el fraccionamiento de madera proveniente de diferentes especies de árboles para extraer biopolímeros (especialmente hemicelulosas), operando con diferentes tipos de reactor (batch, semi-continuo y continuo).

El objetivo final era investigar la posibilidad de acercar a nivel industrial el proceso de agua caliente líquida, para la extracción de hemicelulosa y transformación en compuestos de alto valor, empezando con un sistema a escala laboratorio y llegando a diseñar y construir una planta piloto multilecho para fraccionamiento de biomasa.

Para alcanzar el propósito, se definieron los siguientes objetivos parciales, descritos en los 6 capítulos que constituyen la tesis:

Objetivos

1. Búsqueda de condiciones operativas óptimas para la extracción de hemicelulosa mediante un reactor semi-continuo a escala de laboratorio.
    - Fraccionamiento e hidrólisis de hemicelulosa y celulosa procedente de madera de *Eucalyptus globulus*, a diferentes temperaturas y diferentes caudales líquidos.
2. Procesamiento de oligómeros y monómeros derivados de la biomasa lignocelulósica, con agua subcrítica y supercrítica, para obtener productos de alto valor.
    - Desarrollo de un proceso hidrotermal que combina el fraccionamiento de la biomasa lignocelulósica en un reactor semi-continuo, con la hidrólisis de los componentes extraídos utilizando agua supercrítica.
    - Ajuste de las temperaturas de funcionamiento y de los caudales de líquido para aumentar la selectividad hacia diferentes productos de degradación.

3. Investigación sobre la extracción de hemicelulosa de 10 especies diferentes de árboles; Detección de una correlación entre la composición de la biomasa, su estructura y el rendimiento de hemicelulosa extraída.
    - Proceso llevado a cabo a temperatura constante en un reactor batch a cascada, situado en Åbo Akademi (Finlandia). El sistema permitió recoger muestras líquidas, con 5 tiempos de residencia diferentes, durante el mismo experimento. Algunas características técnicas del reactor fueron utilizadas en el diseño de la planta piloto, el objetivo final de esta tesis.
4. Escalado del reactor semi-continuo desde escala de laboratorio a escala piloto para la extracción de hemicelulosas de biomasa lignocelulósica con agua caliente líquida presurizada. La implementación de innovaciones tecnológicas permitió diseñar y construir una planta piloto multilecho con una operatividad continua, tiempos de parada mínimos y facilidad en la operación.
    - Estudiar la eficiencia del escalado y la capacidad del sistema en operar a diferentes temperaturas.
    - Estudiar la evolución de las características del extracto líquido al cambiar su tiempo de residencia dentro del sistema.
5. Descripción detallada de la planta piloto y su funcionamiento. Reivindicaciones sobre la novedad de la invención con respecto a sistemas existentes para la extracción de compuestos solubles en agua a partir de biomasa lignocelulósica.
    - Redacción de una patente nacional.
6. Diseño de un modelo de negocio y evaluación de la viabilidad de una empresa creada en torno a la producción y venta de xilitol.

- El modelo de negocio considera la producción de xilitol por hidrogenación catalítica del extracto líquido obtenido con la planta piloto desarrollada durante la tesis. Se centra en las características saludables, sociales y ecológicas de un edulcorante producido con agua como único disolvente.

Discusión de los resultados

En el **capítulo uno**, titulado como "Optimal conditions for hemicelluloses extraction from *Eucalyptus globulus* wood: hydrothermal treatment in a semi-continuous reactor", se constituye la primera etapa para acercarse a un proceso industrial rentable y verde.

En este trabajo se han evaluado condiciones óptimas para la extracción de hemicelulosa a partir de madera procedente de *Eucalyptus globulus*, en un sistema semi-continuo (Figura 1).

El eucalipto fue seleccionado como materia prima debido a su abundancia en el sur de Europa, a su bajo consumo de agua, a su rápido crecimiento y su eficiencia en la producción de material lignocelulosico.

Se fraccionaron muestras de 5 g de chips de madera, utilizando agua caliente presurizada, para producir azúcares (pentosas y hexosas) y un residuo sólido enriquecido en lignina. Se evaluaron cinco caudales entre 2.5 y 20 mL/ min y cuatro temperaturas entre 135 y 285 ºC, con el objetivo de maximizar la producción de azúcares, evitando la formación de productos de degradación. Se identificaron condiciones óptimas para la extracción de hemicelulosa a 185 ºC y 5 mL/min, lo que dio lugar a un rendimiento de pentosas de 67 % en peso, con 0,702% en peso de productos de degradación. Se pudo concluir que con una temperatura de 185ºC, tiempos de residencia de los sólidos en el reactor entre 20 y

40 min son perfectos para extraer hemicelulosas, y que tiempos de residencia de líquidos por debajo de 2,00 min evitan la formación de subproductos.

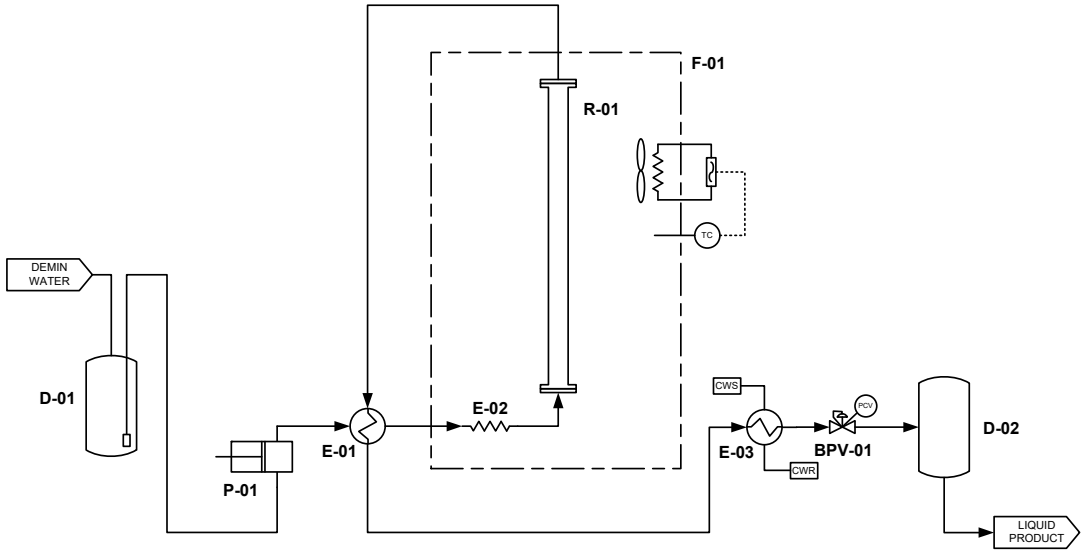


Figura 1. Diagrama de flujo esquemático del sistema experimental para el fraccionamiento de biomasa. Equipo: D-01 tanque de agua, P-01 bomba, E-01 pre-calentador de agua de alimentación, R-01 reactor, F-01 horno de convección de aire, E-02 capilar de precalentamiento, BPV-01 Valvúla de contrapresión, E-03 refrigerador del producto, D-02 recipiente de recogida del producto líquido.

En el **capítulo dos**, titulado como "Online integrated fractionation-hydrolysis of lignocellulosic biomass using sub- and supercritical water", se investigó la posibilidad de degradar selectivamente los productos extraídos con el sistema semi-continuo, para obtener productos químicos de alto valor.

El reactor semi-continuo (de fraccionamiento) se conectó en serie con un reactor continuo (de hidrolisis), como se representa en Figura 2. Se utilizó encina como biomasa lignocelulósica para ser tratada. Este proceso combinado realiza una valorización

selectiva de la biomasa lignocelulósica real, evitando el costoso proceso de molienda extrema necesario para la fluidización en un proceso hidrotérmico continuo.

En el reactor de fraccionamiento, la hemicelulosa y la celulosa se solubilizaron y se hidrolizaron parcialmente con el objetivo de alimentar el reactor de hidrólisis con concentraciones elevadas de C5 o C6. El fraccionamiento se realizó en dos etapas: a 180 ºC optimizando la extracción de hemicelulosa, y a 260 ºC extrayendo celulosa y la hemicelulosa que permanecia en la estructura de la biomasa. Se analizaron tres caudales de agua: 11, 17 y 26 mL/min. Se alcanzaron rendimientos de azúcar del 71 al 75%, compuestos principalmente de oligómeros de xilosa y glucosa y cantidades menores de otros productos químicos, como productos retroaldólicos, ácido acético o 5-HMF.

La corriente de salida del reactor de fraccionamiento se mezcló directamente con agua sub o supercrítica en el mezclador de entrada de un reactor continuo, donde el tiempo de reacción se podía controlar con precisión.

La temperatura, la presión y el tiempo de reacción se modificaron para obtener una idea de su efecto sobre el rendimiento de productos de condensación retroaldolica. Mediante este proceso se solubilizó hasta 64,2% de material lignocelulosico, principalmente como oligómeros de hexosas y pentosas, en parte como monosacárido y con una pequeña fracción de compuestos retroaldólicos.

Los principales productos de la hidrólisis adicional en el segundo reactor fueron glicolaldehído, piruvaldehído y ácido láctico.

Con largos tiempos de reacción (350 ºC, 160bar y 8.6 s) se encontró un rendimiento de 24% en peso de glicolaldehído-piruvaldehído, mientras con tiempos cortos de reacción se encontró un 25% en peso de ácido láctico (400ºC, 250 bares y 0.23 s).

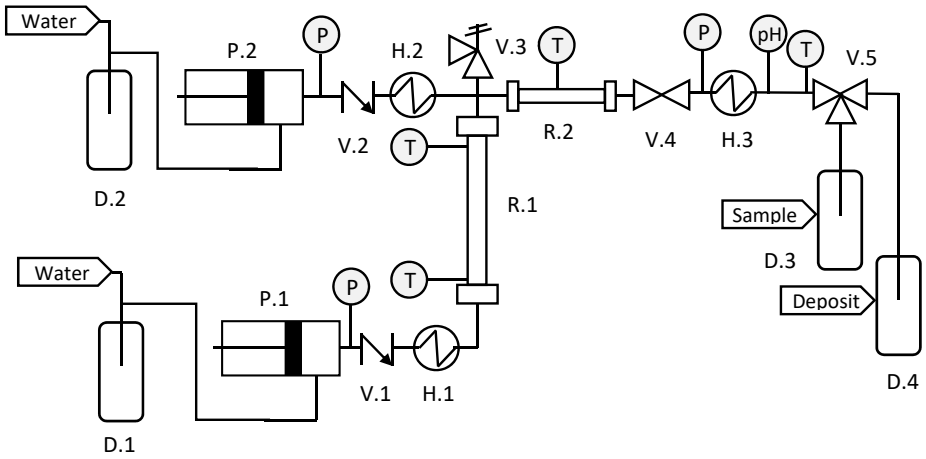


Figura 2. Configuración del sistema experimental acoplado para fraccionamiento e hidrólisis de biomasa. D.1, D.2: Depósitos de agua desionizada. P.1: Bomba de pistón de alta presión. P.2: bomba de membrana. V.1, V.2: válvula de retención. H.1: calentador eléctrico de baja temperatura (100 cm de tubería SS316 de 1/8 pulgada y resistencia eléctrica de 2 kW). H.2: calentador eléctrico de alta temperatura (1800 cm de tubería SS316 de 1/8 pulgada y resistencia eléctrica de 10 kW. R.1: reactor de fraccionamiento (40 cm de longitud, ½ pulg. Tubería SS316). V.2: válvula de alivio. R.2: segundo Reactor (SHR) construido con ¼ in O.D. SS316. Se utilizaron dos tamaños de reactores de 11 cm y 100 cm de longitud. V.3: válvula de alivio. V.4: válvula de alta temperatura Autoclave Engineers. IE: 200 cm de intercambiador de calor de tubo concéntrico ½ pulg. ¼ pulg. V5: válvula de tres vías. D.3: frascos Falcon®. D.4: depósito de recogida del producto líquido.

El **capítulo tres**, se titula "Raw material effect on hemicellulose extraction yield and molecular weight during hot pressurized water pretreatment by autohydrolysis". Se realizó un estudio exhaustivo sobre la extracción de hemicelulosa a partir de 10 especies arbóreas diferentes, típicas de la zona de Castilla y León. La extracción hidrotermal se realizó a 160 ºC mediante un reactor batch en cascada (Figura 3), ubicado en Åbo

Akademi (Finlandia). El objetivo fue buscar las especies con madera que permitieran obtener una alta concentración, rendimiento y / o peso molecular de hemicelulosas.

Se realizaron extracciones a varios tiempos de residencia (de 5 a 80 min). Se demostró que existe una relación entre el rendimiento de extracción (entre 9,7 y 40,3%), la composición de la materia prima y la estructura inicial de la biomasa, determinada indirectamente a través de análisis TGA. El mayor rendimiento se obtuvo con madera de eucalipto.

El peso molecular de los oligómeros variaba de una especie a otra (de 3,4 a más de 100 kDa). Se observaron tres tendencias: decaimiento de la masa molar con tiempo, máximo y mínimo de masa molar.

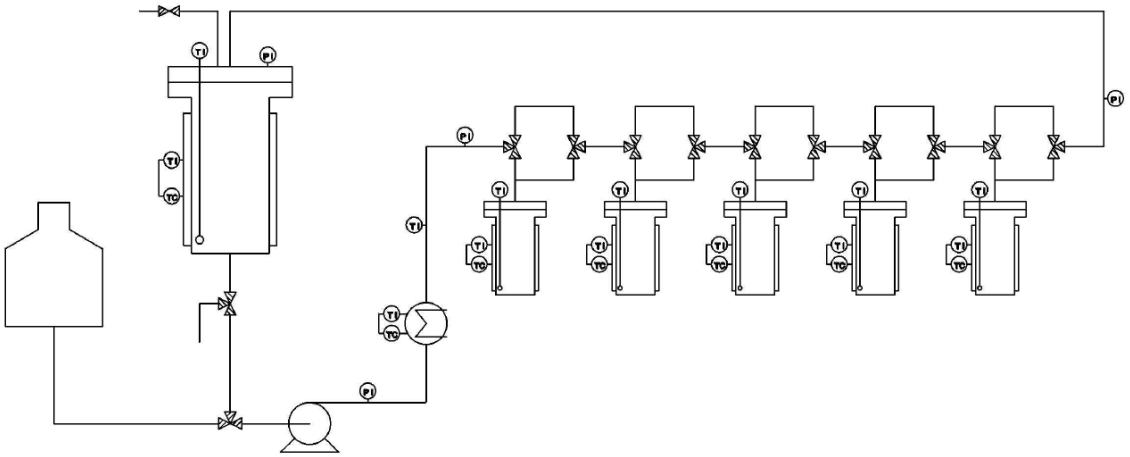


Figura 3. Esquema simplificado del reactor en cascada discontinuo utilizado en los experimentos. Equipo: V-01 Tanque de agua, V-02 Recipiente colector, P-01 Bomba, H-01 Calentador, R-01 / R-05 Reactores.

El **capítulo cuatro**, titulado “Hydrothermal extraction of hemicellulose from lab to pilot scale” es el capítulo central de esta tesis de doctorado. En este apartado se explica cómo se llevó a cabo el escalado de un reactor a escala laboratorio para la extracción de

hemicelulosas con agua caliente presurizada, para diseñar y construir una planta piloto multilecho.

Uno de los objetivos fue minimizar las dificultades de sustitución de la biomasa en el reactor, con el fin de facilitar el funcionamiento y mantenimiento del reactor piloto.

A escala de laboratorio, existen muchos métodos que pueden utilizarse para extraer el sólido gastado (algunos de ellos son métodos de "fuerza bruta", por ejemplo, perforación, empuje con aire comprimido, etc.) que son difíciles de usar a nivel piloto o industrial.

En un primer momento, se han establecido los criterios básicos para escalar un reactor semi-continuo con un volumen de 27.5 mL (cargado con 5 g de chips de madera) a un reactor piloto de 2000 mL (cargado con 400 g de astillas de madera), donde la biomasa podía ser reemplazada gracias a la inserción y extracción de un cartucho a través de una válvula de bola colocada en el fondo del reactor.

La unidad del reactor constaba de 3 partes principales (Figura 4a):

1. Un cilindro abierto, construido con una malla de metal (7) que se podía abrir longitudinalmente. El diámetro interno del cilindro era de 4 cm y la longitud era de 159 cm.
2. Dos cilindros de acero inoxidable del mismo diámetro (4 y 5), uno de los cuales (5) tenía varios orificios (con un diámetro de 1 mm) en el fondo (6), y funcionaba como filtro. Se podía colocar una capa de lana de vidrio sobre los orificios para disminuir la dimensión de los huecos.

   La malla metálica se insertaba entre los dos cilindros, formando un cartucho, que se llenaba con biomasa. El diámetro interior de los dos cilindros era de 4.3 cm; De modo que la malla adhirió perfectamente a las paredes. El grosor de los cilindros era de 2 mm.

3. Un cilindro exterior de acero inoxidable (2) con dos orificios (A y B) cerrados en el extremo superior con una brida mecanizada (1) con una abertura (C) y con una válvula de bola (3) atornillada en el extremo inferior. El diámetro interno de la válvula y del cilindro era de 5.1 cm, de manera que el cartucho podía introducirse completamente desde el fondo.

Este sistema facilitó en gran medida la sustitución de la biomasa, que podía ser retirada del sistema simplemente abriendo la válvula y sacando el cartucho. La abertura longitudinal de la malla de alambre, además, redujo el esfuerzo requerido para retirar la biomasa húmeda del cartucho.

Para determinar la efectividad del escalado, se realizaron experimentos con los dos sistemas a 160 y 170 ºC, utilizando madera de *Catalpa bignonioides* como materia prima, y comparando los rendimientos de hemicelulosa extraída y xilosa monomérica. Un parámetro de forma de L / D = 40, con una porosidad de lecho de 0.71 y un tiempo de residencia de líquido de 6.0 min han demostrado ser eficientes para tal fin.

Después de establecer la efectividad del escalado, se realizaron varias modificaciones en la planta piloto con 1 reactor, dirigidas a realizar un sistema que pudiera realizar la extracción hidrotermal de manera continua y rápida. El diseño completo de la planta se muestra en la Figura 4b. Se trata de una planta compuesta por cinco reactores semicontinuos, cada uno capaz de trabajar en serie con los demás o con la opción de poder ser excluido del sistema. Un sistema de válvulas y cartuchos hizo posible extraer la hemicelulosa de la biomasa sin necesidad de detener la planta. Incluso era posible enfriar y desmontar los reactores por separado durante las fases de carga y descarga, minimizando así el tiempo de inactividad. En este trabajo hemos demostrado la operación

con 3 reactores, los experimentos se realizaron a cuatro temperaturas diferentes (140, 150, 160 y 170 ºC), con un flujo de agua constante de 15 L/ h.

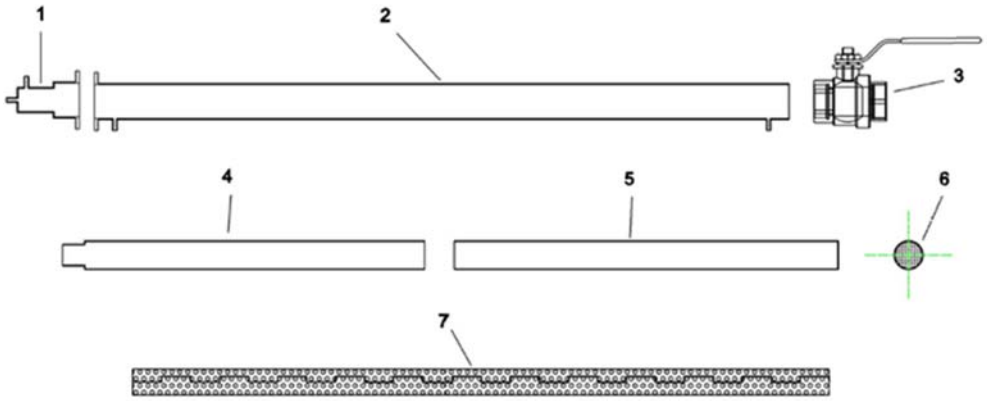
El proceso de extracción podía llevarse a cabo de una manera pseudo-continua, reemplazando rápidamente la materia prima y recogiendo totalmente el sólido agotado.

El sistema fue ampliamente probado mediante un estudio de temperatura, analizando el rendimiento de extracción de hemicelulosa y su peso molecular. Esta operación permitió estudiar las variaciones en las características de las hemicelulosas extraídas variando el tiempo de residencia del producto líquido dentro del sistema. También se estudió la despolimerización y degradación de los oligosacáridos variando su tiempo de residencia dentro de la planta.

Se verificó que los reactores funcionaban de forma homogénea y que, en las condiciones de funcionamiento adoptadas, no había degradación o modificación sustancial del producto líquido al aumentar su tiempo de residencia dentro del sistema. Por lo tanto, es posible trabajar continuamente, reemplazando la biomasa, sin cambios en la composición del producto. El mayor rendimiento en hemicelulosas, con una distribución homogénea de peso molecular, fue de alrededor del 40% obtenido al operar a 170 ºC.

Aunque se necesitan algunas mejoras en las operaciones, para obtener productos más puros en hemicelulosa, la planta descrita en este documento ha demostrado ser versátil y adecuada para su finalidad prevista y puede ser considerada en la industria.

a)



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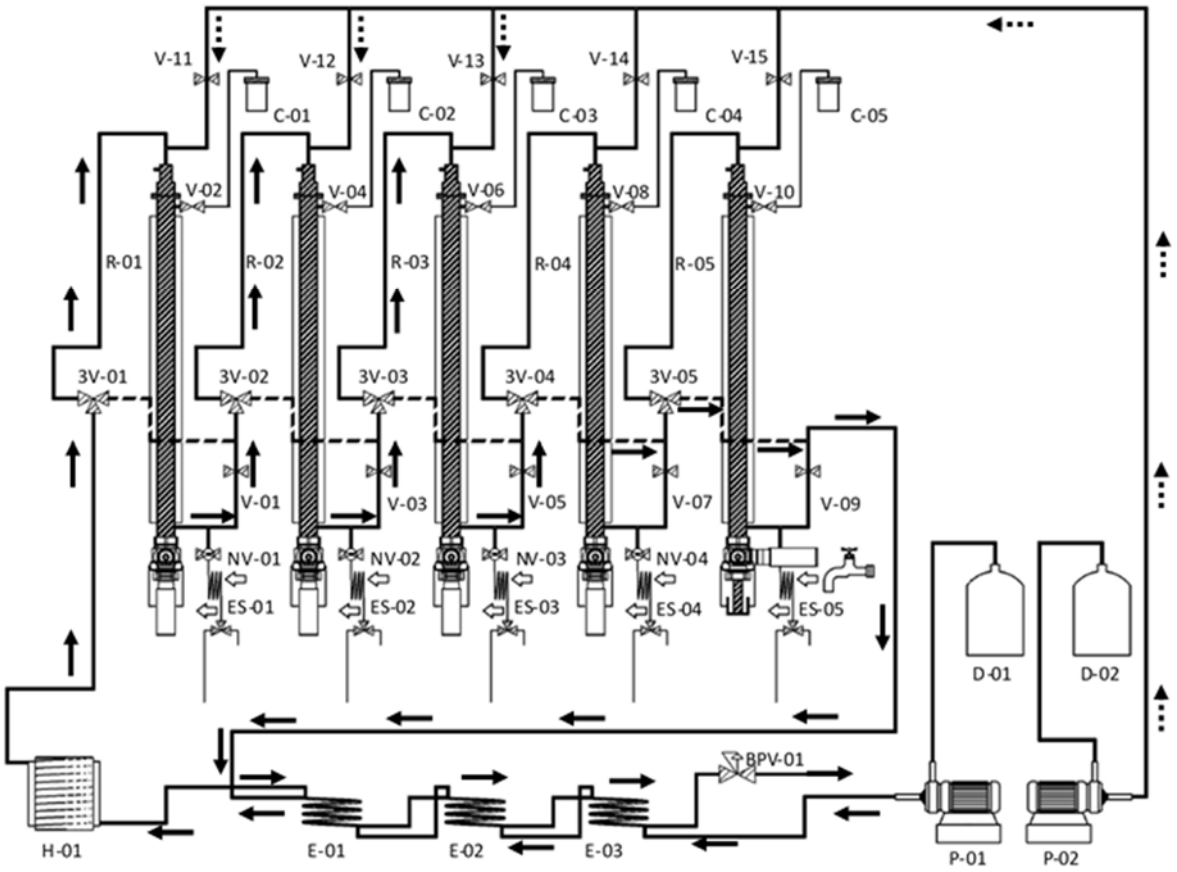

Figura 4. a) Sección del reactor: 1. Tapa del cilindro exterior; A, B, C. Aberturas; 2. Cilindro externo; 3. Válvula de bola; 4. Cilindro interno superior; 5. Cilindro interno inferior; 6. Filtro con orificios del cilindro inferior, 7. Malla metálica.

b) Diagrama de flujo del reactor multilecho donde tres unidades están operando en serie. Equipos: DX depósitos de agua, PX bombas, EX intercambiadores de calor de tubos concéntricos, ES-X intercambiadores de calor de tubos concéntricos para muestras retiradas, HX calentadores eléctricos, RX reactores, 3V-X válvulas de tres vías, BPV-X válvulas de contrapresión, VX válvulas de bola, NV-X válvulas de aguja, CX contenedores de plástico. Flujo de líquido que entra en los reactores R-01, R-02 y R-03 conectados en serie.

En el **capítulo cinco**, con título "Proceso y planta piloto multilecho para fraccionamiento de biomasa", hemos identificado las características y ventajas que distinguen la planta piloto mulilecho (Figura 5) de las instalaciones existentes, con el mismo propósito. Se ha redactado una patente para proteger nuestra tecnología y aumentar su valor a nivel industrial.

Figura 5. Imagen de la planta piloto multilecho.

Por último, en el **capítulo seis**, con título "Buisness plan. Sweetgreen, el edulcorante perfecto", se ha explorados la potencial comercialización de uno de los posibles productos que se pueden obtener mediante el procesamiento del efluente obtenido con nuestra tecnología.

La idea es producir un edulcorante a base de xilitol, obtenido por hidrólisis e hidrogenación de la hemicelulosa extraída con agua caliente presurizada, a partir de biomasa lignocelulósica. Se elaboró un plan de negocios basado en la venta de este edulcorante a industrias productoras de repostería, refrescos y caramelos. Los beneficios mutuos se consideran: los agricultores, pueden vender sus residuos, de lo contrario destinado a ser quemado, mientras que los clientes pueden obtener productos sin azúcar con beneficios para la salud y el medio ambiente.

Figura 7. Logotipo y nombre de la empresa propuesta.

Trabajos futuros

De los estudios desarrollados en este doctorado se puede concluir que el proceso de fraccionamiento de la biomasa lignocelulósica con agua caliente presurizada puede ser llevado a nivel industrial y la planta piloto diseñada y construida durante esta tesis es una buena opción para obtener un mecanismo rápido y versátil. A pesar de ello, se necesitan algunas mejoras en las operaciones, para obtener productos más puros en hemicelulosa.

Nos gustaría obtener un producto real que se pueda utilizar para la fabricación de materiales tales como plásticos o hidrogeles, o que se puedan convertir en xilitol por hidrólisis catalítica e hidrogenación. Una primera solución para mejorar el procedimiento podría ser realizar un pretratamiento hidrotermal en dos etapas: el primero a una temperatura entre 120 y 140 ºC, durante el cual se eliminaría parte de los compuestos solubles indeseados y el segundo a una temperatura entre 160 y 180 ºC para extraer hemicelulosas.

Además, se deberían ensayar diferentes biomasas, procedentes por ejemplo de salvado de trigo, pulpa de remolacha y café agotado, para obtener hemicelulosas de diferente composición y peso molecular respecto a las hemicelulosas procedentes de la madera.

También se necesitarían post-tratamientos para concentrar y fraccionar el hidrolizado, mediante el uso de membranas y tecnologías de ultrafiltración.

Una vez que se obtiene un producto puro, el siguiente paso sería la creación de hidrogeles a base de hemicelulosas, y la producción de alcoholes de azúcar mediante hidrogenación catalítica.

Todos estos objetivos se realizarán durante la tesis de un nuevo estudiante de doctorado perteneciente a nuestro grupo de investigación.

AKNOWLEDGEMENTS

AGRADECIMIENTOS

RINGRAZIAMENTI

We got to the queue titles. A moment a little happy, a bit melancholy. A sense of accomplishment and insecurity. And this is also done, and now?

But stop, for a moment do not look to the future, but to the past, and to the present.

A countless number of people have volunteered and involuntarily contributed to the realization of this thesis. Not only in a literal way, but also in words (one or many, said or thought), gestures, love, disappointments, drinking, sorrows, glances, smiles, handshakes, hugs.

Unfortunately, until now, no technology has been invented that converts thoughts into words, and as the title of this section is "Acknowledgments" and not "The life of Gianluca", let's go through a list of names. Thanks.

Thanks to Juan and Pierdomenico, THE Doctors, my directors and friends. Thank you for supporting and open mind, encouraging me to overcome and reach new goals.

Thanks to Maria José Cocero and to all the professors, who allowed me to join this group, supporting me scientifically and humanly.

Thanks to Tapio Salmi, who welcomed me in Finland before and after the beginning of this thesis; a wonderful person and scientist. A pillar in my path.

Thanks to Henrik Grénman, who directed me with passion, consistency and dedication during my stay in Finland

Thanks to the lab technicians who helped me, from whom I learned a lot. They are an essential part of this work and all the research.

Thanks to my friends, everyone, in equal measure. During this time, we spent fun moments and made me feel in a big family.

AKNOWLEDGEMENTS

Grazie ad i miei genitori. Vito e Luciana. Un grazie profondo. Per ogni cosa. Grazie per tutto il bene che mi volete e che mi avete voluto. Sempre.

Grazie a Nerea, mia compagna, amica e sostenitrice. Il premio più grande di questo percorso.

And finally the sponsor.

MEng. Gianluca Gallina wish to acknowledge the Spanish Economy and Competitiveness Ministry for the scholarship/predoctoral contract BES-2013-063556.

Project CTQ2015-64892-R (MINECO/FEDER).

ABOUT THE AUTHOR

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Gianluca Gallina (Montebelluna-Italy, 1987) started the studies of Chemical Engineering at the University of Padova in 2006 and graduated in 2011. Afterwards he gained a scholarship financed by an industry (SCAM), about "granulation of fertilizers and scale up of a high shear mixer" to study the operative conditions of an industrial plant for the production of organomineral fertilizers. Suddenly he gained another scholarship, from another company (ALPRETEC), which produced a special silk tissue with an antimicrobial. His aim was to characterize this material through some biochemical analysis.

On June 2013 he started a scholarship (summer job) in Åbo Akademi University in Turku (Finland). The project was about the direct synthesis of Hydrogen Peroxide with a trickle bed reactor. This experience has been very interesting and educative: he learned a lot about green chemistry, plant engineering, catalysis, chemical processes and kinetics.

His interest for green processes and environmental engineering convinced him to start his PhD in the High Pressure Processes Group of the Department of Chemical Engineering (University of Valladolid) in February 2014.

His PhD was focused in the extraction of hemicellulose from different residual biomasses as for example wood branches from pruning or beet pulp.

He used different kinds of reactors: batch, semi-continuous or continuous, in order to evaluate the efficiency on the extraction of hemicellulose, until he designed and built a pilot plant for the extraction of hemicellulose consisting in a multistage semi-continuous reactor, which allowed to operate in a continuous way thanks to an innovative system for the loading and downloading of the biomass. The idea of this project was born as a scale-up of a laboratory scale semi-continuous which he used at the beginning of my PhD. With

this system he won the prize Prometeo (promoting market-oriented prototypes) which offered the possibility to patent the technology.

From April to July 2015 he went back to Åbo Akademi University in Turku (Finland); where he worked in the extraction of hemicellulose from lignocellulosic biomasses using a batch cascade reactor. Åbo Akademi is one of the first universities which started to study lignocellulosic biomasses, and he could learn from the experience of many "guru" of the topic.

His interest in the transference of knowledge from academia to industry made him participate in the Vivero competition (2016), which aimed to train entrepreneurs and to help creating new technology-based companies based on the results of research projects and technologies of the universities of Castilla y León. He won the first price. Consequently, he participate in the Yuzz Valladolid program, which offered training, support and advice throughout the development of his business plan. He won the elevator pitch competition and the prize for the best technology.

ABOUT THE AUTHOR

ACADEMIC TRAINING

- Bachelor's degree in Chemical Engineering at University of Padova (Italy), 2006-2009.
- Master's degree in Chemical Engineering and Industrial Processes at University of Padova (Italy), 2009-2011.

SCHOLASHIPS

- Project financed by SCAM: study on granulation of organomineral fertilizers, determination of optimum operating conditions and scale up of high shear mixer. University of Padova (Italy), 2012 (8 months)
- Project financed by ALPRETEC: development of methods for the characterization of powder of silk fibroin treated with an antimicrobial. University of Padova (Italy), 2013 (2 months)

STAYS IN FOREIGN RESEARCH INSTITUTES

- Johan Gadolin Process Chemistry Centre, Laboratory of Industrial Chemistry and Reaction Engineering (Åbo Akademi, Finland), in 2013 (8 months). Topic: Direct synthesis of hydrogen peroxide with a trickle bed reactor.
- Johan Gadolin Process Chemistry Centre, Laboratory of Industrial Chemistry and Reaction Engineering (Åbo Akademi, Finland), in 2015 (3 months). Topic: Lignocellulosic Biomass fractionation in batch cascade Reactor.

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- **G. Gallina**, Á. Cabeza, P. Biasi, J. García-Serna, "Optimal conditions for hemicelluloses extraction from Eucalyptus globulus wood: hydrothermal treatment in a semi-continuous reactor" *Fuel Processing Technology*, 2016, 350–360.
- C. M. Piqueras, Á. Cabeza, **G. Gallina**, D.A. Cantero, J. García-Serna, M. J. Cocero, Online integrated fractionation-hydrolysis of lignocellulosic biomass using sub- and supercritical water" *Chemical Engineering Journal*, 308, 2017, 110–125.
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SUBMITTED

- **G. Gallina**, Á. Cabeza, H Grénman, P. Biasi, J. García-Serna, T. Salmi, Raw material effect on hemicellulose extraction yield and molecular weight during hot pressurized water pretreatment by autohydrolysis, *Journal of supercritical fluids.*
- **G. Gallina**, E. Regidor-Alfageme, P. Biasi, J. García-Serna, Hydrothermal extraction of hemicellulose from lab to pilot scale, *Bioresources Technology*.

BOOK CHAPTERS

- **G. Gallina**, P. Biasi, C.M. Piqueras and J. García-Serna, Processing of Lignocellulosic Biomass Derived Monomers using High-pressure CO2 and CO2–H2O Mixtures, *High Pressure Technologies in Biomass Conversion, RSC.*

PATENTS

- **G. Gallina** and J. García-Serna. **Patente Nacional solicitada.** Proceso y planta piloto multilecho para fraccionamiento de biomasa

CONTRIBUTIONS TO CONGRESSES

ORAL COMUNICATIONS

- A. Zamuner, **G. Gallina**, P. Brun, G.M. Messina, G. Iucci, I. Castagliuolo, G. Polzonetti, G. Marletta and M. Dettin. HYDROGEL DECORATED WITH ADHESIVE SEQUENCES AND GROWTH FACTORS AT TISSUES-PROTHESIS

INTERFACE". 1th International Conference on Materials in Medicine (MiMe). 8-11 October 2013, Faenza (Italy).

- **G. Gallina**, P. Biasi , J. García-Serna, T. Salmi, J-P Mikkola. Bromide and acid effect elucidation for the hydrogen peroxide direct synthesis". 4th International Congress on Green Process Engineering (GPE-4th). 7-10 Abril 2014, Sevilla (Spain).
- **G. Gallina**, J. García-Serna, P. Biasi and M. J. Cocero. Pilot-scale multistage semi-continuous reactor for the extraction of hemicellulose. 10th European Congress of Chemical Engineering (ECCE10). 27 September – 1 October 2015, Nice (France).
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- **G. Gallina**, J. García-Serna, P. Biasi, H. Grenman, T. Salmi. How structure and composition of raw material influence hemicellulose extraction during hot pressurized water pretreatments. 13th International Conference on Renewable Resources & Biorefineries (RRB13). 7-9 June 2017, Wroclaw (Poland).
- **G. Gallina**, J. García-Serna, P. Biasi and M. J. Cocero. New pilot plant for hemicellulose extraction with hot pressurized water. 10th World congress of Chemical Engineering (WCCE10). 1-5 October 2017, Barcelona (Spain).

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- **G. Gallina**, J. García-Serna, P. Biasi and M. J. Cocero. "Fractionation of biomassfrom *Eucalyptus globulus* using subcritical water" 10th International Conference on Renewable Resources & Biorefineries (RRB10). 4-6 June 2014, Valladolid (Spain).

- **G. Gallina**, J. García-Serna, P. Biasi and M. J. Cocero. "Pilot-Scale multistage semi-continuous reactor for the extraction of hemicellulose". 11th International Conference on Renewable Resources & Biorefineries (RRB11). 3-5 June 2015, York (UK).
- **G. Gallina**, J. García-Serna, P. Biasi and M. J. Cocero. Selective fractionation and depolimerization of lignocellulosic biomass using subcritical and supercritical water to produce hemicellulose, cellulose and lignin". 5th International Congress on Green Process Engineering (GPE2016). 19-24 June 2016, Mont Tremblant, Quebec (Canada).

PRIZES

- **Premio Prometeo convocatoria 2016** (organized by Fundación General de la Universidad de Valladolid), "Planta Piloto Multilecho para Fraccionamiento de Biomasa".
- **1° Premio Vivero 2016** (organized by Fundación Universidades y Enseñanzas Superiores de Castilla y León –FUESCYL and Banco Santander), **"**HEMICELULOSA**"** Fraccionamiento de biomasa para la obtención del biopolímero hemicelulosa y una amplia gama de productos a base de la misma con diferentes aplicaciones.
- **Finalista Tecnología Disruptiva Yuzz Valladolid** (organized by Banco Santander, CISE and Parque Cientifico UVA), "SWEET GREEN, el edulcorante perfecto"

SUPPLEMENTARY MATERIAL

HEMICELLULOSE PRODUCTION USING HOT PRESSURIZED WATER: FROM LAB TO PILOT SCALE.

CHAPTER 2

ONLINE INTEGRATED FRACTIONATION-HYDROLYSIS OF LIGNOCELLULOSIC BIOMASS USING SUB- AND SUPERCRITICAL WATER.

Figure S1. Milled Holm oak utilized as model lignocellulosic biomass

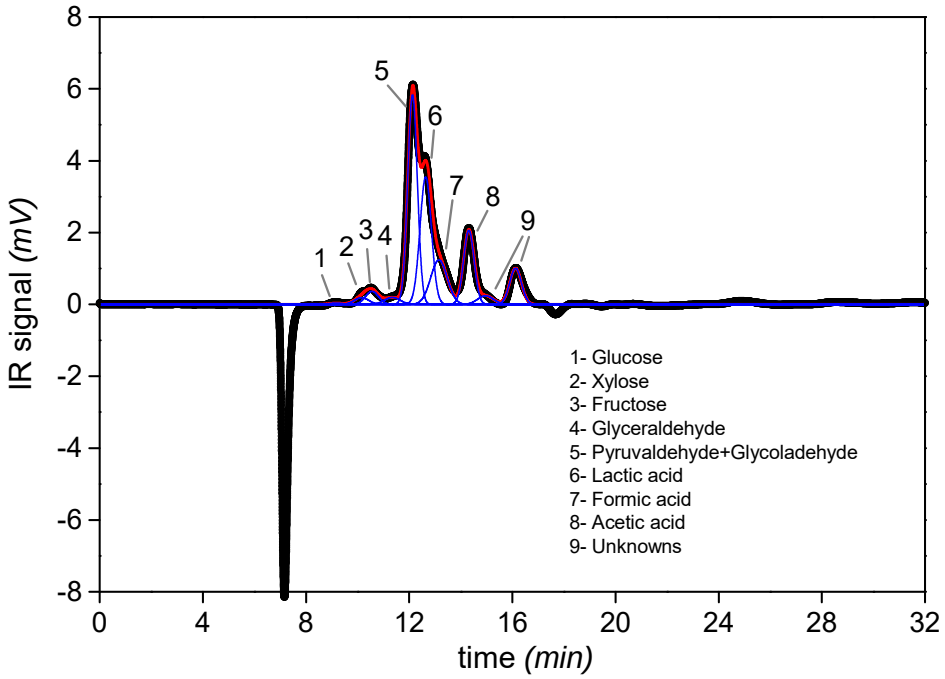


Figure S2. HPLC chromatogram including peaks deconvolution and peaks assignation

CHAPTER 3

RAW MATERIAL EFFECT ON HEMICELLULOSE EXTRACTION YIELD AND MOLECULAR WEIGHT DURING HOT PRESSURIZED WATER PRETREATMENT BY AUTOHYDROLYSIS.

Appendix 1 - Calculation of yields

Reaction yields in the reactors was calculated as follows:

Volume of the system

At the beginning of the experiment (t_0) we measured the volume of the water contained in the system (V_0). At every sampling time, the volume of the system is calculated as:

$V(t)=V_0-\sum Vr_i(t-1)$

Where $Vr_i(t-1)$ is the volume of water contained in the unit removed after the previous sampling time.

So, at a sampling time of 5 min, the volume of the system is V_0; at a sampling time of 10 min, the volume is $V_0-Vr_{(5min)}$ and so on.

Mass of wood in the system

Mass of wood at the beginning of the experiment correspond to the sum of the mass of dry wood contained in every unit.

$Mw_0=\sum Mwr_i$

At every sampling time, the mass of wood in the system is calculated as:

$Mw(t)= \sum Mwr_i(t-1)-Msext(t-1)$

Where $\sum Mwr_i(t-1)$ indicates the mass of wood contained in the reactors at the sampling time, while Msext(t-1) correspond to the total mass of compounds extracted, measured at the previous sampling time.

Mass of hemicellulose extracted

Mass of hemicellulose extracted is calculaed as:

$Mhext(t)= Chext(t)*V(t)- Mhext(t-1)$

Where Chext(t) indicated the concentration of hemicellulose measured in the unit removed at time (t), while Mhext(t-1) indicates the mass of hemicellulose extracted at the previous sampling time.

Mass of hemicellulose in the wood

Concentration of hemicellulose contained in the wood at time 0 (Chw_0) was measured. Mass of hemicellulose contained in the wood at time 0 is calculated as:

$Mhw_0 = Chw_0 * Mw_0$

At sampling time, mass of hemicellulose extracted is subtracted from the mass of hemicellulose contained in the wood at the previous sampling time.

$Mhw(t) = Mhw(t-1) - Chext(t)$

Concentration of hemicellulose contained in the wood at sampling time is calculated as

$Chw(t) = Mhw(t) / Mw(t)$.

Yield of hemicellulose extracted

Yield of hemicellulose extracted is equal to:

$Yhext = (Mhw_0 - Mhw(t)) / Mhw_0$.

Appendix 2 - Figures

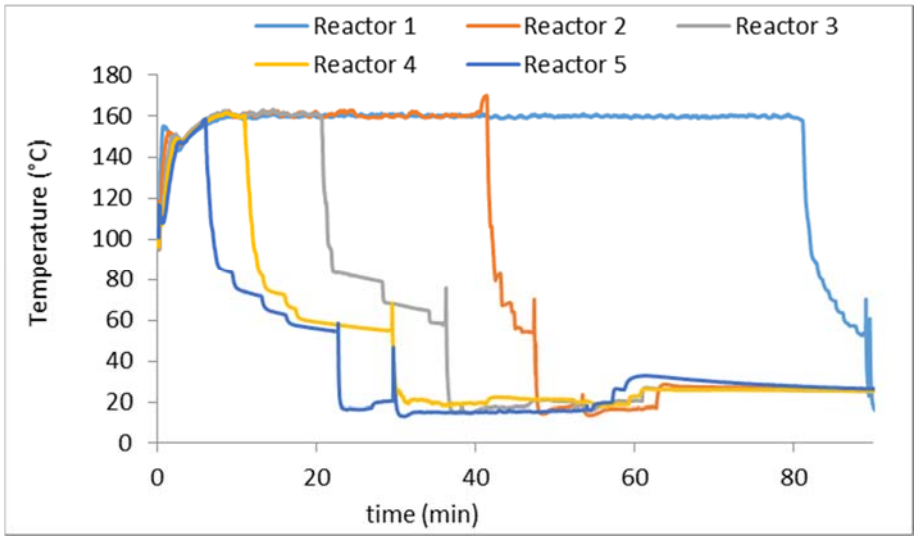
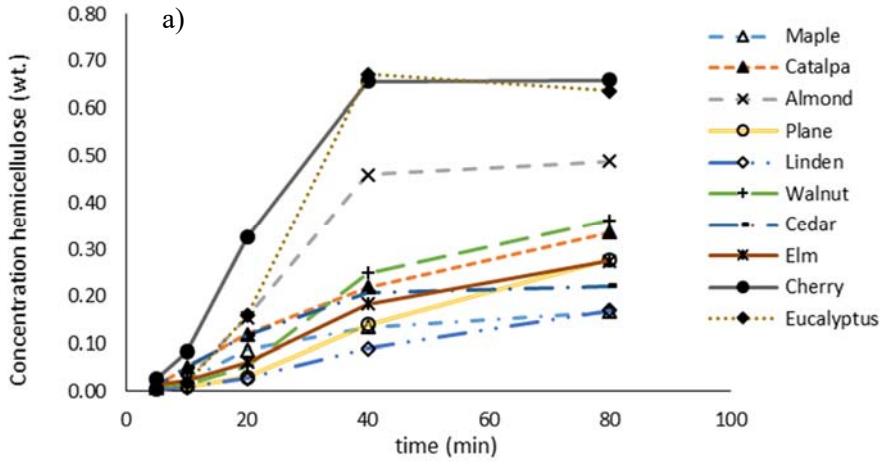


Figure S1. Standard temperature profile followed during the experiments.



b)

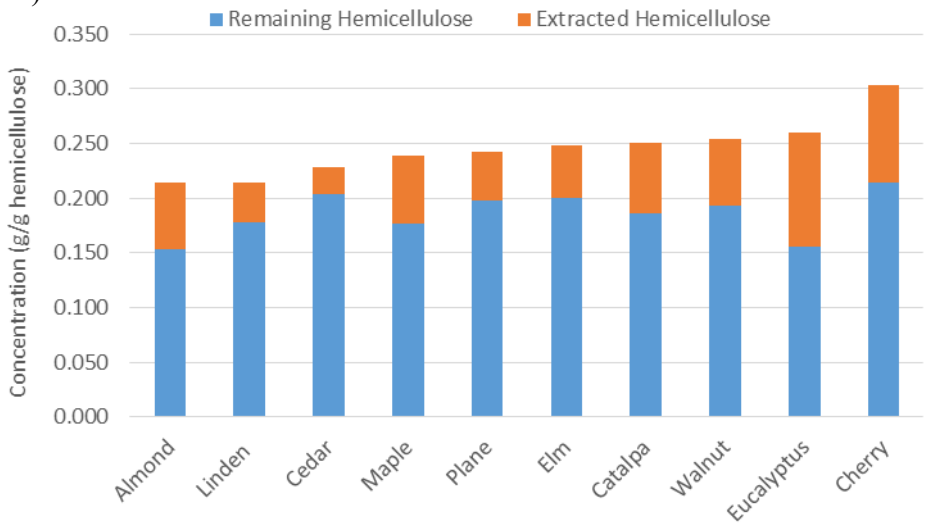


Figure S2. a) Concentration of hemicellulose extracted at different extraction times from the different raw materials. b) extracted hemicellulose vs total hemicellulose content for different raw materials.

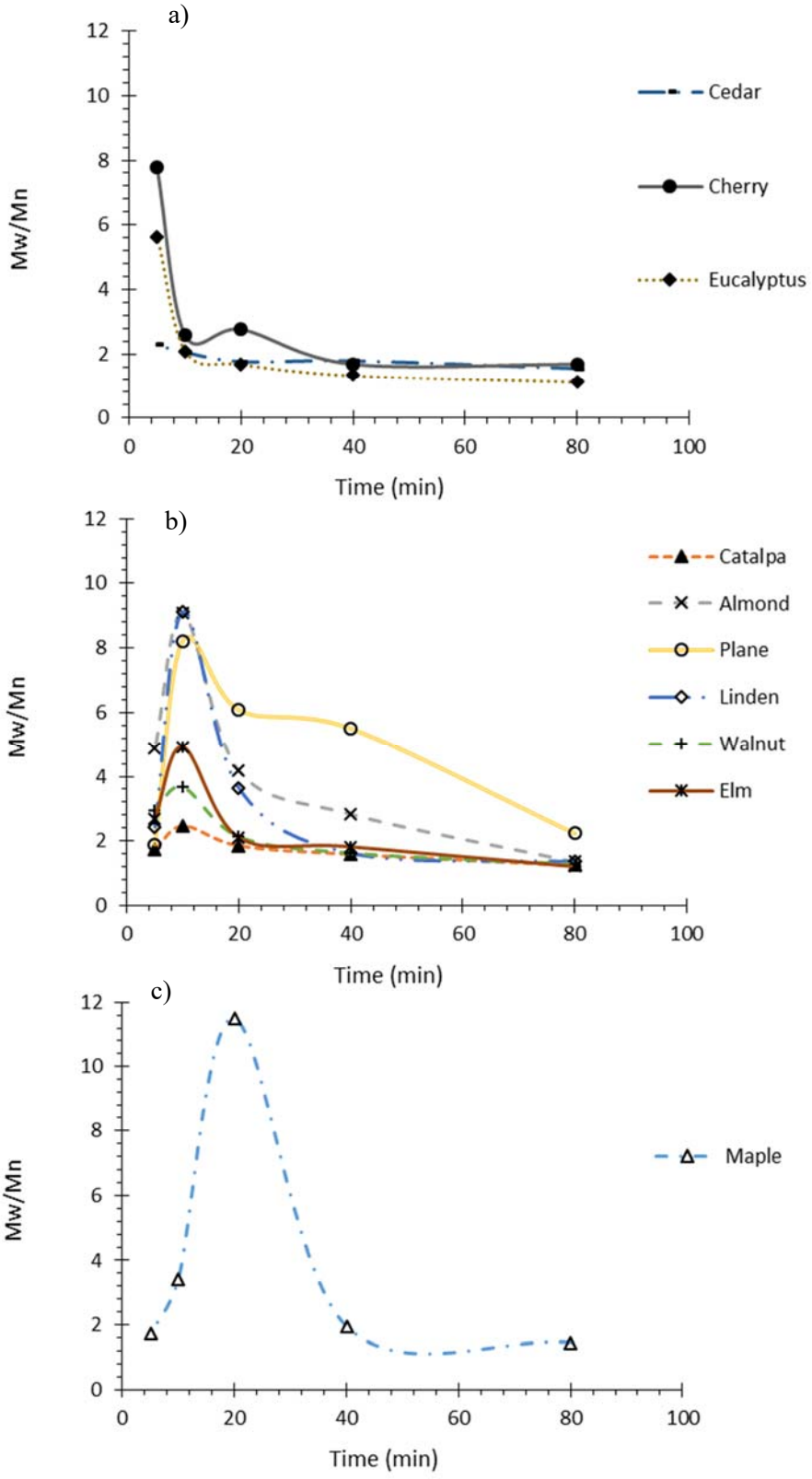


Figure S3. Polidispersity of oligomers extracted from different species of tree.

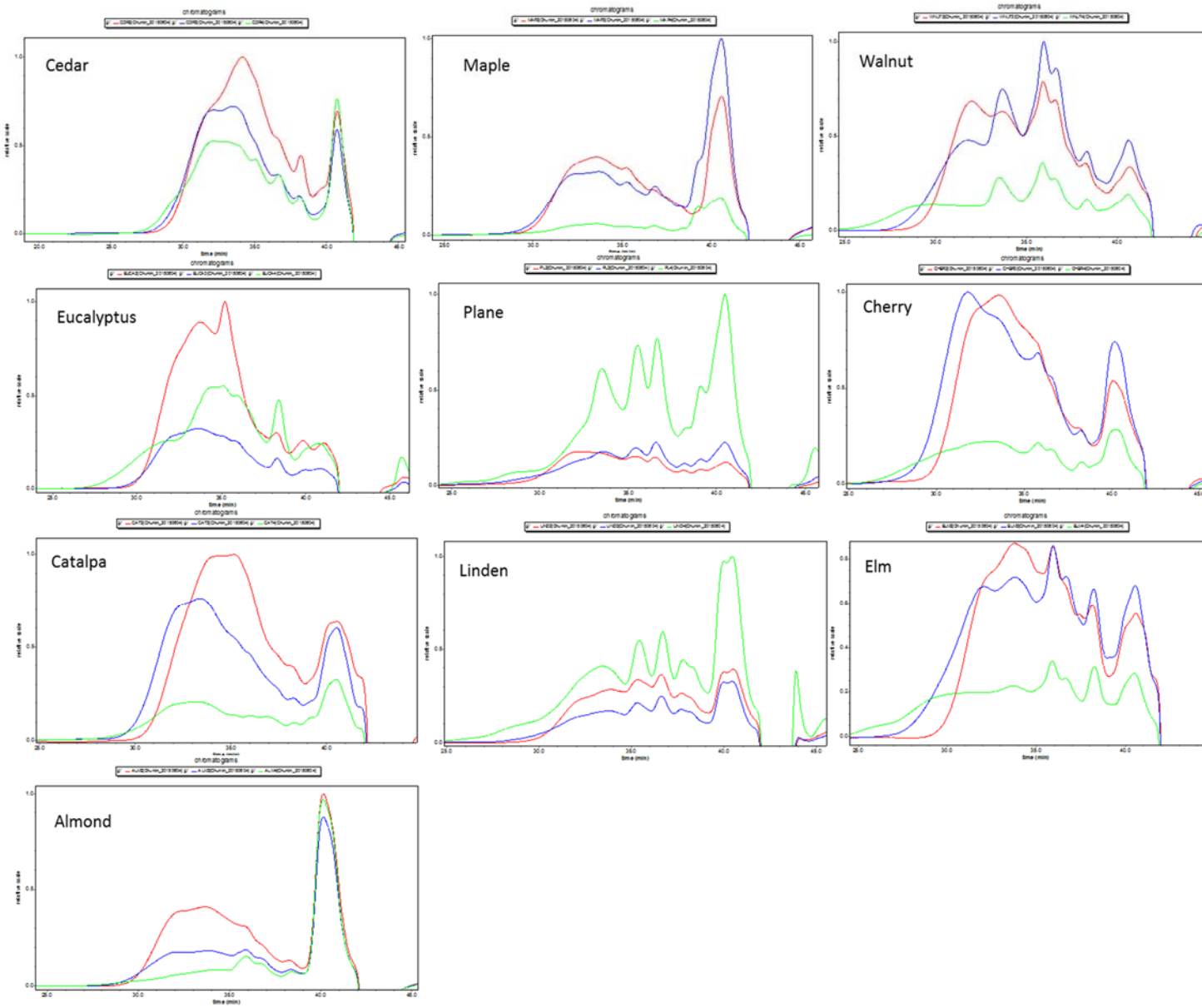
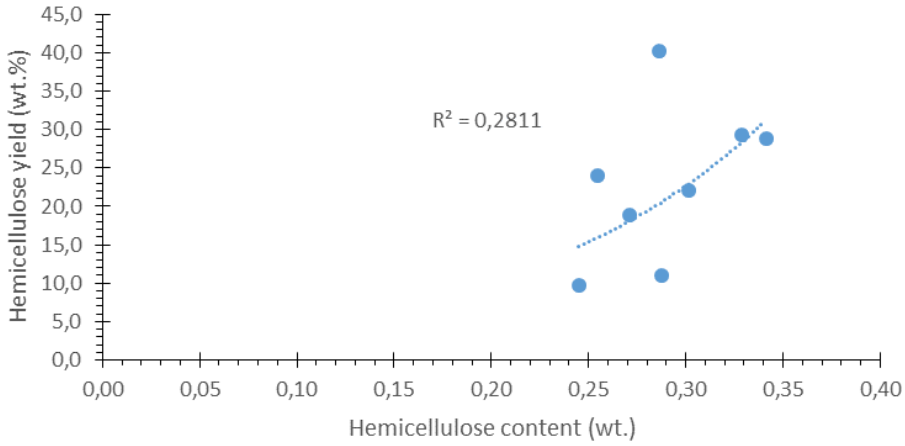
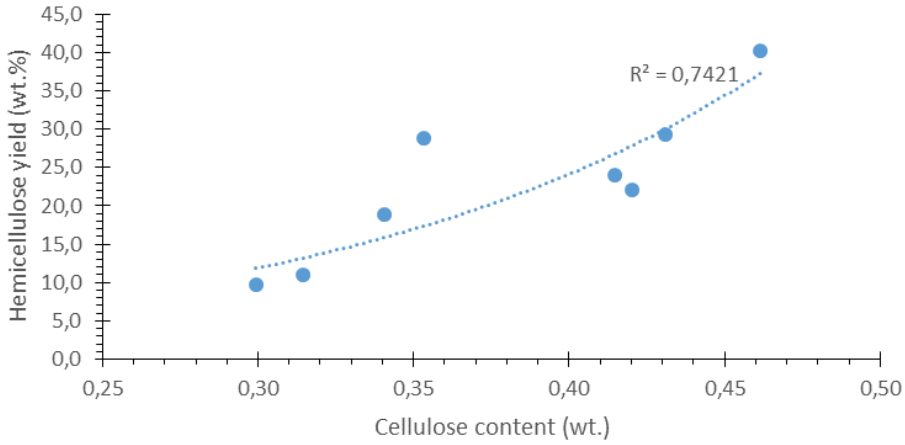


Figure S4. Molecular weight distributions of oligomers extracted from different species at 10 min (green), 20 min (blue) and 40 min (red).

a)


Hemicellulose yield (wt.%)
45,0
40,0
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
R² = 0,2811
0,00
0,05
0,10
0,15
0,20
0,25
0,30
0,35
0,40
Hemicellulose content (wt.)

b)


Hemicellulose yield (wt.%)
45,0
40,0
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
R² = 0,7421
0,25
0,30
0,35
0,40
0,45
0,50
Cellulose content (wt.)

c)

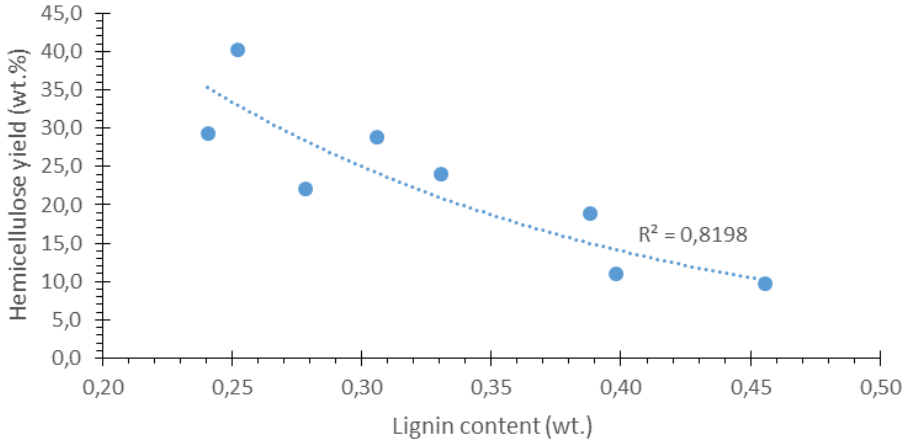

Hemicellulose yield (wt.%)
45,0
40,0
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
R² = 0,8198
0,20
0,25
0,30
0,35
0,40
0,45
0,50
Lignin content (wt.)

Figure S5. Hemicellulose yield evolution with cellulose (a) and lignin content (b) without *catalpa* and *elm*.

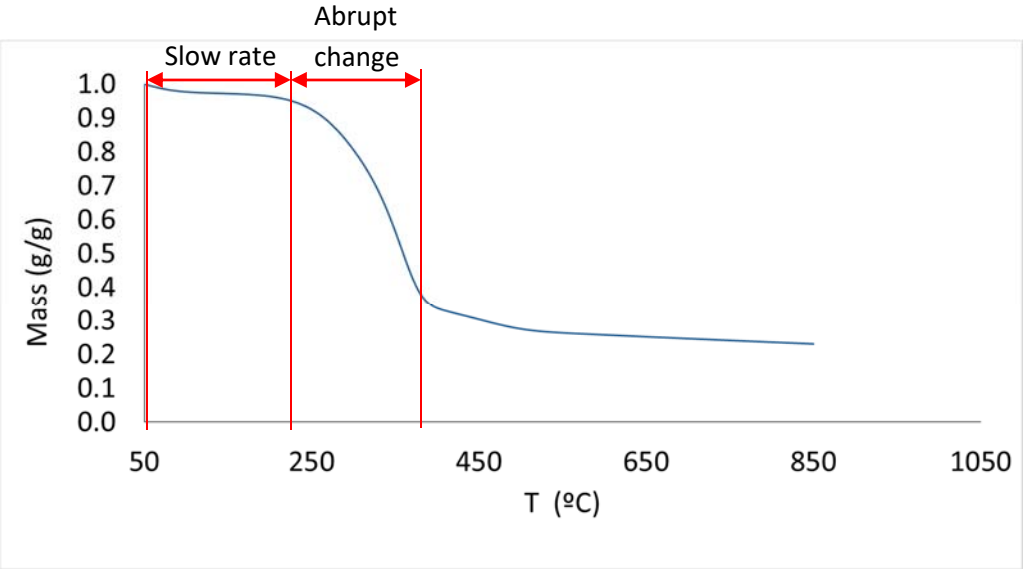


Figure S6. Almond experimental TGA

Appendix 3 – TGA modelling data and results

Table 1S. Absolute deviation for the TGA fittings and hemicellulose extraction yield estimation

	AAD[1] %	AAD[2] %
Walnut	3.70	1.71
Linden	32.27	1.09
Plane	12.31	1.44
Elm	0.01	0.84
Eucalyptus	16.47	0.94
Cherry	12.82	0.85
Cedar	28.20	1.45
Catalpa	0.01	1.56
Maple	0.41	1.19
Almond	20.56	0.82
	12.68	1.19

[1] Yield estimator fitting.

[2] TGA fitting.

Table 2S. Kinetic parameters obtained from the TGA fitting

	k_1*	k_2	k_3	k_4	k_5	k_6	k_7	k_8	k_9	Ea_1/R	Ea_2/R	Ea_3/R	Ea_4/R	Ea_5/R	Ea_6/R	Ea_7/R	Ea_8/R	Ea_9/R	β_1	β_2	β_3	β_4	β_5	β_6	β_7	β_8	β_9
	min^{-1}	min^{-1}	min^{-1}	min^{-1}	min^{-1}	min^{-1}	min^{-1}	min^{-1}	min^{-1}	K	K	K	K	K	K	K	K	K	-	-	-	-	-	-	-	-	-
Walnut-RM**	22,430	9,074	25,377	19,274	0.030	17,769	3,821	0.299	0.398	6,745	12,055	8,119	7,242	347	10,059	8,456	399	109	0.6909	1.477	1.544	1.701	0.524	2.005	2.226	0.839	2.605
Almond	22,041	8,614	25,139	18,977	0.030	17,791	3,923	0.299	0.398	6,546	12,143	9,185	7,934	346	9,995	8,393	399	109	0.689	1.472	1.550	1.702	0.518	2.005	2.226	0.839	2.605
Maple	25,828	5,818	25,897	20,469	0.042	17,656	4,495	0.303	0.385	6,514	11,942	7,166	7,012	349	10,365	8,141	399	111	0.718	1.338	1.532	1.684	0.537	2.006	2.229	0.839	2.607
Catalpa	20,543	6,375	25,467	19,437	0.046	17,435	1,004	0.308	0.431	6,791	12,215	7,945	7,239	349	10,988	10,058	399	110	0.729	1.465	1.526	1.698	0.550	2.010	2.228	0.839	2.605
Cedar	22,364	8,660	25,607	19,330	0.033	17,754	3,851	0.299	0.398	6,485	12,362	7,656	7,247	347	10,100	8,437	399	109	0.687	1.464	1.542	1.702	0.532	2.005	2.226	0.839	2.605
Cherry	22,339	7,913	25,122	18,924	0.030	17,843	3,852	0.298	0.398	6,549	12,083	9,335	8,045	347	9,842	8,437	399	109	0.688	1.436	1.547	1.704	0.525	2.004	2.226	0.839	2.605
Eucalyptus	22,324	8,713	25,209	18,953	0.028	17,831	3,851	0.299	0.398	6,617	12,018	9,211	7,995	347	9,878	8,437	399	109	0.688	1.454	1.547	1.703	0.531	2.004	2.226	0.839	2.605
Elm	24,039	8,850	25,080	18,431	0.005	17,832	3,886	0.299	0.112	6,692	11,814	11,134	8,504	342	9,877	5,172	399	120	0.744	1.452	1.552	1.704	0.499	2.004	2.267	0.839	2.662
Plane	22,467	8,932	25,685	19,448	0.039	17,745	3,821	0.299	0.398	6,656	12,018	7,329	6,895	347	10,129	8,456	399	109	0.690	1.461	1.539	1.701	0.532	2.006	2.226	0.839	2.605
Linden	22,467	9,048	25,348	19,283	0.034	17,769	3,821	0.299	0.398	6,656	12,078	8,279	7,255	347	10,057	8,456	399	109	0.690	1.474	1.545	1.701	0.525	2.005	2.226	0.839	2.605

*Note: sub-index "i" refers to the reaction in which this kinetic parameter is involved. Therefore, 1: hemicellulose gasification, 2: cellulose gasification, 3: lignin gasification, 4: lignin char production, 5: lignin char gasification, 6: cellulose char production, 7: hemicellulose char production, 8: cellulose char gasification and 9: hemicellulose char gasification.

** An overall mass transfer parameters was used for both, water and organic liquid. Its value is 3,000 $g \cdot m \cdot min^{-1} \cdot mol^{-1}$ and 123 $g \cdot m \cdot min^{-1} \cdot mol^{-1}$, respectively.

CHAPTER 4

HYDROTHERMAL EXTRACTION OF HEMICELLULOSE FROM LAB TO PILOT SCALE.

Figure S1. Wood particles used in the pilot reactor

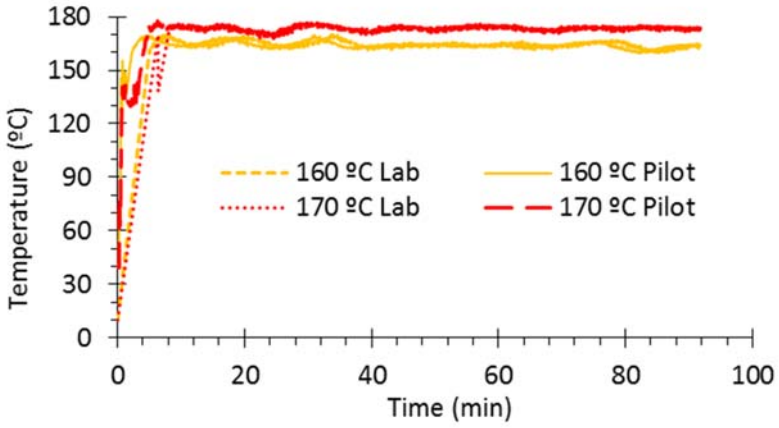
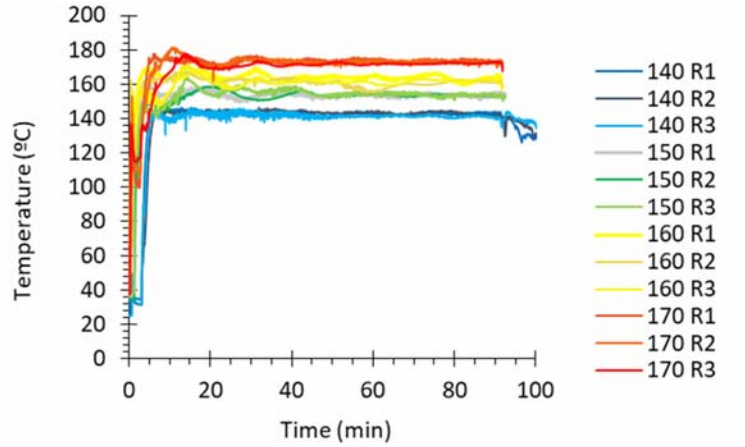


Figure S2. Temperature profile along time in Lab-scale and Pilot reactors in experiments at 160 and 170 ºC.

a)



b)

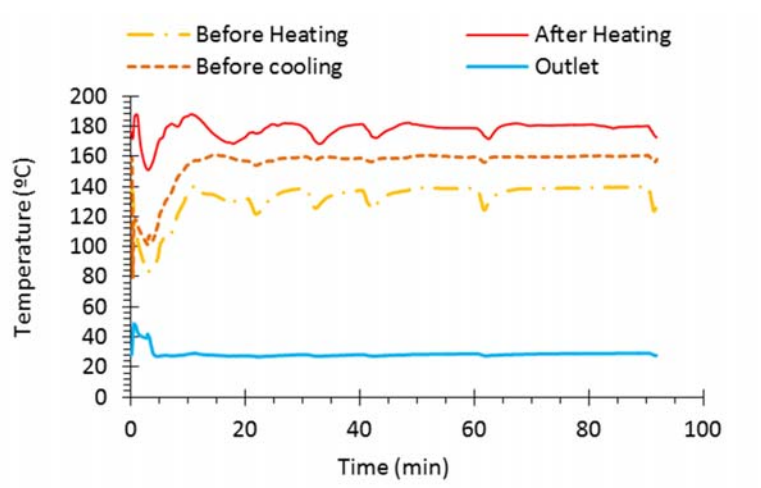


Figure S3. a) Temperature profile along time in reactors R1, R2 and R3 reactors in experiments at 140, 150, 160 and 170 ºC. b) Temperature of water before entering the heater, before entering in the reactors, after the reactors an in the outlet of the system.

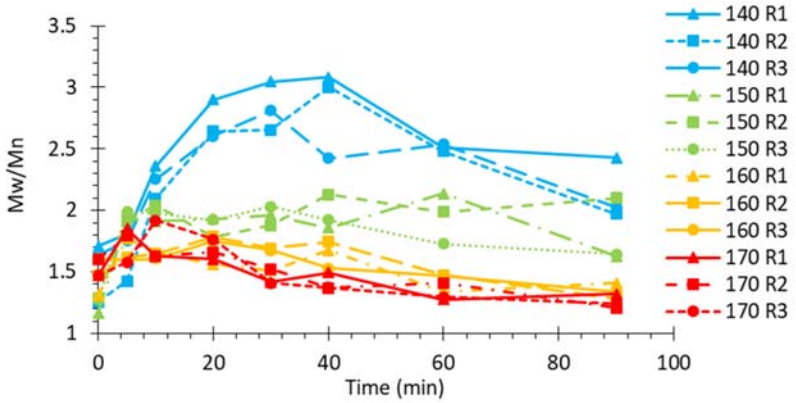


Figure S4. Polydispersity of oligomers in the extracted solutions obtained at 140, 150, 160 and 170 ºC.

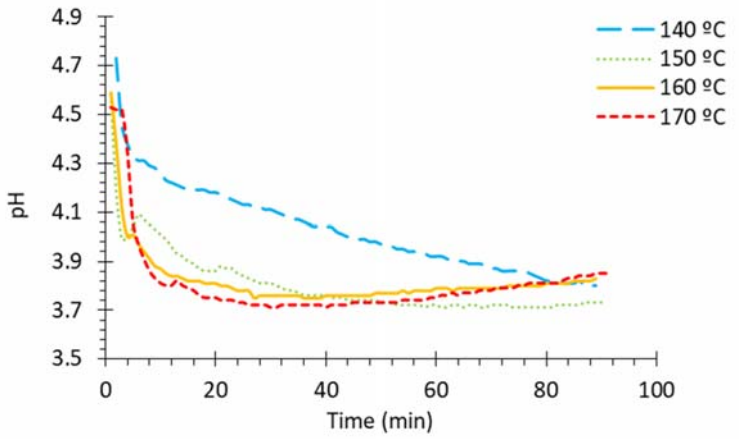


Figure S5. pH in the extracted solutions obtained at 140, 150, 160 and 170 ºC.

Table S1. Composition of the raw material.

Extractives water (wt.)	Extractives in ethanol (wt.)	Lignin (wt.)	Ashes (wt.)	Glucose (wt.)	Xylose (wt.)	Arabinose (wt.)	Acetic acid (wt.)	Galacturonic acid (wt.)
9.6%	11.2%	16.2%	0.3%	32.3%	16.7%	2.3%	4.7%	6.8%

Table S2. Yields of extracted compounds from lab-scale and pilot-scale reactors at 160 and 170 °C.

160 °C

Lab-Scale	time (min)	0	5	10	20	30	40	60	90	**Total**
Yield (wt. %)	Glucose	0.00%	0.32%	0.76%	1.84%	1.47%	1.39%	1.57%	2.33%	**9.67%**
	Xylose	0.00%	3.00%	3.35%	6.42%	4.77%	4.10%	5.68%	6.56%	**33.88%**
	Arabinose	0.00%	5.14%	10.19%	18.69%	19.17%	8.12%	12.69%	10.81%	**84.81%**
	Acetic ac.	0.00%	2.78%	4.28%	12.76%	9.74%	9.99%	11.13%	10.48%	**61.17%**

Pilot-Scale	time (min)	0	5	10	20	30	40	60	90	**Total**
Yield (wt. %)	Glucose	0.18%	0.66%	0.87%	1.00%	0.90%	1.02%	0.86%	1.25%	**6.73%**
	Xylose	0.32%	5.32%	4.15%	5.84%	4.65%	3.58%	5.51%	6.30%	**35.68%**
	Arabinose	2.30%	15.86%	11.79%	11.61%	11.11%	8.87%	13.60%	8.16%	**83.30%**
	Acetic ac.	0.00%	10.01%	7.84%	8.49%	8.81%	6.74%	11.18%	12.33%	**65.41%**

170 °C

Lab-Scale	time (min)	0	5	10	20	30	40	60	90	**Total**
Yield (wt. %)	Glucose	0.00%	0.56%	0.98%	1.39%	1.14%	1.29%	1.92%	2.77%	**10.05%**
	Xylose	0.00%	2.86%	4.55%	8.14%	5.69%	5.16%	5.58%	6.83%	**38.81%**
	Arabinose	0.00%	9.86%	14.68%	10.01%	7.45%	13.52%	11.87%	10.64%	**78.03%**
	Acetic ac.	0.00%	8.38%	10.87%	10.36%	12.66%	11.36%	12.07%	11.13%	**76.83%**

Pilot-Scale	time (min)	0	5	10	20	30	40	60	90	**Total**
Yield (wt. %)	Glucose	0.17%	0.98%	0.74%	0.69%	1.06%	1.06%	1.82%	0.83%	**7.36%**
	Xylose	0.32%	7.94%	5.20%	7.39%	4.81%	4.86%	4.81%	6.33%	**41.66%**
	Arabinose	10.45%	12.46%	13.40%	10.97%	7.43%	9.00%	9.15%	9.95%	**82.80%**
	Acetic ac.	1.97%	15.71%	9.71%	11.41%	8.93%	10.73%	11.12%	8.49%	**78.09%**

Table S3. Yields of extracted compounds from multistage reactor reactors at 140, 150, 160 and 170 °C.

140 ℃	**R1**	time (min)	0	5	10	20	30	40	60	90	**Total**
Yield (wt. %)		Glucose	0.10%	0.70%	0.49%	0.65%	0.75%	0.65%	1.11%	1.20%	**5.65%**
		Xylose	0.13%	0.48%	0.43%	0.77%	0.73%	0.86%	2.04%	4.25%	**9.70%**
		Arabinose	0.00%	5.61%	4.77%	6.43%	4.90%	4.88%	8.47%	10.19%	**45.25%**
		Acetic ac.	0.00%	0.20%	0.83%	2.13%	1.53%	1.41%	3.29%	10.25%	**19.64%**
	R2										
Yield (wt. %)		Glucose	0.07%	0.51%	0.44%	0.81%	0.76%	0.65%	1.18%	1.51%	**5.93%**
		Xylose	0.11%	0.50%	0.40%	0.78%	0.81%	0.87%	1.86%	4.41%	**9.74%**
		Arabinose	0.62%	5.13%	3.93%	6.87%	6.31%	5.32%	7.72%	12.14%	**48.03%**
		Acetic ac.	0.00%	0.89%	0.81%	1.37%	1.72%	0.91%	3.69%	7.27%	**16.67%**
	R3										
Yield (wt. %)		Glucose	0.07%	0.68%	0.40%	0.78%	0.67%	0.56%	0.94%	1.23%	**5.33%**
		Xylose	0.09%	0.27%	0.41%	0.75%	0.75%	0.76%	1.79%	3.63%	**8.44%**
		Arabinose	0.00%	3.55%	4.20%	7.69%	6.36%	5.10%	7.95%	10.44%	**45.30%**
		Acetic ac.	0.03%	0.47%	0.77%	1.50%	1.57%	1.60%	3.72%	7.83%	**17.49%**

150 ℃	**R1**	time (min)	0	5	10	20	30	40	60	90	
Yield (wt. %)		Glucose	0.13%	0.98%	0.75%	0.98%	0.89%	0.94%	1.12%	1.16%	**6.93%**
		Xylose	0.11%	1.94%	1.72%	1.89%	2.38%	1.88%	3.96%	3.80%	**17.68%**
		Arabinose	0.00%	9.89%	8.49%	8.63%	8.52%	4.62%	7.30%	8.40%	**55.86%**
		Acetic ac.	0.00%	4.00%	2.82%	2.18%	9.08%	3.52%	7.85%	7.93%	**37.37%**
	R2										
Yield (wt. %)		Glucose	0.06%	0.76%	0.72%	0.99%	0.83%	0.69%	1.12%	1.61%	**6.78%**
		Xylose	0.06%	1.93%	2.64%	2.86%	3.07%	2.25%	4.81%	4.90%	**22.51%**
		Arabinose	0.00%	9.48%	8.60%	5.35%	8.67%	6.92%	8.39%	11.70%	**59.12%**
		Acetic ac.	0.00%	3.90%	4.46%	5.87%	5.77%	4.97%	8.55%	11.42%	**44.94%**
	R3										
Yield (wt. %)		Glucose	0.00%	0.53%	0.57%	1.01%	0.81%	0.56%	1.25%	1.52%	**6.25%**
		Xylose	0.12%	1.21%	2.05%	3.84%	2.55%	2.58%	4.78%	4.76%	**21.90%**
		Arabinose	0.51%	6.31%	8.00%	8.12%	9.27%	6.42%	9.03%	8.89%	**56.54%**
		Acetic ac.	0.00%	2.07%	3.26%	6.44%	5.59%	5.21%	9.48%	10.62%	**42.68%**

160 °C	R1	time (min)	0	5	10	20	30	40	60	90	
Yield (wt. %)		Glucose	0.18%	0.66%	0.87%	1.00%	0.90%	1.02%	0.86%	1.25%	**6.73%**
		Xylose	0.32%	5.32%	4.15%	5.84%	4.65%	3.58%	5.51%	6.30%	**35.68%**
		Arabinose	2.30%	15.86%	11.79%	11.61%	11.11%	8.87%	13.60%	8.16%	**83.30%**
		Acetic ac.	0.00%	10.01%	7.84%	8.49%	8.81%	6.74%	11.18%	12.33%	**65.41%**
	R2										
Yield (wt. %)		Glucose	0.20%	0.66%	0.64%	0.89%	0.90%	0.67%	1.15%	1.43%	**6.54%**
		Xylose	0.23%	5.16%	4.70%	6.46%	5.67%	4.06%	5.50%	4.50%	**36.27%**
		Arabinose	1.68%	15.92%	13.57%	19.11%	11.56%	9.03%	10.71%	6.25%	**87.82%**
		Acetic ac.	0.20%	9.36%	8.55%	12.77%	10.72%	7.09%	10.76%	8.44%	**67.88%**
	R3										
Yield (wt. %)		Glucose	0.13%	0.41%	0.66%	1.12%	0.96%	0.93%	0.87%	0.92%	**6.00%**
		Xylose	0.16%	2.84%	4.19%	7.60%	5.07%	3.95%	5.36%	4.12%	**33.29%**
		Arabinose	1.10%	9.41%	12.22%	21.50%	13.37%	10.03%	9.52%	7.68%	**84.83%**
		Acetic ac.	0.14%	5.49%	8.38%	14.56%	10.02%	8.25%	10.17%	7.84%	**64.84%**

170 °C	R1	time (min)	0	5	10	20	30	40	60	90	
Yield (wt. %)		Glucose	0.17%	0.98%	0.74%	0.69%	1.06%	1.06%	1.82%	0.83%	**7.36%**
		Xylose	0.32%	7.94%	5.20%	7.39%	4.81%	4.86%	4.81%	6.33%	**41.66%**
		Arabinose	10.45%	12.46%	13.40%	10.97%	7.43%	9.00%	9.15%	9.95%	**82.80%**
		Acetic ac.	1.97%	15.71%	9.71%	11.41%	8.93%	10.73%	11.12%	8.49%	**78.09%**
	R2										
Yield (wt. %)		Glucose	0.18%	0.77%	0.54%	0.89%	0.61%	0.62%	1.08%	1.40%	**6.10%**
		Xylose	0.56%	5.35%	4.93%	10.66%	6.06%	4.30%	5.09%	4.25%	**41.20%**
		Arabinose	1.21%	10.97%	13.39%	16.86%	9.55%	9.42%	9.44%	9.34%	**80.19%**
		Acetic ac.	0.78%	9.62%	9.89%	11.34%	13.07%	9.90%	10.66%	8.47%	**73.72%**
	R3										
Yield (wt. %)		Glucose	0.14%	0.82%	0.47%	0.88%	1.04%	0.56%	0.99%	0.94%	**5.84%**
		Xylose	0.19%	3.84%	4.22%	9.22%	7.07%	4.58%	5.51%	4.39%	**39.01%**
		Arabinose	0.00%	11.30%	11.96%	19.43%	16.65%	10.99%	8.29%	6.02%	**84.63%**
		Acetic ac.	0.30%	7.13%	8.16%	18.45%	14.66%	10.34%	11.85%	7.52%	**78.41%**

Table S4. Molecular weights and polidispersityof samples extracted at different residence times with the multistage system at 140, 150,160 and 170 °C.

140 ℃									
	time (min)	0	5	10	20	30	40	60	90
Mw (Da)	R1	2705.1	2605.1	3624.8	4209.1	4389.6	4594.2	4872.2	4085.6
	R2	1706.8	2022.7	3598.0	4174.0	4361.4	4754.9	4468.1	4096.2
	R3	2524.8	2751.1	3892.0	4448.2	4696.1	4465.1	4683.2	4051.8
Mw/Mn	R1	1.7	1.8	2.4	2.9	3.0	3.1	2.5	2.4
	R2	1.3	1.4	2.1	2.6	2.7	3.0	2.5	2.0
	R3	1.6	1.8	2.3	2.6	2.8	2.4	2.5	2.0
150 ℃									
Mw (Da)	R1	1455.5	3206.0	3342.1	3154.4	3764.8	2961.8	3665.8	3005.5
	R2	1782.7	3058.4	3668.3	3380.2	3491.1	3789.7	3717.8	3078.9
	R3	1627.0	3223.4	3352.8	3566.5	3711.2	3487.5	3393.1	2804.5
Mw/Mn	R1	1.2	1.9	1.9	1.9	2.0	1.9	2.1	1.6
	R2	1.3	1.9	2.0	1.8	1.9	2.1	2.0	2.1
	R3	1.3	2.0	2.0	1.9	2.0	1.9	1.7	1.6
160 ℃									
Mw (Da)	R1	1549.2	2807.8	2789.1	2409.4	2366.1	2566.9	1926.1	2031.7
	R2	1912.3	2379.6	2437.5	2782.0	2542.1	2676.0	2194.7	1873.8
	R3	2145.4	2609.0	2488.2	2287.7	2520.0	2430.3	2216.1	1860.2
Mw/Mn	R1	1.3	1.8	1.7	1.6	1.5	1.7	1.3	1.4
	R2	1.5	1.6	1.6	1.8	1.7	1.7	1.5	1.3
	R3	1.6	1.6	1.6	1.7	1.7	1.5	1.5	1.3
170 ℃									
Mw (Da)	R1	1806.5	2929.9	2635.8	2493.2	2016.2	2085.4	1554.5	1452.4
	R2	2237.5	2846.2	2432.7	2478.6	2206.7	1905.5	1693.9	1372.5
	R3	1954.9	2616.4	2866.8	2529.0	1965.3	1805.6	1542.6	1427.4
Mw/Mn	R1	1.5	1.8	1.6	1.6	1.4	1.5	1.3	1.3
	R2	1.6	1.8	1.6	1.7	1.5	1.4	1.4	1.2
	R3	1.5	1.6	1.9	1.8	1.4	1.4	1.3	1.2