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DEMONSTRATION OF A SELECTIVE PROCESS TO TRANSFORM BIOMASS INTO SUGARS BY ULTRAFAST HYDROLYSIS IN SUPERCRITICAL WATER

Presentada por Celia María Martínez Fajardo para optar al grado de Doctor/a por la Universidad de Valladolid

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CERTIFICAN QUE:

CELIA MARÍA MARTÍNEZ FAJARDO ha realizado bajo su dirección el trabajo ***"DEMONSTRATION OF A SELECTIVE PROCESS TO TRANSFORM BIOMASS INTO SUGARS BY ULTRAFAST HYDROLYSIS IN SUPERCRITICAL WATER***", en el Departamento de Ingeniería Química y Tecnología del Medio Ambiente de la Escuela de Ingenierías Industriales de la Universidad de Valladolid. Considerando que dicho trabajo reúne los requisitos para ser presentado como Tesis Doctoral expresa su conformidad con dicha presentación.

Valladolid a____de_____de 2018.

Reunido el tribunal que ha juzgado la tesis doctoral "***DEMONSTRATION OF A SELECTIVE PROCESS TO TRANSFORM BIOMASS INTO SUGARS BY ULTRAFAST HYDROLYSIS IN SUPERCRITICAL WATER***" presentada por Celia María Martínez Fajardo 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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PRESIDENTE SECRETARIO

VOCAL

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Abstract

Demonstration of a selective process to transform biomass into sugars by ultrafast hydrolysis in supercritical water

To accomplish the challenge of a chemical industry based on biorefineries, research efforts must be focused on developing sustainable processes able to maximize profit out of each biomass fraction with the lowest cost. Hydrothermal processes are an attractive technology to produce valuable products from biomass. However, this technology is still under development to reduce operational costs and at the same time, provide high yields and selectivity towards desired products. To overcome those limitations, the aim of this PhD thesis is to develop an efficient technology to transform agrofood biomass into sugars by the developed FASTSUGARS technology (ultrafast hydrolysis in supercritical water), moving from laboratory to pilot plant scale.

To do so, a continuous laboratory scale plant was initially used. That experimental unit, developed in a previous thesis, is able to work with reactor temperatures up to 400 ºC, pressures up to 30 MPa and reaction times between 0.004 and 5 s with a maximum total flow up to 8 kg/h (max. 3 kg/h of biomass). Chapters 1 and 2 were developed working with this facility. Then, the process was scaled up as the main focus of this thesis, moving from laboratory to pilot plant scale. The continuous pilot plant developed in the present work, able to work with reactor temperatures up to 400 ºC, pressures up to 30 MPa and reaction times from 0.07 s, was designed to operate with total flows up to 30 kg/h (up to 10 kg/h biomass). Chapters 3 and 4 were carried out using this experimental setup.

In **Chapter 1**, the effect of inlet concentration on cellulose hydrolysis in supercritical water was evaluated in the laboratory scale plant. To do so, the experiments were carried out at 400 ºC and 25 MPa with reaction times between 0.07 and 1.57 seconds and suspensions concentrations between 5 and 20 % w/w (corresponding to 1.5 – 6 % w/w at reactor inlet). It was found necessary to increase the reaction time to achieve total cellulose conversion when using highly concentrated suspensions. However, increasing reaction time also favored the degradation of glucose. Therefore, cellulose could be selectively hydrolyzed to sugars by using short reaction times and low concentrations of biomass. Indeed, the best result for sugars production (79 % w/w) was obtained working with a cellulose inlet concentration of 1.5 % w/w and 0.07 s reaction time. On the other hand, if the

desired products were glucose derivatives, such as glycolaldehyde, higher reaction times (>1s) were needed. For glycoaldehyde production, the best result (42 % w/w) was obtained working with 6 % w/w cellulose inlet concentration and 1.57 s reaction time. It was also found that the inlet concentration of biomass affects the conversion rate of cellulose in supercritical water. Increasing the inlet concentration up to 4 % w/w at reactor inlet, the cellulose solubility in supercritical water was lower so that the reaction occurred in a heterogeneous media where the mass transfer resistances limited the reaction rate.

In **Chapter 2**, sugar beet pulp was hydrolyzed in supercritical water for sugars recovery, operating at 390 ºC, 25 MPa and reaction times between 0.11 and 1.15 s in the laboratory scale plant. Sugar beet pulp is the major by-product in sugar industry and to make profit out of this undervalued residue, the FASTSUGARS process was proposed as a competitive alternative, combining the advantages of supercritical water as hydrolysis medium with very short reaction times. It was possible to achieve a selective and simultaneous recovery of both cellulose and hemicellulose fractions as C-6 and C-5 sugars. In this way, a liquid effluent suitable for further conversion into ethylene glycol and sorbitol was obtained. On the other hand, the solid product obtained could be used as additive for composites production. The highest yields of C-6 and C-5 sugars (61 and 71 % w/w, respectively) were obtained at 0.11 s with the lowest yield of degradation products. The solid product obtained at 0.14 s was thoroughly analyzed by TGA and FTIR analysis to prove its enhanced thermal properties and aromaticity.

In **Chapter 3**, the FASTSUGARS process for sugars' recovery from agricultural biomass was scaled up from laboratory to pilot plant scale. System performance was evaluated by comparing the results obtained from sugar beet pulp and wheat bran in both laboratory and pilot plants. When comparing the performance of these biomasses, similar trends were found, as selectivity to sugars decreased with reaction time and then, conversion and degradation yield increased with reaction time. Differences between the results obtained for each biomass were due to composition and reactor conditions. To bring the FASTSUGARS process closer to industrial applications, a bigger particle size was used in the pilot plant (250 μm)

compared to the laboratory scale plant (≤ 150 μm). It was found that the particle size acted as a mass transfer resistance, slowing down the hydrolysis of biomass, providing lower conversion and therefore reducing sugars' degradation (degradation yield was always lower than 15 % in the pilot plant). In that way, higher selectivity to sugars was obtained, reaching values around 90 % for both sugar beet pulp and wheat bran in the pilot plant. Therefore, this slowing down effect in the pilot plant resulted to be positive, since selectivity was increased and at the same time, the degradation was remarkably reduced.

In **Chapter 4**, the hydrolysis of commercial inulin with a polymerization degree comparable to fructooligosaccharides (FOS) was hydrolyzed in supercritical water to evaluate the reaction mechanisms. The hydrolysis reactions were performed in the pilot plant at 385 ºC, 25 MPa and reaction times between 0.12 and 0.74 s. It was observed that the conversion of fructose to glyceraldehyde, 5-HMF and furfural was slower than the subsequent production of pyruvaldehyde and formic acid. As it happened for cellulose, it was also found that reaction time affects selectivity, since as reaction time increased, the sugars production decreased due to their degradation into further products (mainly pyruvaldehyde and formic acid). On the other hand, it was demonstrated that increasing the inlet concentration, the conversion of inulin was reduced, providing higher sugars yield and lower degradation rate. Jerusalem artichoke was selected as an inulin-rich biomass for the production of fructo-sugars via supercritical water hydrolysis. It was observed that the hydrolysis of Jerusalem artichoke was similar to that of FOS at high concentration, yielding up to 68 % w/w of sugars. It was concluded that lower conversion was achieved compared to FOS because initial degree of polymerization was higher and acted as a limitation for the dissolution step. Then, results from Jerusalem artichoke were also compared to those of lignocellulosic substrates obtained in Chapter 3 (sugar beet pulp and wheat bran). Higher conversion was achieved in the case of Jerusalem artichoke due to its composition, since its main constituent was inulin, which was much more easily converted than cellulose under the selected conditions.

Abbreviations

5-HMF	5-hydroxymethylfurfural
AIF	Acid-insoluble fraction
AL	Aldol reaction
BR	Benzilic acid rearrangement reaction
C-5 sugars	Sugars derived from hemicellulose
C-6 sugars	Sugars derived from cellulose
Cin	Inlet concentration
DE	Dehydration reactions
DLS	Dynamic Light Scattering
DP	Degree of polymerization
DTG	Derivative thermogravimetric
FOS	Fructooligosaccharides
FTIR	Fourier Transformed Infrared
HD	Hydration reactions
HPLC	High Performance Liquid Chromatography
HPLC-SEC	Size exclusion chromatography
IL	Insoluble lignin
JA	Jerusalem artichoke (*Helianthus tuberosus*)
MW	Molecular weight
PS	Particle size
RAC	Retro-aldol condensation products

SL	Soluble lignin
SBP	Sugar beet pulp
SCW	Supercritical water
sCW	Subcritical water
% sups	Percentage of suspended solids in the final product
TGA	Thermogravimetric analysis
TOC	Total organic carbon
t_R	Reaction time
WB	Wheat bran

Introduction

Valorization of agricultural biomass by supercritical water hydrolysis as a step towards biorefineries

1. Biorefineries

Countless studies have emphasized the need for shifting the chemical industries away from fossil resources towards renewable raw materials and sustainable processes in the so-called biorefineries [1, 2]. The term biorefinery is widely used nowadays and several definitions have been developed over the past years, but the underlying concept in all definitions is the conversion of biomass into marketable products and the integration of various technologies and processes in the most sustainable way [3]. Biorefineries would be playing an essential role in the biobased economy which goal, according to the European Union, is "a more innovative and low-emissions economy, reconciling demands for sustainable agriculture and fisheries, food security and the sustainable use of renewable biological resources for industrial purposes, while ensuring biodiversity and environmental protection" [4, 5]. So that, the bioeconomy would provide solutions for the upcoming challenges, by generating jobs and business opportunities, reducing the fossil fuels dependence and improving the environmental, social and economic sustainability [6].

The basis for biomass refining processes is the knowledge of biomass structure and composition, since its complexity to generate marketable products is restraining the development of a bio-based economy [2, 7]. Around 1.5 billion dry tons of lignocellulosic biomass are available annually, counting agricultural, forestry residues and woody biomass [8]. Considering that fact, lignocellulosic biomass is an abundant world-wide distributed source of carbon and it is composed of three main bio-polymers, being cellulose (20 – 50%), hemicellulose (15 – 35%) and lignin (10 – 30%), in addition to other fractions such as proteins, pectin or starch and minor but valuable compounds such as polyphenols. It represents a sustainable source that could support large-scale, low-cost production of fuels, chemicals and materials for the future industries, i.e. biorefineries [9, 10]. However, despite its potential, the recalcitrance of the lignocellulosic matrix is the major hurdle restraining the industrial implementation of biorefineries [11].

A functional biorefinery should be able to use a wide variety of raw materials, making profit out of each biomass fraction with the lowest energy cost. Several

technologies have been developed during the last decades to carry out the conversion of biomass into fuels, chemicals, materials and/or energy. These technologies can be classified in two groups: biochemical and thermochemical conversion technologies. First group degrades biomass with enzymes and microorganisms, meanwhile thermochemical processes are based on the thermal breakdown of biomass [12]. Biochemical methods usually need a hydrolysis pretreatment to convert lignocellulosic biomass. Moreover, biochemical processes occur in the range of days to complete the biomass conversion. On the contrary, thermochemical processes require lower reaction times to convert the entire biomass with any pretreatment steps [13]. They convert biomass into intermediate products, so that they can be further converted into value added hydrocarbons [14]. Thermochemical processes include pyrolysis, hydrothermal processing, combustion and gasification. Hydrothermal technology involves using water at high temperature and pressure and it is applied for hydrolysis, liquefaction, extraction, gasification and carbonization of lignocellulosic materials [12, 15].

2. Hydrothermal processing

Hydrothermal technologies are defined as chemical and physical transformations in water at high temperature (from 120 ºC) and high pressure (from 5 MPa) [16]. Within this wide range of operating conditions, the hydrothermal processing has been given a variety of labels, including: liquid hot water, hydrothermolysis, compressed hot water, subcritical and supercritical water, among others [8]. However, each terms implies using different operational conditions [17]. Regardless of the name, the major advantages that these processes offers are: (1) the addition and/or recovery of chemicals is not necessary; (2) the ability to use wet biomass without prior dewatering; (3) the same reaction medium can be used for the fractionation and/or conversion of different biomass fractions; (4) mass transfer limitations are substantially reduced thus reaction rates are faster [16, 18, 19].

The terms “liquid hot water”, “compressed hot water” and “subcritical water” are equivalent and describe water above its boiling point without reaching the critical point (374 ºC, 22.1 MPa). So that, subcritical water (sCW) temperatures are ranging from 100 ºC to 374 ºC under sufficient pressure to maintain water in the liquid phase

[8]. On the other hand, water above its critical point is defined as supercritical water (SCW) and it shows properties very different from those of liquid water. In fact, above the critical point the fluid exists as a single phase having some of the advantageous properties of both a liquid and a gas [20]. Moreover, around the critical point of water density drastically decreases, affecting properties such as solvation power, degree of hydrogen bonding, polarity, dielectric strength, molecular diffusivity and viscosity [16]. All these physical properties can be finely tuned by varying temperature and pressure. Another important property changing around the critical point is the ionic product of water (Kw). Around 300 ºC the Kw reaches its maximum, providing a high concentration of H^+ and OH^- ions. That highly dissociated medium favors acid/basis catalyzed reactions. However, above 374 ºC (critical temperature of water), Kw dramatically decreases. As dissociation can be tuned by changing temperature and pressure, water can simultaneously act as a reactant and/or as catalyst at around the critical point [17]. Additionally, SCW has higher diffusivity and lower viscosity compared to liquid state, facilitating in that way the mass transport. In the case of lignocellulosic biomass, these enhanced properties facilitate the penetration of water into the complex structure of biomass [2].

Both sCW water and SCW can be used as a pretreatment for the selective fractionation of biomass or as reaction medium to directly hydrolyze biomass into its constituent building blocks [17]. Both options (i.e. fractionation and hydrolysis) were studied under hydrothermal conditions in different systems: batch, semi-continuous and continuous. Batch type reactors are the simplest equipment but the process control is poor and easily leads to degradation products due to high reaction times [18]. On the other hand, the key of the semi-continuous process is the water flowthrough system that rapidly removes the liquid products out of the reaction zone, therefore limiting the degradation [8]. For the biomass hydrolysis, the semi-continuous process allows high recovery of hemicellulose as sugars. However, if temperature is increased in order to also recover cellulose, the yield of recovered sugars decreased [21]. The continuous reactors provide a precise control of reaction time, since it can be easily changed by modifying the reactor volume and/or the

flow rate [22]. Therefore, continuous reactors are a promising alternative in the scale-up of the process, since they allow increasing the amount of treated material compared to the semi-continuous processes, maintaining at the same time a very precise control of reaction time. However, the technology is not yet fully developed and its application to agricultural residues is still a challenging task [23].

3. HPPG biorefinery concept

Nevertheless, hydrothermal processes are an attractive possibility leading to the biorefinery concept since they allow converting and/or fractionating biomass into high added-value products [15]. The High Pressure Processes Group (HPPG, University of Valladolid) has focused its research efforts in a hydrothermal-based biorefinery approach, where biomass would be used as feedstock and water would be the solvent used to produce added-value products from the different biomass fractions. Under that approach, each biomass would go through several steps to make profit out of each fraction as shown in Fig. 1. First step would be the extraction of added-value compounds, such as polyphenols. To do so, several extraction techniques could be used: microwave assisted extraction using ethanol as co-solvent allowed extracting polyphenols form grape pomace [24]; β-glucans could be extracted from barley using water as solvent in an ultrasound assisted extraction [25]; then, a hydrothermal extraction could be performed in a semi-continuous reactor, allowing to extract polyphenols and oil from grape seeds [26]. Once added-value products were extracted, they could be transformed into marketable products by means of formulation processes to produce cosmetic and/or pharmaceutical additives. Next step in this hydrothermal biorefinery would be the hydrothermal extraction of hemicelluloses: sCW was used as solvent in a semi-continuous process to extract hemicellulose from woody biomass [27, 28]; on the other hand, hydrothermal extraction assisted by heterogeneous catalyst could also be used to obtain arabinoxylans from destarched wheat bran [29]. With the arabinoxylans obtained from hemicellulose extraction and by means of hydrogenation, attractive products such as xylitol or arabitol could be obtained and used as food additives [30]. Third step in the HPPG biorefinery would be cellulose hydrolysis in SCW which would yield sugars and building blocks such as

glycolaldehyde from biomass [31]. The product obtained from SCW hydrolysis of cellulose and/or biomass could be then transformed into fuels via fermentation or industrial products such as sorbitol and ethylene glycol by hydrogenation over Ru/MCM-48 catalyst [32, 33]. Finally, lignin depolymerization could be also carried out under SCW conditions, yielding aromatic products that would be then transformed into chemicals.

The fractionation of biomass into its individual building blocks is a major challenge to the biorefinery concept due to the recalcitrant nature of the lignocellulosic matrix [2]. Nevertheless, in the proposed hydrothermal biorefinery, biomass could be transformed step by step into marketable products that would compete to petroleum-derived products. However, each step in this biorefinery concept should be individually optimized. The present work is focused in the third stage showed in Fig. 1, meaning the cellulose and biomass hydrolysis in SCW to produce sugars. This process will be identified as FASTSUGARS from now on.

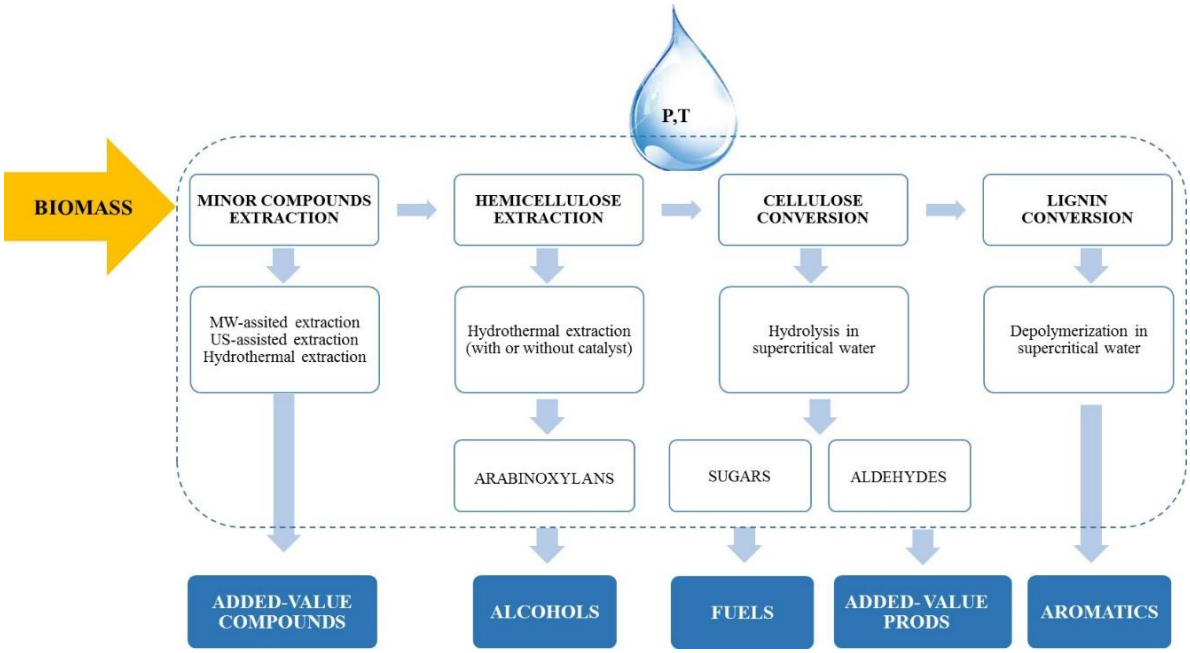

Figure 1.High Pressure Processes Group (HPPG) biorefinery concept to obtain valuable products from biomass using water hydrothermal processes. Mw = microwave, US = Ultrasounds.

4. FASTSUGARS process

4.1. Thesis framework: FASTSUGARS project

The current work is related to the FASTSUGARS project, financed by the Spanish Ministry of Economy and Competitiveness (MINECO, CTQ2013-44143-R) and entitled *"Demonstration of an efficient biomass to sugars transformation process by ultrafast reactor in supercritical water"*. This project is leaded by Prof. Dr. María José Cocero, who is the director of this PhD thesis. FASTSUGARS project had a total duration of 3 years, starting from October 2014 and the total funding received was 224.000 €. The FASTSUGARS project responds to the challenge of developing a selective technology to transform biomass into sugars and chemicals using SCW as hydrolysis medium, with reaction times below 1 second.

In a previous National project (MICINN, CTQ2010-15475) entitled *"Depolymerization and valorization of biomass to obtain high added-value products"*, a laboratory scale plant for the hydrolysis of cellulose and biomass in SCW was developed. The thesis of Dr. Danilo Cantero, who is the co-director of the current thesis, was arisen in parallel with that project [34]. By using that continuous laboratory plant it was demonstrated that complete cellulose conversion into sugars and oligosaccharides could be achieved in just 0.03 seconds [35]. Apart from reaction time, pressure and temperature effect were also studied [36], together with the kinetics of the process [22]. The main goal of that project was the high selectivity achieved, based on the fast hydrolysis of cellulose in SCW. The hydrolysis of cellulose occurs with reaction times of milliseconds, whereas the transformation of sugars into derived products (such as glycolaldehyde or lactic acid) needs higher reaction times [37, 38]. Therefore, the selective production of sugars can be controlled by controlling the reaction time [39]. This fact was also validated working with real biomass (wheat bran), giving as a result a high recovery of cellulose and hemicellulose derived sugars, with a reaction time of 0.2 s [31].

Once it was found the key to selectively transform the biomass into sugars via SCW hydrolysis, the FASTSUGARS project proposed the scale up of the laboratory plant with the construction of a pilot scale plant to convert agricultural biomass into sugars and added-value products. Then, the specific objectives of the FASTSUGARS project were:

1. Design, build and operate a pilot plant to hydrolyze lignocellulosic biomass in SCW, able to operate with up to 35 kg/h, 400 ºC and 30 MPa.
2. Study biomass pretreatments for the hydrolysis process. Microwave assisted extraction would allow recovering polyphenols and added-value compounds from lignocellulosic biomass.
3. Apply the obtained results to biomass of industrial interest: local agricultural biomass as a model of European agricultural food processing wastes.
4. Selective transformation of high fructose content biomass into added-value products such as pyruvaldehyde.

This thesis is focused on objectives 1, 3 and 4 of the FASTSUGARS project. Objective 2 was already developed by other authors [24]. Then, the main aspects of the FASTSUGARS project applied in this work would be further elaborated in next sections, meaning SCW hydrolysis of biomass, ultrafast reactors and the scale of the process.

4.2. SCW hydrolysis of biomass

The majority of literature reports on acid and/or enzymatic hydrolysis to obtain valuable compounds from biomass [40-43], meaning sugars from cellulose and hemicellulose fractions and aromatic units from lignin. However, both technologies have important drawbacks. Acid hydrolysis easily leads to the production of degradation products, it also requires subsequent neutralization and a significant capital expense for special equipment to resist corrosion [44]. On the other hand, as main drawbacks for the enzymatic hydrolysis, the high dosage of enzymes and/or chemicals for pretreatment represents a concern in the operating side cost [45]. To overcome these limitations, SCW technology demonstrated being a promising alternative to valorize biomass with several advantages over those conventional processes: it produces less sugars degradation, less corrosion and no toxic solvents are used compared to acid hydrolysis. Also, it allows equipment and time reduction compared to enzymatic hydrolysis [17]. In the recent years, the use of SCW technology to hydrolyze biomass has been gaining increasing interest, but literature is still limited [34, 44, 46, 47]. Particularly, the major bottleneck for the process is

that hydrolysis performance (kinetics and yields) highly depends on the characteristics of the residue (i.e. composition, structure and interactions of the cell wall) [48]. To improve the understanding of biomass hydrolysis in SCW, the hydrolysis of model compounds (meaning cellulose, hemicellulose and lignin) has been intensively investigated in the recent decades.

Cellulose represents the major component of lignocellulosic biomass, representing up to 50 % in mass and is considered a renewable, cheap and worldwide-distributed polymer with very promising applications for the future biorefineries. It is a linear polysaccharide composed of units of glucose linked by β-(1,4)-glycosidic bonds, which structure is shown in Fig. 2. Cellulose chains aggregates producing fibers, which form the foundations of the cell walls [2].Due to its structure, cellulose is not soluble in water at ambient conditions, but it was proved that it was partially dissolved in sCW and completely dissolved at temperatures above 330 ºC [49]. Cellulose hydrolysis in SCW water has been intensively studied, using different ways of operation as mentioned in Section 2 (meaning batch [50, 51], semi-continuous [52, 53] and/or continuous systems [34, 54, 55]). Those studies indicated that most important parameter affecting the sugars yield is the combination of reaction time and temperature [23]. In sCW, the reactions occur on the surface of the biomass and the randomness of reactions depends on temperature [56, 57]. However, in SCW dissolution and hydrolysis of cellulose are produced simultaneously, implying a homogenous process and therefore improving hydrolysis rates. Because of the different behaviors of cellulose solubilization, sugars yield in SCW are much higher than those from sCW. Indeed, in sCW, the sugars degradation rates are higher than the hydrolysis rate of cellulose, so that high sugars yield cannot be obtained. Around the critical point, the hydrolysis rate increase by more than one order of magnitude and becomes faster than the sugars decomposition, enhancing sugars production in SCW [17, 35].

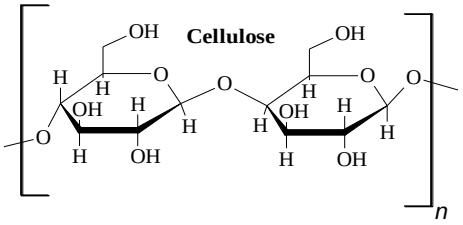

Figure 2.Cellulose chemical structure.

Hemicellulose is the second most common polysaccharide in nature, representing about 15 – 35 % of lignocellulose. It is a heteropolymer consisting of five-carbon (C-5) and six-carbon (C-6) sugars, including: xylose, arabinose, mannose, glucose, galactose and others, which are represented in Fig. 3 [19]. The ratio of these monomers can change dramatically depending on the feedstock sources. Given the lack of repeating β-(1,4)-glycosidic bonds and its random structure, it does not from crystalline and resistant structures as cellulose does, and therefore is much more susceptible to hydrothermal hydrolysis [16]. Its extraction from biomass under mild hydrothermal conditions has been extensively studied, concluding that hemicellulose is easily dissolved in water at temperatures above 160 ºC. Several authors reported that up to 60 – 70 % of the initial hemicellulose in hardwood species could be recovered as oligomers and monomeric sugars operating at 180 ºC [28, 58, 59]. Higher yields could be obtained increasing temperature, but undesired degradation products would appear [60, 61].

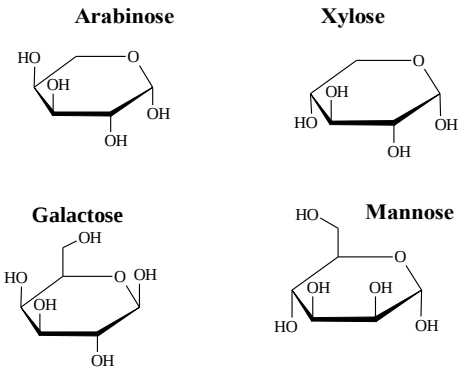

Figure 3. Chemical structure for the main constituent units from hemicellulose.

Lignin is a complex, highly aromatic polyphenolic polymer available in plants in different compositions, molecular weights and proportions [19]. It is widely

accepted that lignin structure comes from the polymerization of three phenylpropane monomers units, being coniferyl, sinapyl and coumaryl alcohol [62], as shown in Fig. 4. Lignin from different biomass are characterized by different percentages of corresponding alcohols and different final networks, which makes difficult to define a regular structure. Despite its non-well known structure, it suggest that lignin can be a valuable source of chemicals if broken into smaller molecular units [2]. There is still little information about the real mechanism of lignin decomposition in near-critical water, compared to the existing knowledge about cellulose and hemicellulose [19]. Recently, the hydrolysis of lignin and its model compounds (vanillic acid, guaiacol, syringol, coniferyl and sinapyl alcohols) in hydrothermal medium has been gaining increasing attention [63-66]. It was concluded that mechanism for lignin decomposition in SCW consisted of a complex network of parallel and sequential fragmentation and re-condensation reactions [67].

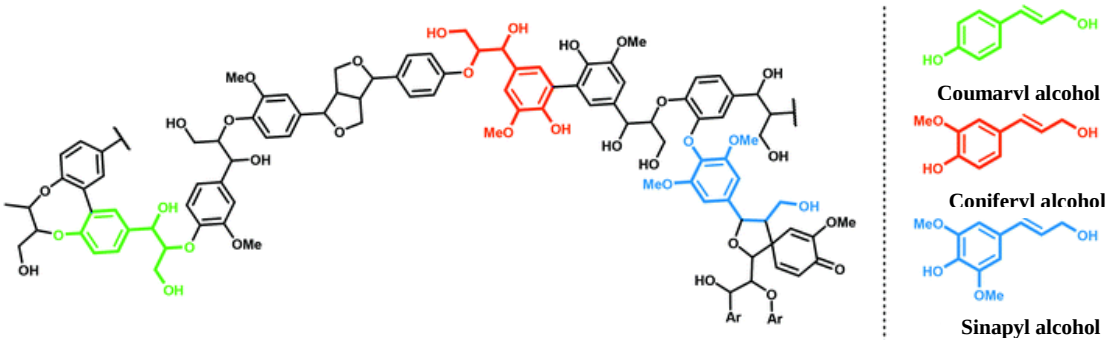

Figure 4. Lignin chemical structure [68].

Even though substantial contributions have been done to better understand the hydrolysis of each individual polymer in SCW, many engineering challenges remain for the processing of whole biomass. The recalcitrant nature of the lignocellulosic biomass is the result of the intricate cell wall architecture, where cellulose, hemicellulose and lignin are linked and interacting [69]. It was already mentioned that each component had its own depolymerization kinetics: hemicellulose is the most labile fraction, meanwhile cellulose hydrolysis requires more severe conditions to be hydrolyzed and finally lignin, being the most recalcitrant fraction, is facing complex and competitive fragmentation and

repolymerization reactions. Then, the selection of reaction conditions is the key to obtain sugars from both cellulose and hemicellulose, avoiding degradation to obtain high yields. To do so, our research group (HPPG) developed a selective technology to hydrolyze biomass in SCW. The so-called FASTSUGARS process (related to both CTQ2013-44143-R and CTQ2010-15475 projects) allows converting biomass into sugars and added-value products such as aldehydes. The lignin abundance in agricultural and food biomass is not so high as in woods [19], so that FASTSUGARS process focused on sugars production, leaving complex lignin reaction pathway to be studied in a different project (CTQ2016-79777-R).

4.3. Ultrafast reactors: the key of the FASTSUGARS process

It was previously mention that biomass hydrolysis in SCW could be carried out using batch, semi-continuous or continuous systems, being the continuous systems the ones providing better control over reactions [18, 21]. Moreover, as carbon and energy recovery efficiency can be favorable, a considerable interest exists for commercial application of SCW technologies in the continuous mode [70]. Previous studies of cellulose hydrolysis in SCW proved that main parameters are reaction time and temperature. It was also proved that around the critical point, the hydrolysis rate of cellulose jumps by more than one order of magnitude and becomes faster than the sugars decomposition rate, providing high yields of sugars [18]. On the contrary, the oligosaccharides ratio and their polymerization degree decreases with temperature, indicating that the glycosidic bond may be easier to break and oligosaccharides may exists for extremely short times before breaking down into monomers [17]. So that, temperatures above the critical point and short reaction times should be combined to obtain high sugars yield in a continuous hydrolysis process.

The FASTSUGARS process allows operating with reactor temperatures up to 400 ºC and reaction times of milliseconds in the so-called ultrafast reactors, shown in Fig. 5 and developed in a previous thesis [34]. In these micro-reactors, the reaction is started and stopped by sharp temperature changes, avoiding heating and cooling slopes that could lead to uncontrolled reactions. The heating is achieved instantaneously by mixing the biomass stream with SCW at the reactor inlet [35,

55]. Then, the reaction is stopped by sudden depressurization through a valve, which immediately cools down the effluent as consequence of the Joule-Thomson effect. Decreasing temperature from 400 ºC to 150 ºC effectively stops hydrolysis reactions. These heating and cooling methods allow a precise control of reaction time, which can be changed by modifying the reactor volume, the flow rate and/or operating conditions [22]. Moreover, the described system is capable of instantaneously cooling the products avoiding their dilution, which would occur if they were cooled down by quenching [71].

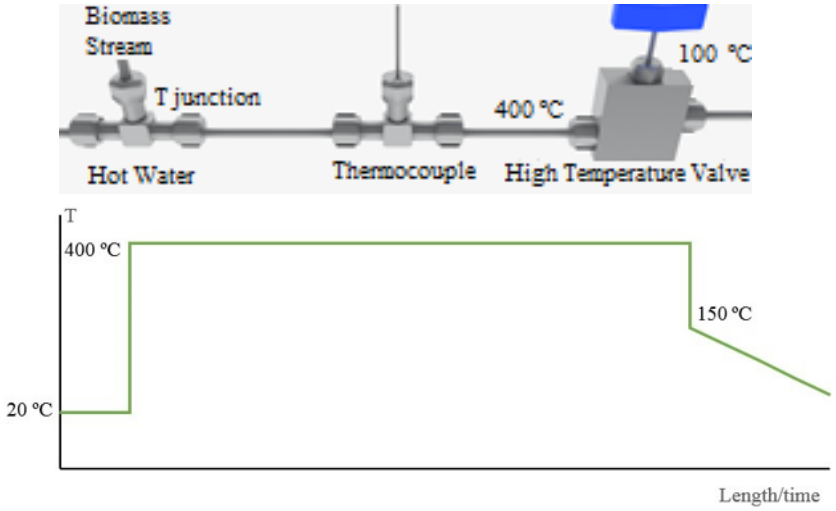

Figure 5. Ultrafast reactor scheme and temperature profile [34].

The ultrafast reactors were already validated in a previous thesis [34], proving that the optimal conditions to obtain soluble sugars from cellulose were achieved operating at 400 ºC and 0.03 seconds. Under those conditions, using the ultrafast reactors allowed stopping the reaction after complete cellulose dissolution but before glucose degradation, recovering up to 98 % w/w of cellulose as sugars [35]. This technology was also validated to hydrolyze agricultural biomass, such as wheat bran [31] and sugar beet pulp [32]. Working with wheat bran, the highest recovery of cellulose and hemicellulose as soluble sugars (76 % w/w) was achieved operating at 400 ºC and 0.19 s reaction time. On the other hand, starting with sugar beet pulp as raw material and working under similar reaction conditions (390 ºC and 0.20 s), up to 10 % w/w of glycolaldehyde was produced. That effluent from sugar beet pulp hydrolysis containing sugars and glycolaldehyde was then

hydrogenated over Ru/MCM-48 catalyst obtaining hexitols and ethylene glycol as products [32]. The reaction mechanism developed for both cellulose and hemicellulose from agricultural biomass hydrolysis in SCW is shown in Fig. 6.

It is clear that working with a real biomass implies not only dealing with cellulose, but also hemicellulose, lignin and other fractions (such as pectins, starch, proteins, etc.). The intricate cell wall of biomass makes the cellulose and hemicellulose fractions less accessible for hydrolysis and therefore higher reaction times were needed compared to pure cellulose hydrolysis. That would explain the need to move optimal conditions from 0.03 s for pure cellulose hydrolysis to 0.2 for biomass hydrolysis in ultrafast reactors [2]. Also, when comparing different biomass it was corroborated that each raw material represents a technological challenge that should be studied separately [17].

4.4. Scale of the process

It was proved that SCW hydrolysis with ultrafast reactors is a promising alternative for the biomass processing, allowing higher selectivity than batch processes [18]. Unfortunately, the technical difficulties associated with pumping biomass slurries made most studies focus on batch and semi-continuous systems [70]. To overcome this problem at the laboratory scale FASTSUGARS plant, the check valve system of the biomass pump was modified. Operating with higher flow rates would also avoid this problem, so that the scale-up of the process could resolve a technical problem and at the same time, expand the future perspectives of the FASTSUGARS process. Indeed, developing a technology to hydrolyze biomass in a rapid and selective way while being cost-effective is still a challenge [17]. The FASTSUGARS process aim to reduce equipment costs taking advantage of the fast kinetics in SCW, which allow dramatically reducing the reaction time and therefore the reactor size [77]. Reducing the reaction time from minutes to milliseconds, allows a reactor reduction from m^3 to cm^3 [18] and thus reducing equipment costs. It is also possible to reduce the operation costs by improving the energy and work recovery [77]. This could be done by recovering the work associated with the depressurization and by means of energy integration [78, 79].

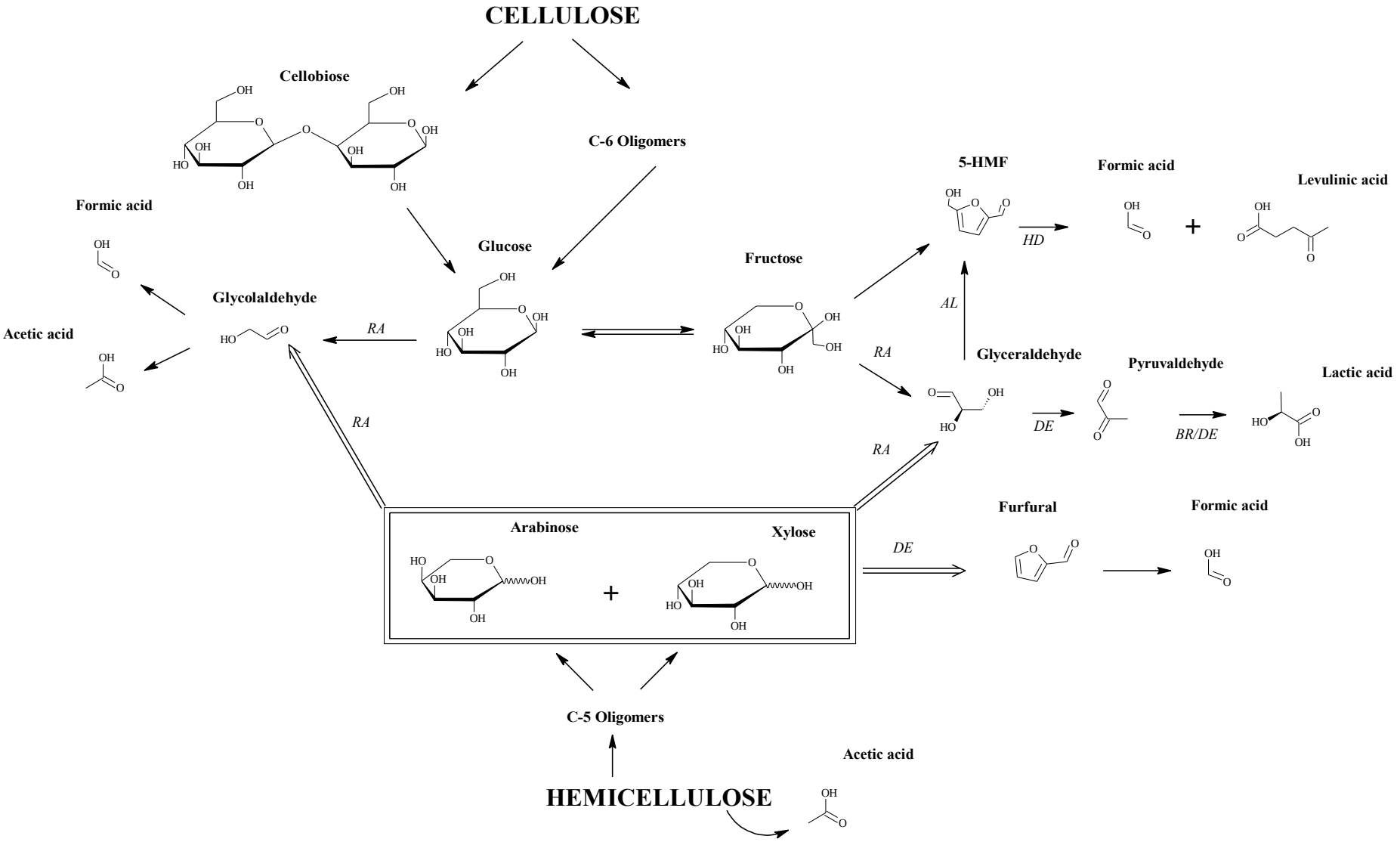

Figure 6. Combined and simplified reaction pathways for cellulose and hemicellulose in biomass under supercritical water hydrolysis conditions [21, 72-75]. Double arrows represent reaction pathways for both arabinose and xylose. RA: retroaldol condensation reactions; DE: dehydration reactions, HD: hydration reactions, BR: benzilic acid rearrangement [75], AL: aldol reaction [76].

Hydrothermal fractionation of biomass is already present in an industrial scale, with Renmatix (USA) producing cellulose-derived products by SCW hydrolysis [80, 81]. Although the available information of the process is limited, the company uses very fast reaction times and small equipment size, reducing the capital and operating expenses [18]. On the other hand, a recent study reported on a pilot scale equipment for SCW hydrolysis of lignocellulosic biomass [46]. In that work, the SCW hydrolysis of woody biomass was carried out at 380 ºC, with reaction times below 1 second (the specific reaction time was not stated by the authors). Sulfuric acid (0.05 %) was added to the feed as a catalyst, producing in that way a maximum sugars yield of 50 % w/w. The cooling method in that work consisted on passing the effluent through a heat exchanger section and a chiller. This cooling method is probably the reason for the uncertainty in reaction time.

In contrast, the FASTSUGARS process is presented as a cleaner alternative where no chemicals are needed to obtain high sugars yield from agricultural biomass. Moreover, it proved to be a versatile technology able to process different substrates obtaining very promising results. The key of the FASTSUGARS process is the effective control of reaction time, which allows obtaining high yields of sugars and/or building blocks (such as aldehydes) just by changing the reaction time.

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Aims and Contents

Demonstration of a selective process to transform biomass into sugars by ultrafast hydrolysis in supercritical water

This PhD thesis is aligned with the FASTSUGARS project, so that the general aim of this work to develop a selective technology to transform agricultural biomass into sugars by ultrafast hydrolysis in supercritical water, moving from laboratory to pilot plant scale. To achieve this holistic aim, the following partial objectives were defined:

- Study the effect on yields and kinetics of biomass concentration on cellulose ultrafast hydrolysis in supercritical water in the laboratory scale plant.
- Study the supercritical water ultrafast hydrolysis of a locally available biomass in the laboratory scale plant: The case of sugar beet pulp valorization.
- Scale-up of the FASTSUGARS process from laboratory to pilot scale plant: Design, build and operate a pilot plant to hydrolyze lignocellulosic biomass in supercritical water, able to operate with up to 35 kg/h, 400 ºC and 30 MPa with reaction times below 1 second.
- Study of the supercritical water hydrolysis of inulin and inulin-rich biomass (Jerusalem artichoke) in the pilot scale plant to produce fructose and pyruvaldehyde: Study the effect of reaction time and biomass reactor concentration on the inulin hydrolysis in supercritical water and determination of the main reaction pathway for inulin hydrolysis in supercritical water.

In order to achieve the specific goals of this thesis, the work was divided in four chapters. The main content of each chapter is detailed below:

In **Chapter 1**, *"Hydrolysis of cellulose in supercritical water: reagent concentration as a selectivity factor"*, the hydrolysis of cellulose was carried out at 400 ºC and 25 MPa with reaction times between 0.07 and 1.6 seconds and suspensions concentrations between 5 and 20 % w/w (corresponding to 1.5 – 6 % w/w at reactor inlet). The experiments were carried out in the laboratory scale plant. The effect of reaction time was studied in terms of products' yields (mainly soluble saccharides and glycolaldehyde). On the other hand, the effect of inlet concentration was related to cellulose conversion kinetics.

In **Chapter 2**, *"Production of saccharides from sugar beet pulp by ultrafast hydrolysis in supercritical water"*, sugar beet pulp was hydrolyzed for the first time in supercritical water for sugars recovery in the FASTSUGARS process. To do so, experiments were performed at 390 ºC, 25 MPa and reaction times between 0.11 and 1.15 s in the laboratory scale plant. The effectiveness of the process was evaluated in terms of cellulose and hemicellulose hydrolysis yields, which were recovered as C-6 and C-5 sugars, respectively. Additionally, the remaining solid after hydrolysis was thoroughly analyzed by TGA and FTIR to study its properties.

In **Chapter 3**, *"Scaling up the production of sugars from agricultural biomass by ultrafast hydrolysis in supercritical water"*, in order to prove the versatility and potential of the FASTSUGARS process as a key step towards functional biorefineries, the process was scaled up from laboratory to pilot plant scale. Moreover, to bring the FASTSUGARS process closer to industrial applications, the particle size of the biomass was increased in the pilot plant. Then, system performance was evaluated by comparing the results obtained from sugar beet pulp and wheat bran in laboratory and pilot plants. The hydrolysis reactions at the pilot scale plant were performed at 380 – 400 ºC, 25 MPa and reaction times between 0.07 and 0.17 s.

In **Chapter 4**, *"Ultrafast hydrolysis of inulin in supercritical water: Fructooligosaccharides reaction pathway and Jerusalem artichoke valorization"*, commercial inulin and Jerusalem artichoke were hydrolyzed in supercritical water for the first time. Commercial inulin was selected as a fructooligosaccharides (FOS) model, studying its degradation pathway in supercritical water. The effects of reaction time and inlet concentration were studied, being the production of fructose and/or pyruvaldehyde the main targets. Once the hydrolysis of FOS was evaluated, Jerusalem artichoke (*Helianthus tuberosus*) results were compared to the results from pure inulin and other biomass hydrolyzed in the FASTSUGARS process. The hydrolysis reactions were performed in the pilot scale plant at 375 – 390 ºC, 25 MPa and reaction times between 0.12 and 0.74 s.

Chapter 1. Hydrolysis of cellulose in supercritical water: Reagent concentration as a selectivity factor [a]

Abstract

In this work, the influence of reagent concentration on the hydrolysis reactions of cellulose in supercritical water was analyzed. The hydrolysis was carried out at 400 ºC and 25 MPa with reaction times between 0.07 and 1.57 s and feeding cellulose concentrations between 5 and 20 % w/w (1.5 to 6 % w/w at reactor inlet). Also, a flash separator was used to separate vapor in the product stream in order to increase the final concentration. The best result for sugars production (79 % w/w) was obtained working with a cellulose concentration of 5 % w/w and 0.07 s of reaction time. For glycolaldehyde production, best result (42 % w/w) was obtained with a concentration of 20 % w/w and 1.57 s. The use of a flash separator allowed reducing the water content in 50 %. It was also observed that increasing the concentration of cellulose into the reactor up to 4 % w/w the hydrolysis took place with a similar kinetic than the hydrolysis in heterogeneous media, reducing in that way the conversion rate of cellulose in supercritical water.

[a] C. M. Martínez, D.A. Cantero, M.D. Bermejo and M. J. Cocero. Hydrolysis of cellulose in supercritical water: reagent concentration as a selectivity factor. *Cellulose*, 22, 2015, pp 2231 – 2243.

1. Introduction

Biomass is a renewable and worldwide-distributed resource of carbon, which can be used to produce energy, chemicals and fuels for the future sustainable industries [1]. Bio based industries, consisting on using renewable energy and materials, promote a decentralized production, which can be an alternative to the centralized petrochemical production plants [2]. Theoretically it is possible to obtain all the chemical materials produced by petroleum from biomass. Biomass can be converted into useful products (chemicals, fuels or energy) by two main processes: thermo-chemical processes and bio-chemical processes [3]. Generally thermo-chemical conversion processes have higher efficiencies than bio-chemical processes in terms of the lower reaction time required and higher ability to decompose most of the organic compounds. The main components of biomass (cellulose, hemicellulose and lignin) could be separated and then used as starting materials to produce interesting compounds via thermo-chemical processes such as hydrolysis [4]. Glucose would be the main product from cellulose hydrolysis, hemicellulose would release its component sugars and lignin would produce phenolic compounds [5].

Taking into account the wide range of derivatives produced from biomass, it is considered a promising source for the sustainable production of sugars and added value products such as glycolaldehyde [6, 7], which can be used then as a raw material to produce two carbons building block molecules. For example, ethylene is one of the most typical starting molecules in petrochemical industries for the production of chemicals. Ethylene can be converted into ethylene glycol, which is a widely applied in plastic and polyester industries. Apart from petroleum it could be obtained through hydrogenation of glycolaldehyde by a transition metal catalyst [8]. Therefore, selective hydrolysis of cellulose to obtain glucose and glycolaldehyde is essential for the effective use of biomass [9].

Cellulose is a major source of glucose not soluble in most conventional solvents [10]. In the recent years there is an increasing interest in using supercritical water as a solvent due to its suitability as environmentally friendly, non-toxic and inexpensive media for chemical reactions [11] since water at around the critical

point (Tc = 374.2 ºC, Pc = 22.1 MPa) shows very different properties from those of ambient liquid water [12]. The main variations in water properties are: (1) around the critical point the dielectric constant is decreased by increasing temperature, enhancing in that way the solubility of small organic compounds; (2) the ionic product (Kw) above the critical point decreases (from 10^{-14} to 10^{-25}) promoting in that way the free-radical reaction mechanisms instead of ionic mechanisms; (3) moreover, interphase mass transfer resistances are substantially reduced or eliminated operating at supercritical conditions, allowing faster reaction rates [13, 14].

Supercritical water technology allows fast conversion of cellulose into sugars being a tunable reaction media for the synthesis of selected chemicals from biomass [15]. In addition, from the point of view of decentralized chemical processes, supercritical water provides fast reaction rate, high selectivity and high conversion yield of many biomass feedstock's, making it possible to carry out chemical transformations in compact devices [16]. The conversion of cellulose and lignocellulosic biomass to valuable chemical intermediates using supercritical water has been previously reported, using different kind of reactors with different inlet concentrations of cellulose. The hydrolysis in batch-type reactors is usually carried out with long reaction times thus favoring decomposition of the produced glucose [17, 18]. The flow-type system makes it possible to shorten the reaction time and therefore reduces the degradation of sugar products. In this way, higher glucose yields could be obtained [12, 17]. Recently, our research group could improve the hydrolysis of cellulose suspensions in supercritical water by using a continuous micro-reactor, giving as a result a total conversion of cellulose in milliseconds and yielding a sugar production of 98% w/w [20]. Results obtained with the aforementioned technologies are shown in Table 1, where it can be seen that concentrations between 2 and 10 % w/w (pumping concentration) of cellulose were hydrolyzed under different operational conditions, obtaining yields of glucose between 10 and 50 % w/w.

Table 1. Literature review about concentrations and yields for supercritical water hydrolysis of cellulose.

Inlet Concentration (% w/w)	Experimental Conditions Reactor, solvent (T, P, t_R)*	Max. Yield (% w/w glucose)	Reference
20 (2 % [a])	FT, sCW (350, 25, 3.5)	25**	[21]
	FT, SCW (400, 25, 0.05)	24**	
~ 3	BT, SCW (380, 100, 5)	23	[17]
2	FT, SCW (380, 40, 0.48)	9	
10	FT, SCW (400, 25, 0.15)	43**	[12]
4	FT, SCW (400, 40, 0.3)	11	[19]
	FT, sCW (280, 40, 240)	15	
	FT, SWC + sCW (0.1 s SCW + 45 s sCW)	29	
~ 2	BT, SCW (380, 22, 17)	27	[18]
~ 2	BT,SCW + sCW (380, 22, 16 + 280, 10, 44)	33	[22]
7.5	FT, SCW (400, 25, 0.05)	50**	[20]
5 (1.5 % [a])	FT, SCW (400, 25, 0.07)	70**	This work
10 (3 % [a])	FT, SCW (400, 25, 0.31)	32**	
15 (4.5 % [a])	FT, SCW (400, 25, 0.13)	34**	
20 (6 % [a])	FT, SCW (400, 25, 0.64)	27**	

* Reactor, as batch-type reactor (BT) or flow-type (FT). Solvent as supercritical water (SCW) or subcritical water (sCW). Temperature (T) in ºC, Pressure (P) in MPa and Residence time (t_R) in s.

** Yield as % w/w of glucose in carbon basis.

[a] Concentration after mixing point

So far, existing models to describe the conversion rate of cellulose are based on the hypothesis that hydrolysis of cellulose particles mainly takes place at their external or inner surface in sub and supercritical water [23, 24], and therefore, the particle size is considered the key parameter for the conversion rate. Several studies have been carried out with the aim of studying the influence of temperature, cellulose concentration and cellulose structure on the primary products of cellulose hydrolysis in hot compressed water [25, 26, 27]. In those works it was determined that an increase in the hydrolysis temperature will improve the production of high molecular weight products (oligosaccharide of glucose) due to the weakening of hydrogen bonds. This effect is particularly high when reaching the supercritical water state, when cellulose hydrolysis seems to take place in a homogeneous phase [23, 24]. In addition, the effect of cellulose concentration was previously evaluated at very diluted systems (up to 60 mg of samples in 2 to 6 l of water), where it was concluded that there is not a solubility effect on the cellulose hydrolysis reactions [25].

In this work it was evaluated the effect of the reagent concentration over the conversion rate and selectivity of cellulose hydrolysis in supercritical water. To do so, cellulose concentrations of 5, 10, 15 and 20 % w/w in the biomass stream (1.5, 3.0, 4.5 and 6.0% in the reactor) were tested with different reaction times in a continuous reactor. In addition, the concentration of sugars in the product stream was an important factor to take into account. In this work two ways to increase the concentration of the products obtained after cellulose hydrolysis in supercritical water were studied: (a) increasing the biomass concentration before the reaction by changing the feeding cellulose concentration and (b) taking out water after the reaction, using a flash separator to maximize the concentration of the products.

2. Materials and Methods

2.1.Materials

Microcrystalline cellulose (99%) used in the experiments was purchased from VWR chemical company. Distilled water was used to carry out the experiments. The standards used in High Performance Liquid Chromatography (HPLC) analysis were: cellobiose (≥ 98%), glucose (≥ 99%), fructose (≥ 99%), erythrose (≥ 75%), glyceraldehyde (≥ 95%), glycolaldehyde dimer (≥ 99%) and 5-hydroxymethylfurfural (≥ 99%) purchased from Sigma. Sulfuric acid (≥ 96%) and calcium carbonate (≥ 99%) supplied by Sigma were used as reagents in the determination of structural carbohydrates. Milli-Q water was also used in this procedure.

2.2.Analysis

The carbon content in the liquid product was determined by total organic carbon (TOC) analysis with Shimadzu TOC-VCSH equipment. The composition of the liquid product was determined by HPLC analysis. The column used for the separation of the compounds was Shodex SH-1011 at 50 ºC, using sulfuric acid (0.01 N) as mobile phase with a flow rate of 0.8 mL/min. A Waters IR detector 2414 was used to identify the sugars and their derivatives and Waters UV-Vis detector was used to determine the 5-hydroxymethylfurfural (5-HMF) concentration at a wavelength of 254 nm.

The solid fraction (cellulose when X < 1) in the final product was separated by centrifugation. Then, it was dried at 60 ºC for 24 h and finally it was weighted. That solid fraction represented the concentration of cellulose at the outlet of the reactor. Then, the cellulose conversion was determined by Equation 1, where *'X'* is the cellulose conversion, *'W_0'* is the concentration of cellulose at the inlet of the reactor (g cellulose/g total) and *'W'* is the outlet concentration of cellulose also as g cellulose/g total.

$$X = \frac{W_0 - W}{W_0} \quad (1)$$

The concentration of soluble oligosaccharides in the liquid samples was determined by acid hydrolysis to glucose and HPLC determination following a Laboratory Analytical Procedure (LAP) from NREL [28] as follows. To 10 mL of filtered liquid aliquots 4 mL of 96 % H_2SO_4 were added. The sample was maintained in an oven at 30 ºC during 60 min. Then 86 mL of Milli-Q water were added and the sample was incubated at 121 ºC for 60 min. Calcium carbonate was added to 20 mL of this sample to neutralize the pH and finally the supernatant was filtered and analyzed by HPLC. Three to six replicates of each experiment were analyzed in order to obtain reliable results.

The yield of the main compounds (C-6 sugars, glycolaldehyde, 5-HMF, erythrose and glyceraldehyde) was determined by Equation 2, where *'Y_s'* is the yield of compound *'s'*, *'C_s'* is the concentration of *'s'* in the liquid product determined by HPLC in carbon basis and *'M_t'* is the total mass of carbon in the product, calculated as shown in Equation 3.

$$Y_s = \frac{C_s}{M_t} \quad (2)$$

$$M_t = \frac{M_{TOC}}{X} \quad (3)$$

In Equation 3, *'M_t'* is the total mass of carbon in ppm, *'M_{TOC}'* is the mass of carbon in the liquid, measured with TOC in ppm and *'X'* is the conversion of cellulose, calculated by Equation 1. Using Equation 3, the TOC values (*'M_{TOC}'*) were

transformed into total mass (*'M_t'*) and HPLC results for each compound were converted into carbon basis concentrations (*'C_s'*), multiplying each value by a carbon factor (C-6 sugars: 0.41; Glycolaldehyde: 0.40; 5-HMF: 0.57; Erythrose and Glyceraldehyde: 0.40) and then divided by total mass to obtain the yield of each product.

2.3.Experimental set-up

The experiments were performed in the continuous plant of the FASTSUGARS process, able to operate at temperatures up to 400 ºC and pressures up to 30 MPa, designed and built in a previous work of our research group [23] and modified for this work as shown in Figure 1.

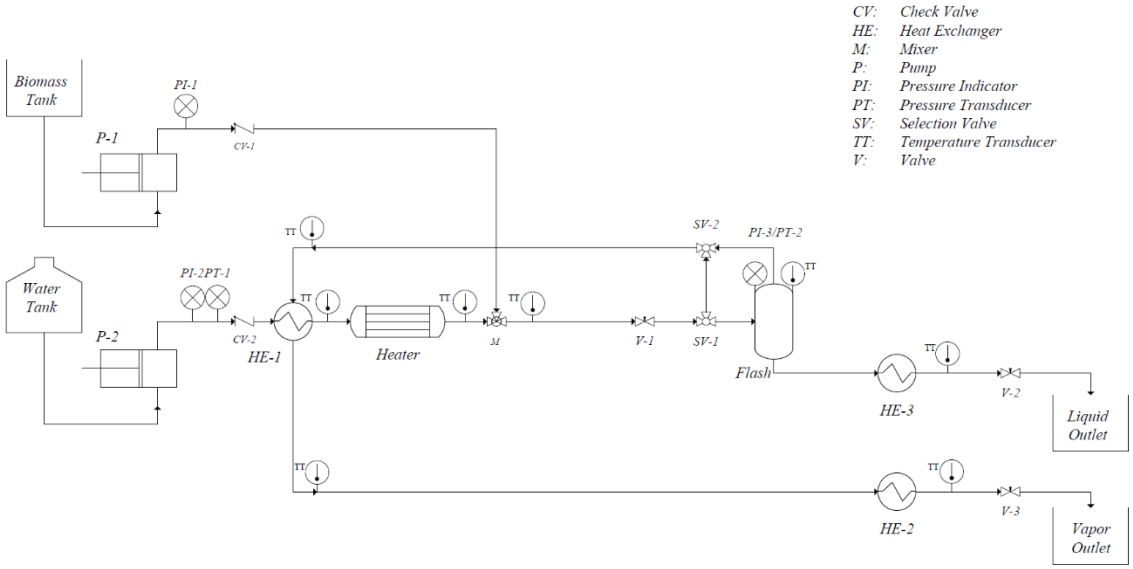

Figure 1. Experimental set up with flash chamber and heat integration. CV: check valve. HE: heat exchanger. M: mixer. P: pump. PI: pressure indicator. PT: pressure transducer. SV: selection valve. TT: thermocouple. V: valve.

The process can be divided in five stages, as follows:

1. Pressurization. Positive displacement pumps (P-1 and P-2) were used to continuously pump water and the cellulose suspension (5, 10, 15 or 20 % w/w) up to the operating pressure (25 MPa) at room temperature. It is important to notice that cellulose is not soluble in water and because of that, particular attention should be paid to biomass or pure cellulose pumping, avoiding clogging problems.

Supercritical water (SCW) was supplied up to a maximum flow rate of 5 kg/h and the cellulose suspension was fed to a maximum flow rate of 3 kg/h. In this set of experiments, cellulose concentration at the inlet of the reactor varied from 1 to 7 % w/w due to the dilution in the mixing point (M).

2. Biomass heating. The water was preheated in a heat exchanger (HE-1) that recovers part of the heat of the products followed by an electric heater with adjustable power up to 10 kW, heating water up to 450 ºC. In that conditions (25 MPa and 450 ºC) water was already in supercritical state. In order to avoid undesired reactions, it was important to heat up the cellulose stream as fast as possible. To do so, the flow ratio biomass/SCW was 1:3, meaning that at the inlet of the reactor a stream of supercritical water at 500 ºC with a flow rate of 5kg/h was mixed with cellulose stream at room temperature and a flow rate of 1.2 kg/h, instantaneously heating up the biomass up to the operating temperature (400 ºC) in a tee junction (M) and simultaneously starting the reaction. In order to avoid heat losses and to keep a constant temperature in the reactor, all the hot elements of the facility were thermally isolated using rock wool.

3. Reaction. Once desired temperature was reached, the reaction time of biomass at reaction conditions became the critical factor to control the reaction. The selectivity of the FASTSUGARS process [23] could be achieved by modifying the flows and the reactor volume. As mentioned above, the reaction started when the suspension and supercritical water met in the mixing point (M), instantaneously heating the biomass stream. The other key point of the reactor is the stopping of the reactions. It was achieved through sudden decompression and cooling.

The reaction times were calculated as the ratio of reactor volume and volumetric flow in the reactor. The volume of the reactor in m^3, *'V'*, was calculated using the dimensions of the reactor (*'D'*, *'L'*) and the flow, *'Fv'*, was calculated using the density of the reaction medium in the reactor at room conditions, considering the fluid as pure water. Since the reactor was thermally isolated and the heating and cooling methods were instantaneous, it could be considered that the temperature along it was constant. Therefore, the density was considered constant through the reactor and $'t_R'$ (reaction time in seconds) was calculated by Equation 4.

$$t_R = \frac{V}{F_v} = \frac{\pi D^2}{4} L \frac{\rho_h}{F_{v,0}\rho_0} \quad (4)$$

In Equation 4, *'ρ_h'* and *'ρ_0'* represents the density at the reaction conditions and ambient conditions in kg/m^3, respectively. *'$F_{v,0}$'* is the flow measured at ambient conditions in kg/s. Using the ratio ρ_h/ρ_0, $F_{v,0}$ is transformed into *Fv.* In a previous work it was studied the influence of the mixing time concerning the overall reaction time in supercritical water reactions. It was concluded that the mixing time between supercritical water and room temperature water takes values between 1 and 3 milliseconds at Ri = $1 \cdot 10^{-2}$ [29]. The Richardson number (Ri = Gr/Re^2) took a value around $1 \cdot 10^{-8}$ in our micro-reactor, suggesting then that the mixing time would be lower than 1 ms which is lower than 1% of the total reaction time considered [30].

4. Depressurization. Sudden depressurization allowed an instantaneous cooling (based on Joule-Thomson effect, the production of a vapor phase will suddenly cool down the effluent, $\approx 1 \cdot 10^{-5}$ s) and therefore stopping the hydrolysis reactions. This was achieved instantaneously by sudden decompression through a high temperature valve Autoclave Engineers 30VRMM4812 (V-1). The cooling method was a key part of the FASTSUGARS process, because it was the mechanism used to effectively stop the reactions, avoiding uncontrolled reactions. With this method it was possible to suddenly decrease pressure from 25 MPa to ambient pressure and temperature of the product from 400 ºC to 150 ºC. The described system was capable of instantaneously cooling the products, while simultaneously avoiding their dilution, which would occur if they were cooled down by quenching.

5. Concentration. The cooling method used in this facility (sudden decompression) represents a flash operation after which two phases are produced, vapor and liquid phases. So, a flash chamber separator was installed after the reactor in the experimental set-up, allowing the separation of the products into two phases: a vapor phase mainly composed of water; and a liquid phase with the concentrated product. After this stage, two heat exchangers were used to cool down the sample to room temperature (HE-2 and HE-3).

Operating with this experimental set-up, in the same conditions (400 ºC, 25 MPa) it was possible to concentrate the product after the reaction, maximizing the concentration of hydrolysis products. Also, by closing or opening the selection valves (SV-1 and SV-2), it could be chosen to bypass or not the flash unit, evaluating in that way the efficiency of the flash separation.

3. Results and Discussion

3.1. Influence of reagent concentration on the yield

The influence of cellulose concentration on the product yield and composition was analyzed at the best experimental conditions obtained in a previous work (400 ºC and 25 MPa) [20]. To do so, a set of experiments was carried out at different reactions times and feeding concentrations, bypassing the flash chamber. The concentration at the inlet of the reactor was varied by changing the concentration of cellulose in the starting biomass suspension. The biomass concentrations used were 5, 10, 15 and 20 % w/w, obtaining in this way cellulose concentration at the reactor inlet between 1.5 and 6 % w/w. The experimental conditions and cellulose conversion after hydrolysis for these experiments are presented in Table 2.

Table 2. Experimental conditions (at 400 ºC, 25 MPa) and cellulose conversion for experiments bypassing the flash.

Exp	Concentration (% w/w)	Reaction time, t_R (s)	Conversion, X (Eq. 1)
1	4.8 ± 0.1	0.13 ± 0.01	1.00 ± 0.01
2	10.0 ± 0.1	0.12 ± 0.01	1.00 ± 0.01
3	15.0 ± 0.1	0.13 ± 0.01	0.86 ± 0.02
4	20.0 ± 0.1	0.15 ± 0.01	0.47 ± 0.09
5	5.0 ± 0.1	0.19 ± 0.01	1.00 ± 0.01
6	10.0 ± 0.1	0.31 ± 0.01	1.00 ± 0.01
7	15.0 ± 0.1	0.17 ± 0.01	0.82 ± 0.04
8	20.0 ± 0.1	0.24 ± 0.01	0.52 ± 0.12
9	5.0 ± 0.1	0.64 ± 0.02	1.00 ± 0.01
10	10.0 ± 0.1	0.64 ± 0.03	1.00 ± 0.01
11	15.0 ± 0.1	0.40 ± 0.01	1.00 ± 0.01
12	20.0 ± 0.1	0.64 ± 0.01	0.97 ± 0.01
13	5.0 ± 0.1	0.07 ± 0.01	1.00 ± 0.01
14	20.0 ± 0.1	1.57 ± 0.01	1.00 ± 0.01

The main hydrolysis reaction pathway for cellulose in supercritical water is shown in Figure 2, based in a previous work [23]. Cellulose is firstly hydrolyzed into

oligosaccharides and then into glucose. Once glucose is produced, it can be converted into dehydrated (5-HMF) or retro-aldol condensation products (glycolaldehyde and glyceraldehyde).

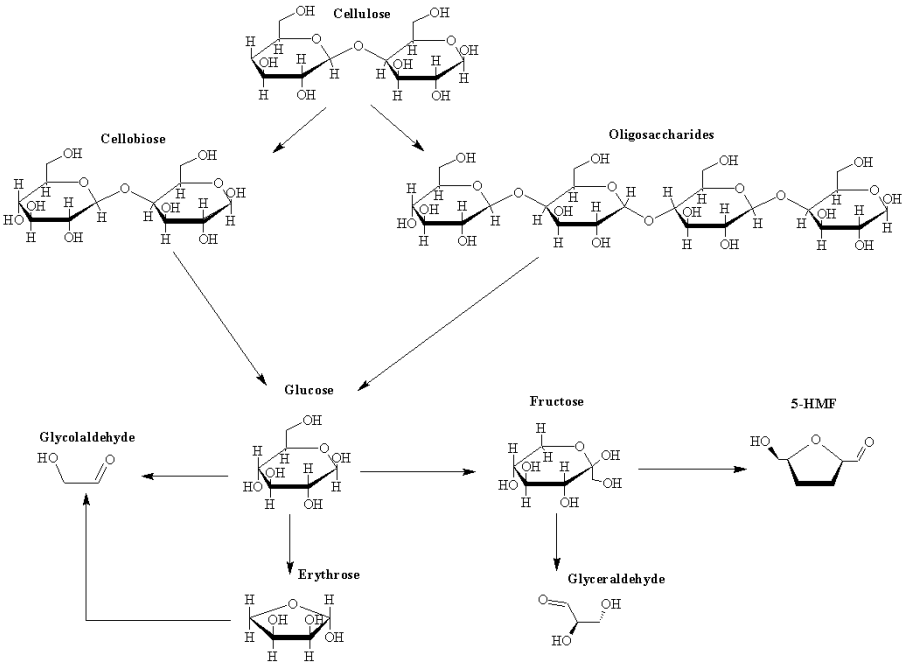

Figure 2. Reaction pathway for cellulose hydrolysis in SCW.

In Figure 3, where it can be seen that the increment of cellulose concentration for a constant reaction time, resulted in lower conversion rates. Also, while increasing the reaction time, the conversion was increased in all cases. These two trends can be explained taking into account that increasing the cellulose concentration, more cellulose particles were present in the same water volume, which meant that a lower amount of solvent was available to dissolve a higher amount of cellulose. This phenomenon represents a limitation in the mass transfer, decreasing the reaction rate. In these conditions, it was necessary to increase the reaction time to obtain complete conversion, increasing from 0.07 s for 5 % w/w to 1.57 s for 20 % w/w. The yields for the main products are shown in Figures 4, 5 and 6 (C-6 sugars, glycolaldehyde and 5-HMF, respectively). Additionally, Table 3 collected the yields of each component.

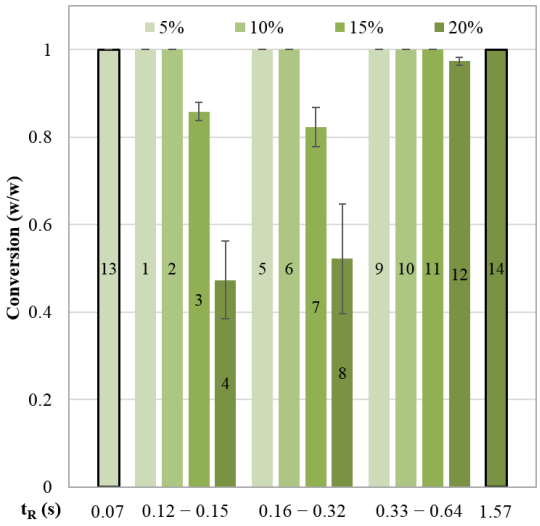

Figure 3. Conversion depending on reaction time and cellulose concentration. Numbers within the bars indicate the number of the experiment.

As a first approach, only experiments 1 to 12 (bars with no borderline) were considered to evaluate the concentration effect on the cellulose hydrolysis. For C-6 sugars (glucose and soluble oligosaccharides up to six units of glucose) it can be seen in Figure 4 that the maximum yield (66 % w/w) was obtained at the lowest concentration (5 % w/w) and lowest reaction time (0.12 s). This dependence with the reaction time was something expected, because as reported in a previous study [20], just 0.02 s were necessary to obtain high yield in sugars recovery (98 % w/w) when hydrolyzing cellulose in supercritical water. Then, an increment in the reaction time favored the degradation reactions by consuming the produced sugars. So, C-6 sugars yield decreased while increasing reaction time. This trend was the same for all the concentrations evaluated, except in the case of 20 % w/w. In that case it can be noticed that by increasing the reaction time (from 0.24 to 0.64 s), the sugars production was increased. This can be explained if it is considered that for a high concentration of cellulose such as 20% w/w, reaction times lower than 0.7 s were not enough to achieve complete conversion of cellulose (see Figure 3). When the hydrolysis was incomplete ($X < 1$) it can be assumed that an increment in the reaction time favored a higher conversion of cellulose into oligosaccharides and glucose and therefore more sugars were produced.

Table 3. Yields for different experiments (% w/w).

Exp	C-6 sugars (olig. fraction)	Glycolaldehyde	5-HMF	Erythrose	Glyceraldehyde
1	65.9 (22.5) ± 3.6	16.9 ± 0.7	0.5 ± 0.1	5.1 ± 0.2	–
2	51.4 (26) ± 2.1	16.2 ± 0.9	0.7 ± 0.1	4.4 ± 0.4	–
3	49.8 (20.4) ± 1.6	11.9 ± 0.5	0.4 ± 0.1	4.2 ± 0.1	–
4	24.6 (28) ± 0.3	6.4 ± 0.3	0.2 ± 0.1	2 ± 0.1	–
5	53.5 (34.6) ± 1.3	16.4 ± 0.5	0.4 ± 0.1	3.5 ± 0.2	–
6	52.3 (28.7) ± 2.9	19.7 ± 0.4	0.5 ± 0.1	–	3.3 ± 0.4
7	47.5 (26.8) ± 1.3	13.8 ± 1.3	0.4 ± 0.1	3.9 ± 0.4	–
8	23.2 (32.6) ± 1.1	7.8 ± 0.5	0.2 ± 0.1	–	1.2 ± 0.1
9	45.1 (30.7) ± 0.7	27.7 ± 1.2	0.6 ± 0.1	–	4.6 ± 0.1
10	50.6 (29.1) ± 45.7	34 ± 1.8	0.8 ± 0.1	–	5.6 ± 0.5
11	45.4 (27.8) ± 1	21.8 ± 0.7	0.6 ± 0.1	–	3.8 ± 0.2
12	43.5 (20.1) ± 1.1	28.7 ± 0.7	1.1 ± 0.1	–	5.9 ± 0.1
13	79.4 (66.1) ± 0.3	13.4 ± 1	0.3 ± 0.1		–
14	11.6 (0) ± 0.9	42.5 ± 1.1	1.9 ± 0.4	–	3.1 ± 0.1

In Table 3 it can be found the fraction of oligosaccharides for each experiment. It was calculated by difference between the concentration of sugars before and after acid hydrolysis. The hydrogen bonds present in cellulose are weakened at supercritical conditions making possible to produce high amounts of oligosaccharides. Once produced, the oligosaccharides yield will depend on the reaction time. In this work, the highest fraction of oligosaccharides (66% w/w) was achieved at the lowest reaction time (Exp. 13). It was something expected, since as shown in Fig. 2 the first step in cellulose hydrolysis is the oligosaccharide production. Working with very low reaction times as 0.07 s, there were not enough time to completely convert oligosaccharides into glucose and therefore the amount of oligosaccharides was higher than glucose. When the reaction time was increased (higher than 0.1s) the main sugar products were monosaccharide.

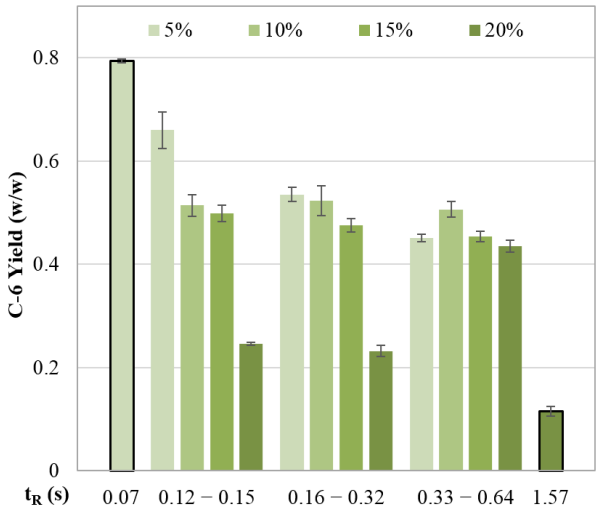

Figure 4. C-6 sugars yield depending on reaction time and cellulose concentration.

Glycolaldehyde is produced via retro-aldol condensation of glucose and it is a promising raw material, which can be used in a variety of industrial processes and applications [31]. In Figure 5 it can be noticed that two trends were observed for glycolaldehyde yield. Working with a constant reaction time, when increasing the concentration of cellulose, the yield of glycolaldehyde decreased (that trend was especially important in reaction times between 0.12 and 0.32 s). For these low reaction times cellulose conversion for high concentrations was incomplete and therefore the production of glucose was relatively low. As glycolaldehyde is the main product of glucose retro-aldol condensation [1], low production of glucose implied low glycolaldehyde yields. On the other hand, by increasing the reaction time, for the full range of concentrations, the yield of glycolaldehyde increased because sugars derived into other products (mainly glycolaldehyde), increasing in that way glycolaldehyde production. The maximum yield for glycolaldehyde in this section (34 % w/w) was achieved at the highest reaction time and 10 % w/w of cellulose.

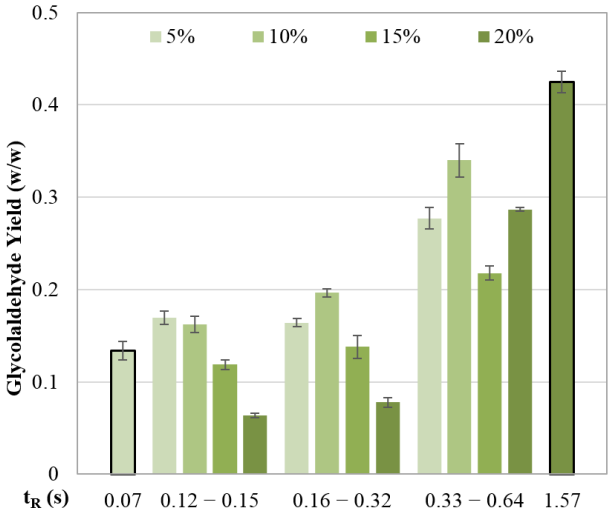

Figure 5. Glycolaldehyde yield depending on reaction time and cellulose concentration.

5-HMF is a dehydration product of fructose and it is an undesired compound in the sugars production if a microorganism post-processing of the product is required [32]. The production of 5-HMF was lower than 2 % w/w in all the experiments (see Fig. 6), being the maximum amount achieved at the highest concentration and highest reaction time. The behavior observed for 5-HMF yield was almost the same that for glycolaldehyde, since at a constant range of reaction times while increasing the concentration, the yield decreased. On the other hand, by increasing the reaction time, the yield of 5-HMF increased. The degradation reactions were favored by increasing the reaction time, enhancing in that way the yield of degradation product such as 5-HMF in addition to glycolaldehyde.

The behavior of other compounds as erythrose and glyceraldehyde (see Table 3) showed a strong dependence on reaction time. For low reaction times (lower than 0.2 s) only erythrose was yielded whereas glyceraldehyde was produced for the rest of experiments. This can be explained by following the reaction pathway shown in Figure 2. It can be seen that glucose could be converted into fructose or erythrose plus glycolaldehyde by isomerization or retro-aldol condensation, respectively [31]. The fructose would also produce glyceraldehyde via retro-aldol condensation [1]. As it was demonstrated before, the production of fructose was favored by increasing the reaction time [33]. So, when working with low reaction times the production of

fructose was low and as a consequence, the yield of glyceraldehyde was negligible. Furthermore, when increasing the reaction time, the erythrose produced was decomposed into glycolaldehyde via retro-aldol condensation [31], so the yield of erythrose for high reaction times was also negligible. Therefore, while increasing the reaction time for the full range of concentrations, the yield of erythrose decreased and the production of glyceraldehyde increased. On the other hand, working with a constant reaction time, the yield of erythrose decreased when increasing the concentration of cellulose. This behavior was also observed for glycolaldehyde yield. In both cases it can be assumed that for low reaction times the conversion of cellulose in highly concentrated suspensions was incomplete and therefore, the production of glucose was relatively low (just 20 % w/w), which implied lower yields of its hydrolysis products (glycolaldehyde and erythrose). In the case of glyceraldehyde, no clear tendency was shown for the different concentrations. Maximum yield of erythrose (5 % w/w) was achieved for the lowest concentration and lowest reaction time. For glyceraldehyde the maximum (6 % w/w) was produced at the highest concentration and the highest reaction time.

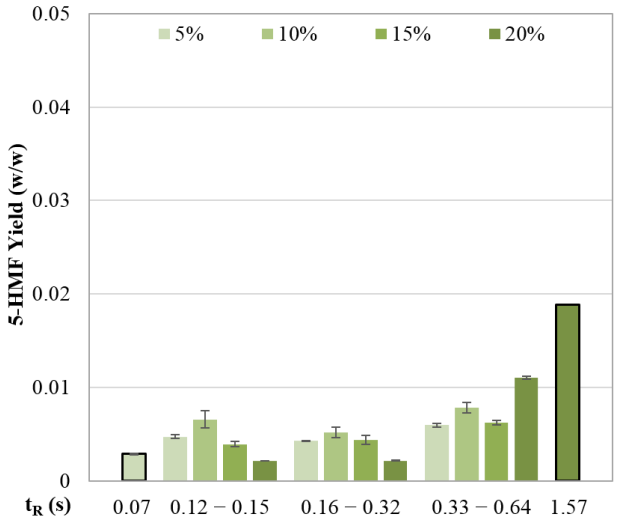

Figure 6. 5-Hydroxymethylfurfural (5-HMF) yield depending on reaction time and cellulose concentration.

Once it was evaluated the influence of the reagent concentration and reaction time over the products yield, the aim was to optimize the yields obtained for both C-6 sugars and glycolaldehyde. As shown before, the maximum yield for C-6 sugars

was achieved at the lowest concentration and lowest reaction time (Exp. 1) and for glycolaldehyde the maximum yield was achieved at the highest cellulose concentration and highest reaction time (Exp. 12). To optimize these extreme conditions, two more experiments were carried out: an experiment with a lower reaction time and the lowest concentration (0.07 s, 5 % w/w) to maximize the production of C-6 sugars and another experiment with the highest concentration and a higher reaction time (20 % w/w, 1.57 s) in order to improve glycolaldehyde yield. These results were also plotted in Figures 3, 4, 5 and 6 (represented by bars with borderline).

The experiment with 5 % w/w of cellulose and 0.07 s (numbered as 13) was performed using a lower reaction time than those used in the previous experiments. So, as expected, when decreasing the reaction time the yield of sugars increased. That confirmed that lower reaction times produced lower glucose degradation and thus higher yield of C-6 sugars. Therefore, it was demonstrated that reaction time worked as a key factor for the reaction selectivity as it was reported in previous studies [20]. In this experiment it was possible to maximize the yield of C-6 sugars, giving as a result a yield of 79 % w/w.

In the case of 20 % w/w of cellulose and 1.57 s (experiment 14) the key factor to optimize the production of glycolaldehyde was achieving total conversion. Following the reaction pathway shown in Figure 2, it can be seen that the first step in cellulose hydrolysis was the production of oligosaccharides, cellobiose and glucose (C-6 sugars). Then as a second step, the glucose turned into glycolaldehyde and other products. So, when the hydrolysis of cellulose was incomplete ($X < 1$), the first sign was the low yield of glucose and as a consequence, the low yield of glycolaldehyde (experiments 4 and 8). Conversion came closer to 1 when the reaction time was increased (experiment 12) and as a result the conversion of cellulose into glucose was enhanced. When total conversion was achieved ($X = 1$) all the glucose produced was rapidly degraded into other products, providing at the same time low yields for C-6 sugars and high yields for degradation products (experiment 14). In this last experiment it can be seen that it was achieved the minimum yield of C-6 sugars (12 % w/w) and also the maximum yields for other

products evaluated (42 % w/w glycolaldehyde and 2 % w/w 5-HMF). The glycolaldehyde yield was as high as expected for this relatively low reaction time. In a previous work it was reported a yield of 12 % w/w for C-6 sugars, 40 % w/w for glycolaldehyde and around 1 % w/w for 5-HMF (carbon basis), working at 400 ºC, 25 MPa and 1 s of reaction time [20]. In the current study, working with a slightly higher reaction time, it was possible to increase the yield of glycolaldehyde when operating at 400 ºC and 25 MPa.

3.2.Concentration with flash chamber

To study the performance of the process when using a flash chamber to concentrate the products in the final effluent, optimal conditions presented before were selected (5 % w/w cellulose, 0.07 s and 20 % w/w, 1.57 s). In both experiments, same conditions of pressure and temperature were used (25 MPa, 400 ºC) and total conversion was achieved (X = 1).

These experiments (numbered as 13 and 14, respectively) were performed using the facility shown in Figure 1, following two steps: (1) first, hydrolysis bypassing the flash chamber was carried out. This part helped to identify and optimize the effect of reaction time over the yield, as commented in the previous section; (2) then, the product stream went through the flash chamber and it was separated into two streams, allowing to take samples of the liquid (L) and vapor (V) phases. In that way the efficiency of using a flash chamber as a way to concentrate the product was evaluated. Results obtained in these experiments were presented in Table 4 and Figure 7. Once the cellulose was hydrolyzed, the product went through the flash and it was separated into two phases (liquid, L and vapor, V). In Figure 7 the results were plotted in ppm in order to compare the effect of the separation over the final concentration of products (C-6 sugars and glycolaldehyde concentrations were selected to follow the separation carried out in the flash).

In the case of the lowest concentration and reaction time (experiment 13) in Table 4 it can be seen than the separation ratio (L:V) was approximately 2:1. Indeed, the separation was taking place in the flash chamber in terms of flow distribution. As it can be seen in Figure 7, the flash allowed increasing the concentration of sugars

from 10000 ppm to 15000 ppm in the liquid phase only by setting a flash separation after the reaction. In the case of derived products it can be observed that the concentrations of glycolaldehyde remained almost the same. The vapor phase is not able to dissolve saccharides and that was the reason why the concentration of hydrolysis products was so low. So, the flash chamber proved to be an effective way to increase the concentration of sugars in the final product.

The use of the flash chamber in this case gave as a result a liquid stream rich in sugars with a relatively low content of glycolaldehyde and a lower concentration of 5-HMF (see Table 4). Indeed, it was possible to increase the concentration of C-6 sugars up to 50 % just by adding the flash separation after the reaction. On the other hand, the vapor stream was mainly composed by water and 10 times less carbon content regarding the initial sample (5165 ppm of carbon bypassing the flash versus 593 ppm in the vapor phase).

Table 4. Results for liquid products operating bypassing or not flash chamber (L for liquid and V for vapor phase)

Experiment	**13**	**13-L**	**13-V**	**14**	**14-L**	**14-V**
t_R (s)	0.07	0.08	0.08	1.57	1.34	1.34
Flow rate (mL/s)	0.60	0.39	0.18	1.71	1.27	0.51
M_{TOC} (ppm)	5165	9303	593	17823	23702	6722
C-6 sugars	10005	14499	135	5064	4845	296
Glycolaldehyde	2084	2402	637	18943	20701	7655
5-HMF	26	29	1	588	550	27
Erythrose	588	666	0	0	0	0
Glyceraldehyde	0	0	0	1390	2228	67

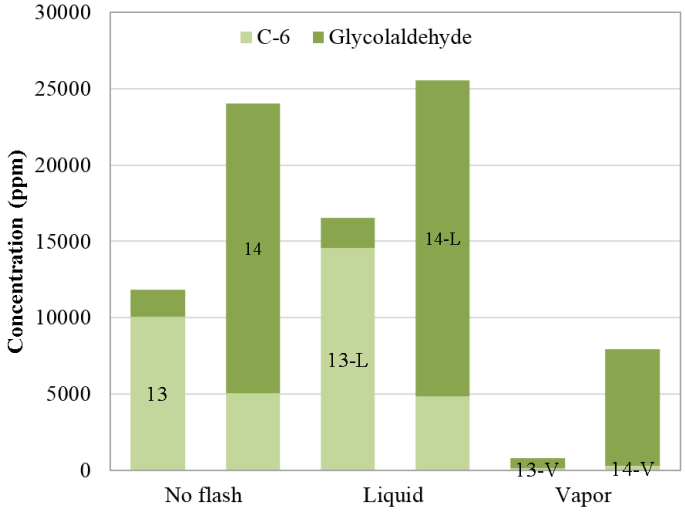

Figure 7. Concentration of sugars and glycolaldehyde for experiments 13 and 14 bypassing the flash (no flash) and using the flash.

For the experiment with the highest concentration and reaction time (experiment 14) the main difference was that the main product yielded was glycolaldehyde instead of C-6 sugars. In terms of flow distribution, the separation ratio (L:V) was approximately 3:1, very similar to the ratio obtained in experiment 13. Regarding the concentration of different products, in Fig. 7 it can be observed that using the flash separator it was possible to increase the concentration of glycolaldehyde from 19000 ppm to 21000 ppm in the liquid phase. This increment was lower than the increment experienced by the C-6 sugars in the previous experiment. That was due to the relatively high amount of glycolaldehyde solubilized in the vapor phase. In both experiments it can be noticed that C-6 sugars and 5-HMF stayed in the liquid phase, but on the contrary, the glycolaldehyde produced went solubilized in the vapor phase, probably due to its low molecular weight.

In this experiment, the addition of a flash separation step allowed to increase the concentration of glycolaldehyde in the liquid stream up to 10 %, obtaining as a result a liquid stream rich in glycolaldehyde with a low content of sugars and derived products as 5-HMF. It was also remarkable the possibility to obtain a vapor phase mainly composed by water and glycolaldehyde.

Carrying out these two experiments helped to identify the efficiency of using a flash chamber to concentrate the products in the final effluent. When obtaining sugars as major product (low reaction time and concentration) the flash chamber allowed to increase the concentration of C-6 sugars in 50 %, giving as a result a liquid stream rich in sugars with low content of degradation products. Nevertheless, when glycolaldehyde was the main product, the addition of a flash separator increased the concentration of glycolaldehyde just in 10 % due to the higher solubility of glycolaldehyde in the vapor phase compared to saccharides one.

3.3.Influence of reagent concentration on kinetics

Existing models to describe the conversion rate of cellulose hydrolysis in supercritical water assumed that hydrolysis of cellulose particles mainly takes place at their surface [23, 24] and implied the use of a no conventional kinetic equation. In this work a conventional first order kinetic was assumed to describe the conversion rate of cellulose in supercritical water. Equation 5 represents the kinetic expression, where *'C_0'* is the inlet concentration in % w/w, *'C'* is the final concentration calculated by Equation 6 where *'X'* is the conversion of cellulose. *'k'* is the kinetic constant, in s^{-1} and *'t_R'* is the reaction time in s.

$$\ln\left(\frac{C}{C_0}\right) = k \cdot t_R \tag{5}$$

$$C = C_0 \cdot (1 - X) \tag{6}$$

Experimental results obtained with feeding concentrations of 5, 15 and 20 % w/w of cellulose (1.5, 4.5 and 6 % w/w at the reactor inlet) were used to calculate the kinetic constant. Experimental data for 5% w/w were taken from a previous work [20]. It should be mentioned that the experiments using a cellulose concentration of 10% yielded a total conversion, so, the hydrolysis kinetic has not been calculated. In Figure 8 it was plotted the logarithm against the reaction time, and a linear dependence was found, where the slope represented the kinetic constant, *'k'*.

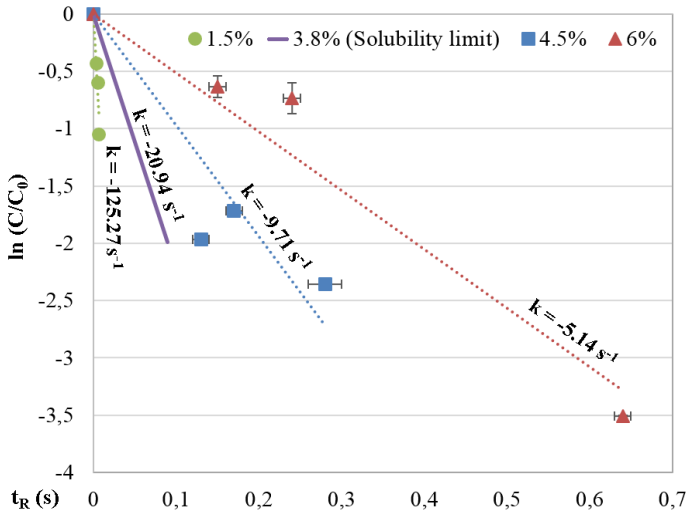

Figure 8. Kinetic analysis for cellulose concentrations of 5, 15 and 20 % w/w. C=1.5%; R^2=0.90. C=4.5%; R^2=0.81. C=1.5%; R^2=0.96.

In a previous work, Arrhenius parameters were calculated for cellulose hydrolysis in subcritical water, being 154.4 kJ/mol the activation energy (E_A) and 29.6 the pre-exponential factor (ln A) [23]. From this data the kinetic constant at 400 ºC was calculated for the heterogeneous kinetic equation validated in the previous study (based on the superficial consumption of the cellulose grain). In that way it was possible to estimate the conversion of cellulose for different reaction times. Then, these values of conversion and reaction times were used to calculate a new kinetic constant (–20.94 s^{-1}) for the first order kinetic, which corresponded to 3.83 % w/w of cellulose at the inlet of the reactor. This concentration can be considered as the solubility limit, representing the limit between homogeneous reaction media and heterogeneous media for cellulose hydrolysis in supercritical water. Assuming that the concentration was lower than 3.83 % w/w, then the cellulose was completely solubilized in supercritical water. In that case, it can be considered that hydrolysis of cellulose occurred in a homogeneous phase and therefore the conversion rate was higher. On the contrary, if the concentration was higher than the solubility limit, then the cellulose behaved as if it had been hydrolyzed at subcritical conditions. Under these conditions the cellulose was not totally dissolved and the hydrolysis reaction occurred in a heterogeneous phase. This fact can be explained assuming that a decrease in the solubility of cellulose in supercritical water implied a

heterogeneous reaction where the mass transfer resistances could limit the reaction rate.

Moreover, beyond 3.83 % w/w of cellulose, the slope decreased when increasing the concentration (see Figure 8). This can be explained considering that the mass transfer resistance increased when the cellulose concentration was higher. As previous studies reported, it can be assumed that the mass transfer rate coefficient was strongly dependent on reagent concentration [34] and mass transfer limited overall conversion yields in systems with high-solids loadings [35]. So, it can be assumed that not only particle size is a key parameter in the conversion rate of cellulose hydrolysis in supercritical water but also cellulose concentration and interphase mass transfer limitations must be considered.

4. Conclusions

Cellulose hydrolysis in supercritical water was studied experimentally to evaluate the effect of biomass concentration on the reactions. It was found that it is necessary to increase the reaction time to achieve total cellulose conversion when using highly concentrated suspensions. This also favors the conversion of glucose into its derived products. So, cellulose (and biomass) can be selectively hydrolyzed in supercritical water to sugars with low reaction times and using low concentrations of biomass. If the desired products are glucose derivatives, such as glycolaldehyde, high reaction times are needed. To increase the concentration of the products it was proposed the addition of a flash separation, which allows an increment of the concentration up to 50 % in sugars and 10 % in glycolaldehyde. It was also found that the inlet concentration of biomass affects the conversion rate of cellulose in supercritical water. Increasing the inlet concentration up to 4 % w/w, the reaction occurs in a heterogeneous media where the mass transfer resistances could limit the reaction rate. In addition, these mass transfer resistances show a strong dependence on cellulose concentration.

Acknowledgements

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Chapter 2. Production of saccharides from sugar beet pulp by ultrafast hydrolysis in supercritical water [b]

Abstract

Sugar beet pulp (SBP) is the major by-product in sugar industry. To make profit out of this undervalued residue, the FASTSUGARS process was proposed as a solution, combining the advantages of supercritical water as hydrolysis medium with very short reaction times in the so-called ultrafast reactors. Operating at 390 ºC, 25 MPa and reaction times between 0.11 and 1.15 s it was possible to convert SBP into sugars and to obtain a lignin-like solid fraction. The highest yields of C-6 and C-5 sugars (61 and 71 % w/w, respectively) were obtained at 0.11 s with the lowest yield of degradation products. The solid product obtained at 0.14 s was thoroughly analyzed by acid hydrolysis, TGA and FTIR analysis to prove its enhanced thermal properties and aromaticity. The FASTSUGARS process demonstrated being a versatile and promising technology to be integrated in the future biorefineries.

[b] C. M. Martínez, D.A. Cantero and M. J. Cocero. Production of saccharides from sugar beet pulp by ultrafast hydrolysis in supercritical water. *Journal of Cleaner Production*, 2018. 204: pp 888-895.

1. Introduction

In the recent years, many studies have focused on the requirements of the future industries to meet the European Union climate and energy targets for the year 2020. The foundation of the chemical industry is the conversion of raw materials into fuels, chemicals, materials and energy. From the past century, fossil resources have been the primary feedstock for chemical industries [1]. However, the global economy tends to shift the chemical industry away from petroleum towards renewable raw materials and sustainable processes in the so-called biorefineries [2, 3].

The success of a biorefineries eventually depends on the extent of integration that can be achieved [4]. Supercritical fluids are a promising alternative to integrate the depolymerization, reaction and separation processes [5]. In fact, using supercritical water (SCW, meaning water above its critical point: 374 ºC and 22 MPa) as reaction or extraction medium for biomass has several advantages over other processes: first obvious reason would be its suitability as solvent, being an environmentally friendly and nontoxic medium for chemical reactions [6]. Moreover, water itself is one of the constituent of biomass so that, using water as solvent would make unnecessary to previously dry biomass, implying an important energy saving [7]. Finally, physical properties of water can be finely tuned by varying temperature and pressure at around its critical point. That would allow fractionation of biomass, since just by changing the reaction conditions it is possible to extract and/or depolymerize the different fractions of biomass. Particularly, operating under SCW conditions, mass transfer resistances are substantially reduced giving as a result faster reaction rates [7]. Indeed, certain biomass fraction face reactions that occurs in the range of milliseconds. Then, changing the reaction time from minutes to milliseconds, allows the reactor volume being reduced from m^3 to cm^3 and therefore implies an important equipment cost reduction [5]. That drastic reaction time reduction is a strong step towards the process intensification of biomass usage. The intensification of biomass use as feedstock is of utmost importance in the development of compact and efficient facilities, consuming local available biomass and providing local needs. Moreover, SCW technology could be integrated with

power generation by gas turbines, injecting the steam produced in the hydrolysis process to the combustor [8]. That integration results in very low extra energy consumption when coupling SCW hydrolysis and heat and power generation.

There is more than one parameter to evaluate when choosing a feedstock to develop the biorefinery concept. When pursuing industrial sugars, like glucose or xylose, plus lignin; it becomes very important to consider as a feedstock a cheap and highly available feedstock. In that sense, the agro-industrial byproducts are considered promising resources for the production of sugars and lignin [9]. This is the case of sugar beet pulp (SBP), which is the major by-product in beet sugar industry. It is composed of 20 – 25 % cellulose, 22 – 30 % hemicellulose, 24 – 32 % pectin, 10 – 15 % protein and 1 – 3% insoluble lignin on a dry basis [10, 11]. Due to its low insoluble lignin and high carbohydrates content, sugar beet pulp is an interesting candidate for both sugars recovery and platform chemical production in the future biorefineries [12]. In some cases, the sugar plants from beet possess internal heat and power generation systems by gas turbines. This fact presents an opportunity to link SCW hydrolysis of SBP with heat and power production by gas turbines.

During the past years, several authors studied the fractionation of SBP to obtain ferulic acid [13, 14], arabinoxylans and/or pectic substances [15, 16]. To do so, enzymatic hydrolysis was the preferred method to release those components. However, the high dosage of enzymes and/or chemicals required to release sugars is still a concern in the operating cost side, presenting a significant barrier to commercialization [17]. Moreover, for SBP being a complex mixture of cellulose, hemicellulose and pectin, the efficient enzymatic conversion of the whole crop is still a problem to be solved [12]. Dilute acid pretreatments are usually presented as a solution [10] but they have important drawbacks such as equipment corrosion, poor catalyst recyclability and sugars degradation [18]. To overcome these limitations, SCW technology has demonstrated being a promising alternative to transform biomass into sugars with several advantages over conventional process. It produces less sugars degradation compared to acid/alkali methods and it allows equipment and time reduction compared to enzymatic routes [18]. In the recent

years near-critical water hydrolysis of agricultural and food industry residues has been intensively studied, but SCW hydrolysis studies are still limited [19-21].

Considering the complexity of the matrix interactions and the diversity of their compositions, each biomass represents a technological challenge that should be studied separately [18]. In this work, sugar beet pulp was hydrolyzed for the first time in supercritical water for sugars recovery in the so-called FASTSUGARS process. The reaction temperature for this study was dropped from previous studies at 400 ºC to 390 ºC to evaluate the ability of the system to still produce high selective hydrolysis while cutting the energy demand. To do so, the hydrolysis was carried out in a continuous flow type reactor setup, called as ultrafast reactor from now on. Since the sugar industry from beet shows a perfect example for the integration of sugars and lignin production from residual biomass with the heat and power production systems by gas turbines, the aim of this work was to optimize the ultrafast SCW hydrolysis to convert SBP into sugars, platform chemicals and lignin-like solid products.

2. Materials and Methods

2.1.Materials

A local sugar industry (ACOR, Spain) provided the SBP used in the experiments. It was milled to obtain an average particle size of 60 µm. Deionized water was used as the reaction medium to run the experiments. The High Performance Liquid Chromatography (HPLC) standards were purchased from Sigma-Aldrich, being: cellobiose, galacturonic acid, glucose, xylose, fructose, arabinose, glyceraldehyde, pyruvaldehyde, glycolaldehyde, lactic, formic and acetic acids and 5-hydroxymethylfurfural (5-HMF). Milli-Q water and sulfuric acid were used as the mobile phase in the HPLC analysis. For the determination of carbohydrates and lignin, sulfuric acid and calcium carbonate supplied by Sigma were employed as reagents. The pectin identification assay kit from Megazyme was used to determine the pectin fraction in biomass. For this purpose, Trizma base and sodium hydroxide pellets were purchased from Sigma and hydrochloric acid solution 5 M was purchased from Fluka. For Kjeldahl determination of protein content, Kjeldahl catalyst (Cu) (0.3% $CuSO_4.5H_2O$) tablets were purchased from PanReac.

2.2.Methods

2.2.1. Chemical characterization of the raw sugar beet pulp

Laboratory Analytical Procedure from the National Renewable Energy Laboratory (NREL) was used to determine the structural carbohydrates (namely, cellulose and hemicellulose) and lignin content in the biomass [22]. This same protocol was described in a previous work in which wheat bran was characterized [19]. Using this procedure, it was possible to quantify the extractives, cellulose, hemicellulose, ash, insoluble and soluble lignin in sugar beet pulp. The particle size of the starting material was measured using a Dynamic Light Scattering (DLS) Mastersizer 2000. The mean particle size was 60 μm. Total Kjeldahl nitrogen was determined according to APHA Sandards Methods and then total proteins were calculated as Kjeldahl N × 6.25 [23], calculated as shown in Eq. 1, where *'N'* was the normality of the sulfuric acid used for the titration (0.05 N in this case), *'V'* is the volume of acid used in the titration in mL and *'m_s'* is the mass of sample used in g.

$$Kjendahl\ \ Nitrogen = \frac{(N \times V)}{m_s} \quad (1)$$

The pectin identification assay kit from Megazyme was employed to determine the pectin fraction in SBP. Using this kit, pectin was dissolved in water at pH 12, yielding polygalacturonic acids through the conversion of pectin into pectate. The pectate was incubated with pectate lyase enzyme which broke the polygalacturonic acid, releasing unsaturated oligosacchariedes which absorbed at 235 nm [24]. As this kit contained pectin from SBP as a standard, the pectin content in the sample was determined considering that the absorbance from the pectin standard equaled to 100 % pectin content and therefore the pectin percentage in raw material was calculated by comparison.

2.2.2. Analysis

The composition of the liquid product was determined by HPLC analysis, using a Shodex SH-1011 column as described in previous works [19, 25]. The carbon content in the liquid product was determined by total organic carbon (TOC) analysis with Shimadzu TOC-VCSH equipment. The solid product was separated by

centrifugation, dried at 105 ºC for 24 h and weighted to determine the suspended solids. Then, its composition was determined following the same NREL procedure used for lignin determination in the raw material [22]. Elemental C-S analyser, using a LECO CS-225 equipment, determined the carbon content of the raw material and remaining solids. The solid fraction was also analyzed by spectroscopy Fourier Transformed Infrared (FTIR) by using a Bruker Tensor 27. Thermogravimetric analysis (TGA) was carried out in a TGA/SDTA RSI analyzer of Mettler Toledo.

2.2.3. Experimental setup

The experiments with SBP were performed in the continuous hydrolysis plant of the so-called FASTSUGARS process. This FASTSUGARS plant was designed and built in a previous work of our research group, which operating procedure was deeply described before [19, 25, 26]. The reaction section was modified for this work as shown in detail in Fig. 1.

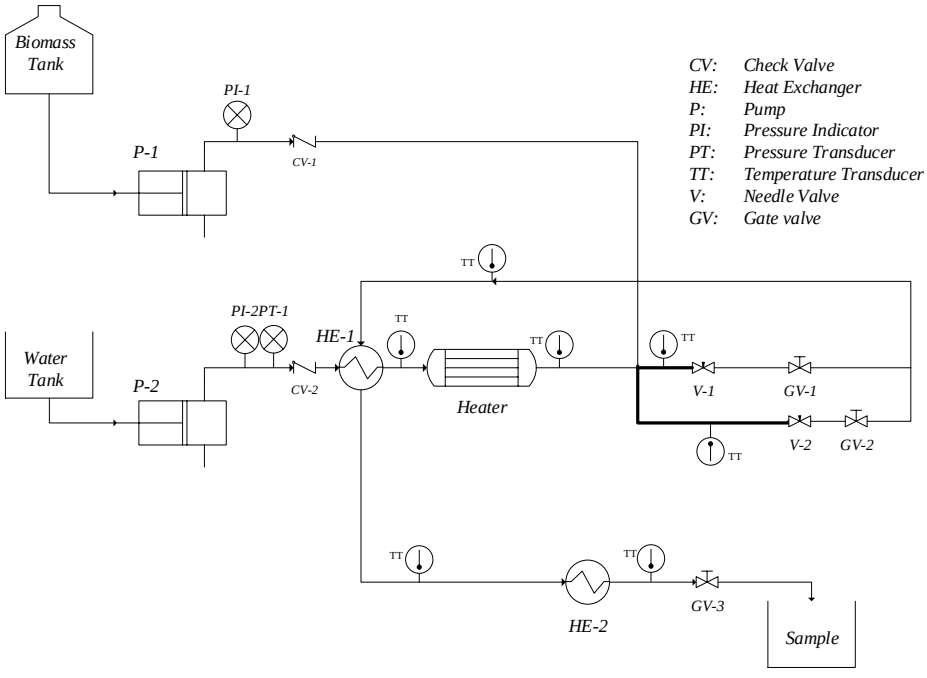


Figure 1. FASTSUGARS setup used to carry out the hydrolysis of sugar beet pulp in supercritical water.

Then, the key factor in the FASTSUGARS process was the accurate control of the reaction time, meaning the time that biomass and SCW spent together between the mixing point and the reactor outlet. This was possible due to the unique characteristics of the FASTSUGARS reactor. The reactions were instantaneously stopped by a sudden cooling generated by decompressing the reactor from ~25MPa to ~0.2 MPa. That pressure drop produced an instantaneous cooling effect by massive steam explosion (also known as Joule-Thomson effect). This cooling mechanism uniquely stopped the reactions. The reaction times, *'t_R'* in seconds, were calculated as shown in Eq. 2. The reactor volume, *'V'* in m^3, was calculated using the dimensions of the reactor. The volumetric flow in the reactor, *'Fv'* in m^3/s, was calculated as a function of the density of the reaction medium at ambient conditions *'ρ_0' and* reaction conditions *'ρ_r'*, both in kg/m^3 and considering the fluid as pure water. Using the ratio *'$\rho r/\rho_0$'*, it was possible to transform the flow measured at ambient conditions, *'$F_{v,0}$'* in m^3/s, into *'F_v'*. Therefore, in order to change the reaction time for the different experiments, either reactor's length, total flow or both were varied.

$$t_R = \frac{V}{F_v} = \frac{\pi \cdot L \cdot D^2}{4} \frac{\rho_r}{F_{v,0} \cdot \rho_0} \tag{2}$$

Therefore, in order to change the reaction time for the different experiments, either reactor's length or total flow should be varied. In the new configuration implemented for this study, two reactors with different lengths were installed. In this way it was possible to work with low reaction times (around 0.2 s) or high reaction times (around 1 s) just by selecting which reactor to use. To do, apart from the needle valve for each reactor (V-1 and V-2), two gate valves were installed (GV-1 and GV-2), so that when using the reactor 1 (short t_R) the valve V-1 was used to control the pressure, GV-1 was opened allowing the continuous flow in the reactor and at the same time V-2 and GV-2 were closed to block the flow in reactor 2. Using this system it was possible to hydrolyze sugar beet pulp in supercritical water at 390 ºC and 250 bar, with reaction times between 0.11 and 1.15 seconds.

3. Results and discussion

3.1.Biomass characterization and calculations

The compositional analysis of the raw material is shown in Table 1. The lignin fraction was a result of the sum of both soluble and insoluble lignin fractions, being 4.4% insoluble lignin and 18.4% soluble lignin (measured at 280 nm in a spectrophotometer, using 17.084 L/g·cm as extinction coefficient obtained as the average of extinction coefficients from literature [27]).

Table 1. Compositional analysis for SBP (dry basis).

Lignin	Ash	Cellulose	Hemicellulose	Proteins	Pectins	Extractives
23.9%	1.3%	16.6%	19.7%	9.5%	27.5%	1.9%

The sugar beet pulp was hydrolyzed in supercritical water at 390±5 ºC and 25±5 MPa at different reaction times in the FASTSUGAR plant. In Table 2, the main parameters used for carbon balance were presented. The carbon balance in the experiments was around 100%. Carbon balance was solved as shown in Eq. 3, where *'carbon inlet'* was calculated by using Eq. 4 being *'Cin'* (% w/w) the concentration of dry biomass at the inlet of the reactor converted into ppm of carbon (ppmC) by multiplying by 10000 and then by *'CFbiomass'* (0.33 g carbon/g biomass) that was the carbon factor of the raw material measured by elemental analysis. Then, *'carbon outlet'* was the sum of the carbon due to the liquid (directly measured by TOC in ppmC) and the carbon due to the solid products, calculated as shown in Eq. 5. Carbon in the solid named as *'carbon solid'* in ppmC was calculated as a function of suspended solids, *'% susp'* in % w/w and converting them to carbon units by using the carbon factor corresponding to the remaining solid *'CFremaining'*, being 0.394 g carbon/g remaining solid.

$$carbon\ balance = \frac{carbon\ outlet}{carbon\ inlet} \tag{3}$$

$$carbon\ inlet\ = Cin \cdot 10000 \cdot CFbiomass \tag{4}$$

$$carbon\ outlet = carbon\ liq + carbon\ solid\ = TOC + \%susp \cdot 10000 \cdot CFremaining \tag{5}$$

Table 2. Carbon balance calculations for SBP experiments in FASTSUGARS process.

EXPERIMENT	1	2	3	4	5
t_R (s)	0.11 ± 0.003	0.14 ± 0.016	0.19 ± 0.007	0.23 ± 0.019	1.15 ± 0.053
Cin (%)	1.90	1.68	1.64	1.72	1.73
Carbon inlet (ppmC)	6624	5546	5428	5690	5713
Susp. Solids (%)	0.15	0.13	0.06	0.12	0.03
Carbon solid (ppmC)	588	526	221	459	111
Carbon liquid=TOC (ppmC)	5883	5093	5189	5092	5386
Carbon outlet (ppmC)	6471	5619	5411	5551	5497
Carbon balance (%)	103 ± 1	101 ± 4	100 ± 2	98 ± 8	96 ± 5
Total hydrolysable basis (ppmC)	4289	3797	3716	3896	3911
Hydrolysable to sugars (ppmC)	2564	2270	2222	2329	2338

Then, for calculating the main hydrolysis parameters it was necessary to define the calculation basis for the liquid effluent. To do so, Eq. 6 was used where the carbon available for hydrolysis, named as *'total hydrolysable basis'* in ppmC, was defined as a function of the *'carbon inlet'* and then multiplied by the hydrolysable fractions of biomass that would yield products detectable by HPLC, namely cellulose, hemicellulose and pectins. The fractions of cellulose and hemicellulose in biomass were translated into their derived sugars, so that *'% cell'*, being 19 % w/w, was obtained by dividing cellulose fraction of biomass by 0.9 and *'% hemicell'*, being 22 % w/w, was obtained by dividing hemicellulose fraction in biomass by 0.88. *'% Pectins'* remained the same, being 28 % w/w (see Table 1 in results section).

$$total\ hydrolisable\ basis = carbon\ inlet \cdot \frac{\%cell + \%hemi + \%pectins}{100} \qquad (6)$$

As mentioned above, this hydrolysable basis was considering the inlet fractions that could yield products detectable by HPLC analysis and therefore cellulose, hemicellulose and pectins were considered. However, previous studies demonstrated that high recoveries of pectins can be achieved under mild hydrothermal conditions (120 – 140 °C and 4 – 30 MPa) [28]. As in this work, the

operational conditions were more severe, pectin were rapidly degraded so that they were not considered for sugars yields. Therefore, a different basis was defined for sugars yield, just considering *'% cell'* and *'% hemicell'* as shown in Eq. 7.

$$hydrolysable\ to\ sugars = carbon\ inlet \cdot \frac{\%cell + \%hemi}{100} \quad (7)$$

Table 3. HPLC concentrations in the liquid effluent transformed into carbon concentrations (ppmC) for each compound by multiplying by each carbon factor. Sugars and degradation out are calculated as the sum of sugars and rest of products, respectively. The carbon factors for each component were: 0.41 for C-6 sugars, 0.40 C-5 sugars, glycolaldehyde, glyceraldehyde, lactic and acetic acids; 0.5 for pyruvaldehyde; 0.37 for galacturonic acid; 0.26 for formic acid and 0.57 for 5-HMF (in g carbon/g component i).

EXPERIMENT	**1**	**2**	**3**	**4**	**5**
t_R (s)	0.11	0.14	0.19	0.23	1.15
C – 6 sugars	711	535	543	496	106
C – 5 sugars	992	822	572	407	198
SUGARS OUT	1703	1357	1115	903	305
Pyruvaldehyde	126	140	107	123	34
Glycolaldehyde	656	523	541	467	588
Glyceraldehyde	142	132	148	117	110
Galacturonic acid	173	141	71	173	68
Lactic acid	329	330	580	444	613
Formic acid	105	170	80	139	64
Acetic acid	292	317	359	307	348
5 – HMF	80	74	72	65	73
DEGRADATION OUT	1903	1827	1958	1835	1898

The HPLC results were collected in Table 3, where the products were divided into sugars and degradation products. In that way, the yield of total sugars *'sugars yield'* in % w/w was calculated in Eq. 8 as the sum of C-6 and C-5 sugars, called as *'sugars out'* in ppmC divided to the *'hydrolysable to sugars'* basis. Independent C-6 and C-5 sugars yield were calculated as shown in Eq. 9, where C-6 or C-5 sugars obtained after acid hydrolysis in the analytical technique (in ppmC) were divided by the carbon fraction corresponding to cellulose (in the case of C-6 yield) or hemicellulose (in the case of C-5 yield) as sugars. In a similar way, the yield of each individual sugar as *'$sugar_i$'* (referred to cellobiose, glucose, fructose, xylose or arabinose) was calculated as shown in Eq. 10, where the HPLC concentration of

each sugar (in ppmC) was divided to the carbon inlet multiplied by the each constituent sugar composition shown in Table 4.

$$sugars\ yield = \frac{sugars\ out}{hydrolysable\ to\ sugars} \tag{8}$$

$$yield\ C-6\,|\,C-5 = \frac{C-6\,|\,C-5\ sugars}{carbon\ inlet \cdot (\%cell\ |\ \%hemi)} \tag{9}$$

$$yield\ sugar_i = \frac{sugars_i}{carbon\ inlet \cdot (\%sugar_{in})} \tag{10}$$

Table 4. Specific yield for each compound in % w/w.

EXPERIMENT	1	2	3	4	5
t_R (s)	0.11	0.14	0.19	0.23	1.15
Cellobiose*	62 ± 3	50 ± 12	51 ± 10	40 ± 12	17 ± 7
Glucose*	64 ± 6	57 ± 9	54 ± 6	51 ± 4	7 ± 5
Fructose*	92 ± 10	49 ± 12	53 ± 12	63 ± 12	45 ± 11
Xylose*	87 ± 8	80 ± 10	44 ± 10	59 ± 3	24 ± 7
Arabinose*	74 ± 5	70 ± 4	36 ± 6	24 ± 8	14 ± 2
Pyruvaldehyde	3 ± 0	4 ± 1	3 ± 0	3 ± 1	1 ± 0
Glycolaldehyde	15 ± 2	14 ± 1	15 ± 1	12 ± 2	15 ± 4
Glyceraldehyde	3 ± 0	3 ± 1	4 ± 1	3 ± 1	3 ± 1
Galacturonic acid	4 ± 1	4 ± 1	2 ± 1	4 ± 2	2 ± 0
Lactic acid	8 ± 1	9 ± 0	16 ± 3	11 ± 1	16 ± 2
Formic acid	2 ± 0	4 ± 1	2 ± 0	4 ± 1	2 ± 1
Acetic acid	7 ± 0	8 ± 1	10 ± 1	8 ± 1	9 ± 1
5 – HMF	2 ± 0	2 ± 0	2 ± 0	2 ± 0	2 ± 0

*Raw material content in sugars: 2 % cellobiose, 14 % glucose, 1 % fructose, 7 % xylose and 14 % arabinose

On the other hand, the rest of the products, including the sum of degradation products yield, names as *'degradation yield'* were calculated by dividing the HPLC results in ppmC to the *'total hydrolysable basis'*, as shown in Eq. 11.

$$\deg radation\ yield = \frac{\deg radation\ out}{total\ hydrolysable\ basis} \tag{11}$$

The conversion achieved for the liquid effluent called as *'conversion'* (% w/w) was calculated in Eq. 12 by subtracting the *'sugars in solids'* from the *'hydrolysable to sugars'* basis and then dividing it by *'hydrolysable to sugars'*. *'Sugars in solids'* was calculated as multiplying the *'carbon solid'* by the amount of sugars in the

remaining solid (see Table 6). Then, selectivity towards sugars named as *'selectivity'* (% w/w) was calculated in Eq. 13 by dividing *'sugars yield'* to *'conversion'*.

$$conversion = \frac{hydrolysable\ to\ sugars - sugars\ in\ solids}{hydrolysable\ to\ sugars} \quad (12)$$

$$selectivity = \frac{sugars\ yield}{conversion} = \frac{sugars\ out}{hydrolysable\ to\ sugars - sugars\ in\ solids} \quad (13)$$

Using these equations, main hydrolysis parameters (yields, conversion and selectivity to sugars) were calculated and collected in Table 5.

Table 5. Main hydrolysis parameters calculated according to equations 8 to 13. C-5 and C-6 sugars are grouped under the label 'SUGARS', the sum of glycolaldehyde, pyruvaldehyde and glyceraldehyde are labeled as 'RAC' (meaning retro-aldol condensation products) and lactic, formic, acetic and galacturonic acids are named as 'ACIDS'. Then, total degradation yield is the sum of RAC, acids and 5-HMF yields. All the results are presented in % w/w.

EXPERIMENT	**1**	**2**	**3**	**4**	**5**
t_R (s)	0.11	0.14	0.19	0.23	1.15
C – 6 yield	61 ± 7	52 ± 8	54 ± 8	47 ± 4	10 ± 3
C – 5 yield	71 ± 4	66 ± 5	47 ± 5	32 ± 5	15 ± 2
SUGARS YIELD	66 ± 2	60 ± 2	50 ± 2	39 ± 2	13 ± 1
CONVERSION	94 ± 2	95 ± 2	99 ± 2	99 ± 2	100 ± 1
SELECTIVITY	70 ± 2	63 ± 2	51 ± 2	39 ± 2	13 ± 1
RAC yield	22 ± 2	21 ± 3	21 ± 1	18 ± 2	19 ± 4
Acids yield	21 ± 1	25 ± 1	29 ± 3	27 ± 1	28 ± 2
Total degradation yield	44 ± 2	48 ± 3	53 ± 3	47 ± 2	49 ± 4

3.2.Liquid product characterization

The main hydrolysis parameters were plotted in Fig. 2, where it can be seen that C-6 and C-5 yields showed the same trend, since both decreased as reaction time increased. That was the expected trend for biomass hydrolysis in supercritical water, since the reactions of cellulose and hemicellulose hydrolysis were very fast in SCW and very short reaction times were required to hydrolyze these fractions to sugars. In fact, as reaction time increased, the produced sugars from both cellulose and hemicellulose would be degraded into other products (see schematic reaction pathway at Fig. 3), therefore decreasing the yields of sugars. So, for SBP it was

possible to recover up to 71 % w/w of hemicellulose as C-5 sugars and at the same time 61 % w/w of cellulose was recovered as C-6 sugars at 0.11 s reaction time. Although hemicellulose was degraded faster that cellulose, it can be seen that both polymers hydrolysis yielded similarly high sugars recoveries. Reaction kinetics for cellulose were highly increased approaching hemicellulose values and both yield were kept high because of the strict control of the reaction time in the FASTSUGARS process. In previous studies, the hydrolysis of pure cellulose in supercritical water was carried out under similar conditions (400ºC, 25 MPa) in the FASTSUGARS plant, allowing to recover up to 98 % w/w of inlet cellulose as C-6 sugar after 0.02 s of reaction time [26]. However, when hydrolyzing a real biomass such as wheat bran under same conditions, it was found that higher reaction times were needed to obtain high recoveries of both cellulose and hemicellulose sugars [19].

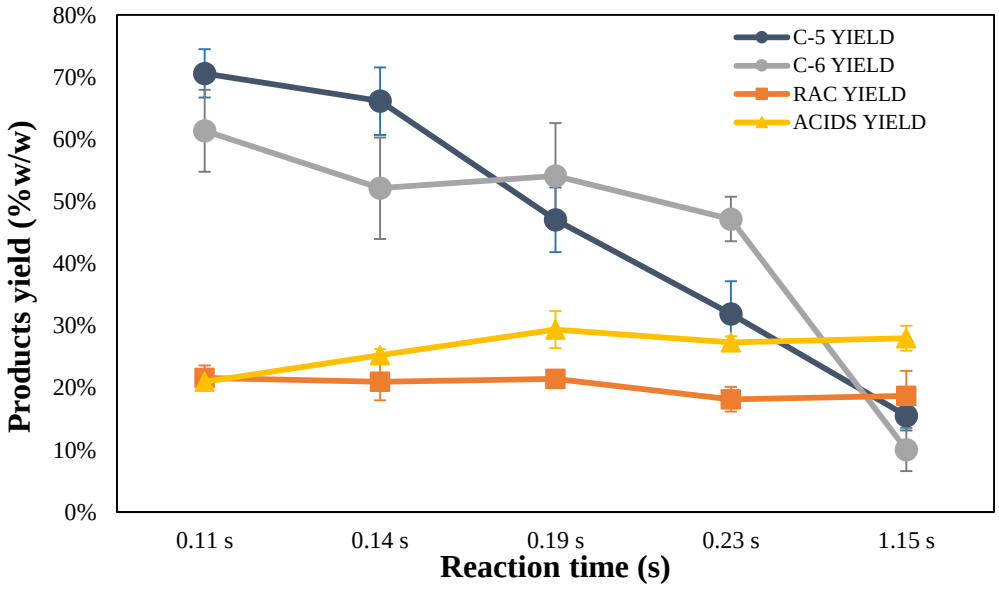


Figure 2. Yields of main compounds after SCW hydrolysis of SBP at 390ºC and 25MPa in the FASTSUGARS plant at different reaction times. The sum of glycolaldehyde, pyruvaldehyde and glyceraldehyde were labeled as 'RAC' and lactic, formic, acetic and galacturonic acids were named as 'ACIDS'.

Comparing SBP to wheat bran results, despite their differences, they showed very similar values for maximum C-6 yield (being 63 % w/w for wheat bran and 61 %

w/w for SBP). However, these results were not obtained under same reaction time conditions, since for wheat bran maximum C-6 yield was achieved at 0.22 s meanwhile for SBP just 0.11 s were necessary. A possible reason might be the particle size of each biomass, being 60 µm for SBP and 125 µm for wheat bran. Wheat bran having a higher particle size would need higher reaction time to get same yield than sugar beet pulp. In fact, taking into account the conversion calculated by Eq. 12 and shown in Table 5, it could be seen that under same reaction time conditions (experiments at 0.19 s were performed for both biomass), the conversion for SBP was 99 %, meanwhile for wheat bran it was lower (93 %), corroborating that having a bigger particle size, higher reaction time was required to achieve same conversion and therefore same C-6 yield from cellulose. It was already proved that biomass particle size significantly affected hydrolysis processes, since smaller particles have larger surface area per unit of volume, improving the accessibility to cellulose and hemicellulose fractions [29].

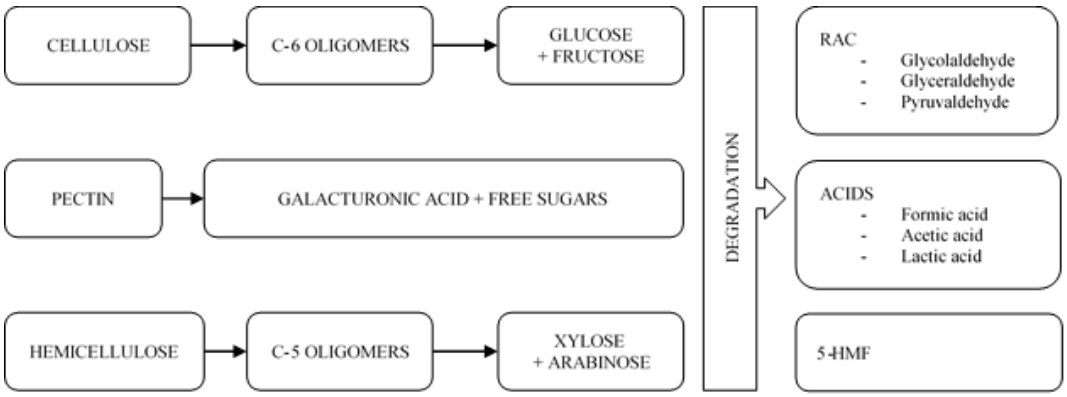


Figure 3. Schematic reaction pathway for cellulose, hemicellulose and pectin in biomass under SCW hydrolysis conditions.

In order to better understand the different reactions simultaneously occurring during SBP hydrolysis in SCW, Fig. 2 represented also the yield of the main components detected by HPLC in the liquid product, also separately shown in Table 5. Moreover, the reaction pathway for both cellulose and hemicellulose hydrolysis in SCW was shown in Fig. 3, where it could be seen that once the monomeric sugars from cellulose (glucose and fructose) and from hemicellulose (xylose and arabinose) were obtained they could yield retro-aldol condensation (RAC) products

and/or acids. Then, C-5 and C-6 sugars, RAC and acids yields were plotted together in Fig. 2. As it can be seen in the figure, for C-6 sugars the yield remained constant (being around 55 %) when reaction times were between 0.11 and 0.23 s and then suddenly decreased to 10 % at 1.15 s. The conversion achieved for reaction times between 0.11 and 0.23 s was close to 100 % but it only reached 100 % at 1.15 s. This fact would suggest that reactions times higher than 0.23 s were needed to get total conversion and therefore complete access to the intricate biomass matrix. Then, as reaction time increased, more severe reaction conditions were achieved and complete conversion was obtained as a result, releasing the most resistant fractions of biomass and making them available for hydrolysis. With a higher reaction time, the hydrolysis of that released cellulose would lead to degradation instead of sugars production, drastically decreasing the C-6 sugars yield. So that, conversion gave an idea of the extent of the hydrolysis reaction. On the other hand, for C-5 sugars, a more pronounced decrease occurred when increasing reaction time. Since hemicellulose is more labile than cellulose, it was more rapidly degraded as reaction time and conversion increased. The behavior of both C-5 and C-6 yields matched the behavior of RAC and acids yields. As reaction time increased, the sugars yields decreased and at the same time the degradation products yields increased, due to the transformation of the sugars into RAC products and/or acids. When looking at Table 5 it can be seen that the overall degradation yield (considering RAC products, acids but also 5-HMF) at 0.11 s was 44 %, due to cellulose and hemicellulose degradation, but also from pectin hydrolysis as shown in Fig. 3. Pectin is representing 28% of the feedstock and is a structural heteropolysaccharide which repeating unit is D-galacturonic acid that forms a hydrated gel that “glues” the cell wall components together [30]. Pectin also contains neutral sugars as rhamnose, arabinose, mannose, galactose, xylose and even glucose in its chains [31]. Those free sugars seemed to be already degraded at 0.11 s, yielding glycolaldehyde and residual galacturonic acid from the very beginning. So that, even though the highest sugars yield was achieved, some degradation was already going on at the shortest reaction time mostly due to pectin hydrolysis. Anyway, as the objective in this work was to obtain the highest sugars yield with the lowest degradation, 0.11 s was found to be the optimal reaction time

for the production of sugars from SBP through supercritical water hydrolysis in the FASTSUGARS plant. In fact, as a real application for the effluent of this process, the liquid product from SBP hydrolysis in SCW by the FASTSUGARS process produced in parallel to this work was hydrogenated over Ru/MCM-48 to obtain a mixture of hexitols and ethylene glycol [32], which is a widely applied feedstock in the plastic and polyester industries. Therefore, it was proved that the liquid effluent obtained via the FASTSUGARS process was a suitable feedstock for the future biorefineries to produce valuable products from biomass that could compete with the petroleum-derived products.

The conventional method for sugars' recovery from SBP consisted on enzymatic hydrolysis, usually with a previous dilute acid pretreatment. The goal of the pretreatment was to solubilize hemicellulose and make residual cellulose more degradable by enzymes [10]. The authors of that previous work obtained a liquid rich in C-5 sugars (arabinose recovery was up to 68 % w/w) and the 5-HMF yield was around 10 % w/w after acid pretreatment. Then, after the enzymatic hydrolysis the total reducing sugars yield was around 60 % w/w. For the current work, Table 4 showed the detailed composition of raw SBP in terms of constituent sugars together with the recovery for each individual sugar at different reaction times. It can be seen that maximum glucose recovery was up to 64 % w/w and 74 % w/w of the arabinose was recovered after the FASTSUGARS process. On the other hand, the maximum total sugars yield was 66 % w/w, with a 5-HMF yield of 2 % w/w. So that, when compared to enzymatic hydrolysis, the FASTSUGARS technology allowed improving both cellulose and hemicellulose recovery as sugars in just one efficient step, increasing total sugars yield and reducing fermentation inhibitors at the same time. Another aspect to take into account when comparing SCW technology to conventional enzymatic hydrolysis would be the thermo-economical and environmental analysis. In a previous study, both processes were compared for sugar cane bagasse hydrolysis and it was concluded that SCW technology allowed reducing the total investment of the biorefinery and the water intake [33]. Moreover, several alternatives were proposed to improve the energetic efficiency of the FASTSUGARS process, from the coupling of the ultrafast reactors to

commercial combined heat and power (CHP) systems [8] to the use of a green desuperheater as an alternative to decompression valve [34]. So that, the improved yields obtained in the case of SBP and the lower cost associated to SCW technology, proved that FASTSUGARS process is a promising and versatile technology to convert biomass into sugars in a sustainable way.

Then, if comparing the current results to the ones obtained from similar technologies involving SCW hydrolysis of agricultural biomass, FASTSUGARS technology also improved existing results. When converting corn stalks under combined supercritical and subcritical conditions in a flow reactor, the maximum recovery of C-6 sugars was 68 % w/w with less than 2 % w/w of C-5 sugars [20]. The supercritical reaction in that study was carried at 380 ºC with a reaction time of 9 seconds. Comparatively speaking, reducing temperature in that work, slowed down the cellulose hydrolysis rate, which allowed obtaining slightly higher C-6 sugars recovery compared to the current work. However, increasing reaction time resulted in total degradation of hemicellulose, which not occurred in the present study. All in all, operating with the FASTSUGARS plant at 390 ºC and 0.11 s it was possible to simultaneously and selectively recover both cellulose and hemicellulose as sugars.

3.3.Solid product characterization

Once the liquid effluent was completely characterized, the solid product composition compared to the raw material composition was shown in Fig. 4. As it was shown in Table 2, the amount of solid obtained after each experiment was almost negligible in terms of mass, but it was important to study the evolution of the hydrolysis process with time regarding the remaining solid composition and also allowed closing the mass balance. Lignin is a complex high molecular weight compound with highly random structure, which makes it difficult to completely liquefy the lignin fraction from biomass [35]. Under the conditions selected for the current work remaining solid was always obtained, so it was not possible to achieve total liquefaction of the initial biomass. That was probably due to the depolymerization and repolymerization reactions that lignin was suffering under supercritical water conditions [36] that produced a solid mostly insoluble in acid.

In fact, as it can be seen in Fig. 4, the main fraction found in the solid product was that acid-insoluble fraction (AIF) in all cases. The AIF content in the remaining solid increased when increasing reaction time, meanwhile the hydrolysable fractions, decreased with reaction time. In Fig. 4 it can be seen that the amount of sugars still trapped in the remaining solid at the shortest reaction time was as high as 25 % w/w, suggesting that higher reaction times were needed to fully hydrolyze cellulose and hemicellulose to sugars. Then, when increasing the reaction time above 0.14 s, the sugars content in the remaining solid continuously decreased from 21 % w/w at 0.14 s to 1 % at 1.15 s. See Table 6 for detailed composition of the solid after reaction.

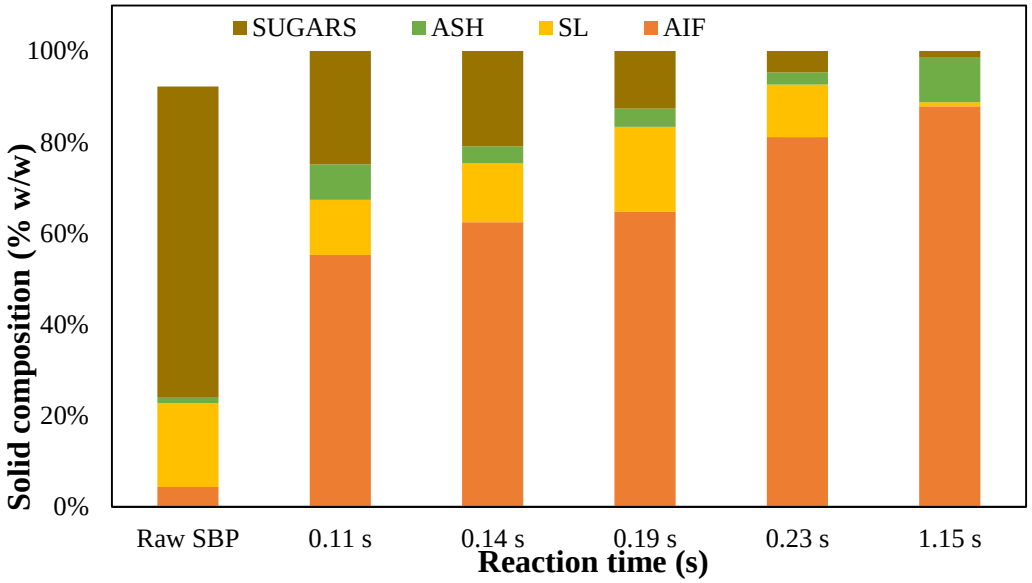


Figure 4. Composition of the solid product obtained after SCW hydrolysis of SBP at 390ºC and 25 MPa in the FASTSUGARS plant at different reaction times, compared to raw material. AIF = acid-insoluble fraction, SL = soluble lignin, SUGARS = sugars from hydrolyzed cellulose, hemicellulose and pectin.

Table 6. Composition and conversion of the solid product after acid hydrolysis (dry basis). AIF = acid-insoluble fraction (meaning insoluble lignin for the raw material), SL = soluble lignin, SUGARS = sugars from hydrolyzed cellulose, hemicellulose and pectin (dry basis).

t_R (s)	AIF (%)	SL (%)	ASH (%)	SUGARS (C-6 + C-5) (%)
0 (raw material)	4.4	18.4	1.3	68.1 (18.4 + 22.4 + 27.5 pectins)
0.11	55.3	12.1	7.8	24.8 (22.2+ 2.7)
0.14	62.4	13.0	3.7	20.9 (19.0 + 2.0)
0.19	64.8	18.6	4.0	12.6 (11.4 + 1.2)
0.23	81.2	11.5	2.7	4.7 (4.1 + 0.5)
1.15	87.9	0.9	9.8	1.4 (1.1 + 0.3)

In order to better understand the effect of SCW hydrolysis on the solid product and the nature of its AIF, thermogravimetric analysis (TGA) was performed to a solid sample obtained after supercritical water hydrolysis, being the operating conditions 392 ºC, 25 MPa and 0.14 s and also to the raw material. TGA and DTG (derivative thermogravimetric) profiles for both raw material and solid after reaction were presented in Fig. 5. In terms of complex biomass, it is widely accepted that its thermal degradation is divided in three stages: first moisture drying, then a devolatilisation that takes place in the range of 200 – 400 ºC which is related to the labile fractions from biomass. This degradation process is then followed by a continuous slight devolatilisation related to lignin [37, 38]. In Fig. 5 it can be seen that the raw material TG curve corroborates the behavior mentioned above, since a first important weight loss was occurring between 200 and 370 ºC, corresponding to first hemicellulose and pectin and then cellulose degradations. After that, a continuous plain decreasing curve started at 370 ºC to 850 ºC that would be related to continuous lignin degradation. The TG curve shown in this work was comparable to those found in literature for SBP [39, 40].

Then, on the DTG curve the peaks describe the maximum rate of weight loss occurred at different temperatures [41]. First peak between 50 – 100 ºC corresponded to moisture drying. Then, having as a reference an study of separated pure hemicellulose, pectin and cellulose pyrolysis [37], the DTG peaks of the raw SBP were identified by comparison to pure compounds curves (see Fig. 5). So that, the first peak of raw material DTG shown at 258 ºC was due to both pectin and

hemicellulose degradation. Next peak at 304 ºC was due to secondary pyrolysis of hemicellulose and the peak at 348 ºC was attributed to cellulose decomposition. That last peak was not only due to cellulose but also to lignin (in a minor proportion).

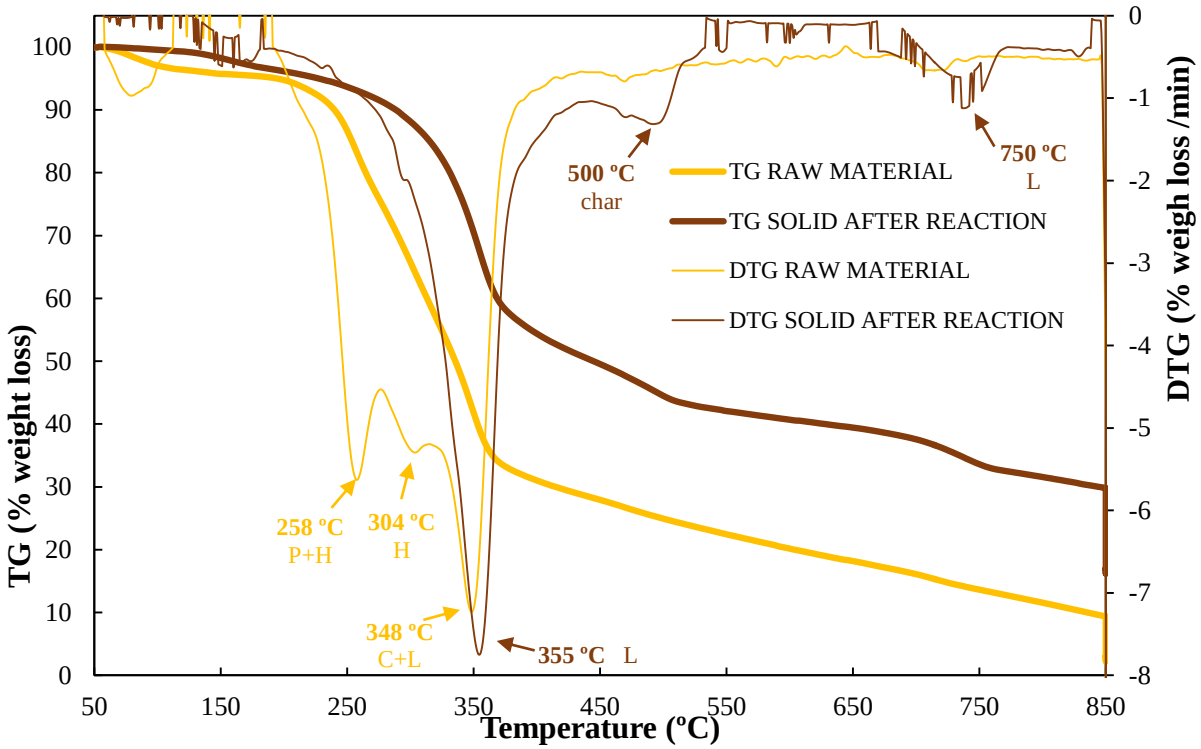


Figure 5. TG and DTG curves for the raw SBP and the solid obtained after SCW hydrolysis in the FASTSUGARS process at 3920ºC, 25 MPa and 0.14 s. (P =pectin degradation; H = hemicellulose degradation; C = cellulose degradation; L = lignin degradation).

On the other hand, when taking a look to the TG curve of the solid product after reaction a similar behavior was found, with the cellulose, hemicellulose and pectins degradation curve from 200 to 370 ºC again. Then, instead of a continuous decreasing curve, two different slopes were found, first one between 370 to 510 ºC and second one between 510 and 740 ºC. DTG curve was also analyzed and compared to previous studies. Both TG and DTG curves obtained from the remaining solid in this work were comparable to those obtained for dealkaline lignin from a previous work [42]. That dealkaline lignin from that previous work showed two main peaks at the DTG curve, at around 350 and 750 ºC. That peaks were found for the solid product in this work, corroborating the lignin-like nature

of the solid obtained after FASTSUGARS process. Once the lignin nature of the solid was confirmed, further explanation for the 500 ºC peak was needed, as it was not observed for other lignin DTG curves in previous works [38, 42, 43]. For sugarcane bagasse it was found that this region was related to the end of cellulose decomposition and the formation of char [41]. So that, it seemed that char was produced during the FASTSUGARS process, probably related to the transformation suffered by both cellulose and hemicellulose trapped inside the cell wall network that could yield to the production of recalcitrant humins from both 5-HMF and furfural [44]. It could be concluded that the most recalcitrant fraction of the solid product, meaning acid-insoluble fraction, was composed of insoluble lignin and char produced during the SCW hydrolysis.

Table 7. Thermal properties for raw SBP and solid collected after SCW hydrolysis at FASTSUGARS process (392 ºC, 25 MPa, 0.14s).

	Raw SBP	**After reaction**
Temperature at 50% degradation (ºC)	335	444
Degradation between 200 – 600 (% w/w)	74.59	55.47
Ash (% w/w)	2.23	16.81

To analyze the thermal behavior, several parameters were calculated through TGA results and shown in Table 7 according to previous works [43, 45]. In first place, the temperature that produced 50 % degradation of the sample was calculated. It can be seen that this temperature was higher for the treated solid, so it could be said that after FASTSUGARS process, the resistance of the solid to thermal degradation and therefore thermal stability was improved (shifting from 335 ºC for the raw SBP to 444 ºC for the treated solid). The temperature to 50 % weight loss for the solid after reaction (444 ºC) was higher to the one found for kraft lignin (430 ºC) [43], corroborating again the lignin-like nature of the solid obtained after FASTSUGARS process. Then, another way to show the thermal stability of the samples was regarding the degradation produced between 200 – 600 ºC. Within this range, all the hydrolysable fractions were degraded and just lignin, char and ash remained. The raw SBP, as it was mainly composed of those hydrolysable fractions, it suffered

an important degradation within that temperature range. On the contrary, as the solid after reaction was mainly composed of an AIF comparable to insoluble lignin, it could better resist thermal degradation within that range. The degradation value for the solid after FASTSUGARS process (56 %) was consistent with values reported for other lignins [46]. In terms of ash content, it could be seen that ash content was around 8 times higher for the solid after reaction compared to the raw material. After the FASTSUGARS process, the hydrolysable fractions were removed from the remaining solid to the liquid product and therefore the solid was concentrated in other compounds such as ash.

Then, FTIR analysis was performed to the raw material and the solid product obtained after reaction (same conditions before: 390 ºC, 25 MPa and 0.14 s) to have some insight about the changes produced by the FASTSUGARS process in the chemical structure of the solid. Both FTIR spectra were shown in Fig. 6 and, in order to compare, several regions were identified and collected in Table 8. When comparing both spectra in Fig. 6, a remarkable difference in the regions related to lignin was observed, since sharper peaks appeared for the solid after reaction compared to the raw SBP (see detailed areas plotted in Fig. 6a, 6b, 6c, 6d and 6e). So that, the enhancement of these peaks meant that the aromatic nature of the solid after FASTSUGARS process was enhanced in detriment of its carbohydrate content. In fact, the reduced carbohydrate content was obvious when comparing the intensity of certain bands in the miscellaneous regions of the raw material spectra (meaning regions related to cellulose, hemicellulose and lignin), so that when removing the polysaccharides from the solid during the FASTSUGARS process, these peaks were considerably reduced.

Table 8. Assignment of wavelength peaks and bands found in biomass FTIR analysis of biomass and solid after reaction with its related chemical structure, polymer and references.L = Lignin, C= Cellulose, H = Hemicellulose. Continue in the next page

Wavelength (cm^{-1})	**Chemical structure**	**Polymer**	**Reference**
700 – 875	C – H out-of-plane vibration on aromatic rings	L	[36, 47-49]
875 – 990	C – H deformation in cellulose	C	[47-49]
1030	C – H aromatic deformations	L	[50]
	C – H stretching of polysaccharides	C + H	[46]
1050	C – O stretching in cellulose	C	[48, 49]
1130	C – H aromatic in-plane deformation	L	[48, 50, 51]
1160	B-glycosidic bond	C + H	[47, 52, 53]
1216	C – C and C – O stretch in aromatics	L	[52]
1230 – 1280	Phenolic stretching (guaiacyl units)	L	[47, 49, 50, 54]
1310 – 1340	C – O stretching in syringyl units	L	[43, 46, 47, 50, 52]
1370	C – H vibrations	C + H + L	[46-48, 52]
1400 – 1490	C – H deformations in lignin and carbohydrates	C + H + L	[46, 47]
	C – H in-plane deformations	L	[52]
	O – H in-plane bending	C + H + L	[52]
1500 – 1600	Aromatic skeletal vibrations	L	[43, 46-48, 50, 52, 53, 55]
1600 – 1700	C = O stretching vibrations (unconjugated groups)	L	[47, 48, 52]
1730	Unconjugated ketone and carbonyl group vibrations	H + L	[47, 49, 50, 52]
2850	C – H stretching in lignin	L	[52, 53]
2850 – 2920	C – H stretching (CH_3 and CH_2 groups)	C + H	[43, 54, 55]
3300 – 3500	Representative of O – H stretching (both phenolic and aliphatic OH)	C + H + L	[43, 46, 48, 53-55]
3430	Indicative of O – H stretching specifically from lignin	L	[36, 52]

Through several analyses (acid hydrolysis, TGA and FTIR) it was proved that the solid product obtained after FASTSUGARS process was mainly composed of an acid-insoluble fraction (AIF), being a combination of insoluble lignin from the raw material and char produced during SCW hydrolysis. Increasing the reaction time, the AIF increased in the solid since the cellulose and hemicellulose were hydrolyzed until hollowing out the cell wall leaving behind the most recalcitrant fractions of biomass: ash and acid-insoluble residue. It was also proved that the FASTSUGARS treatment improved the thermal properties of the solid and enhanced its aromatic nature.

As a step towards integrated biorefineries, coupling the SCW hydrolysis of SBP in the exiting industrial facilities for sugar production would allow the energetic integration of the process with current heat and power generation systems, like the gas turbine processes. So, after sugar production from beet a wet by-product, meaning SBP, would be produced. If that SBP would be directly feed to the FASTSUGARS process, three products would be obtained: (1) a liquid product containing sugars and building blocks such as glycolaldehyde; (2) a solid product with enhanced thermal properties and aromaticity and (3) a high-pressure steam composed almost exclusively of water. Then, both liquid and solid product should undergo downstream processes to obtain marketable products such as ethylene glycol or sorbitol from the liquid and on the other hand, the solid fraction could be purified to produce composite additives. Additionally, the steam could be injected to the combustor of a gas turbine in order to increase the energy (shaft work) production (please refer to previous work for concept evaluation [8]). Therefore, the integration of the FASTSUGARS process would transform the sugar production process into a closed loop system, increasing the value of the ending products (SBP mostly used as animal feed would be converted to valuable building blocks) and reducing the energy demand through the energetic integration developed in a previous work [8].

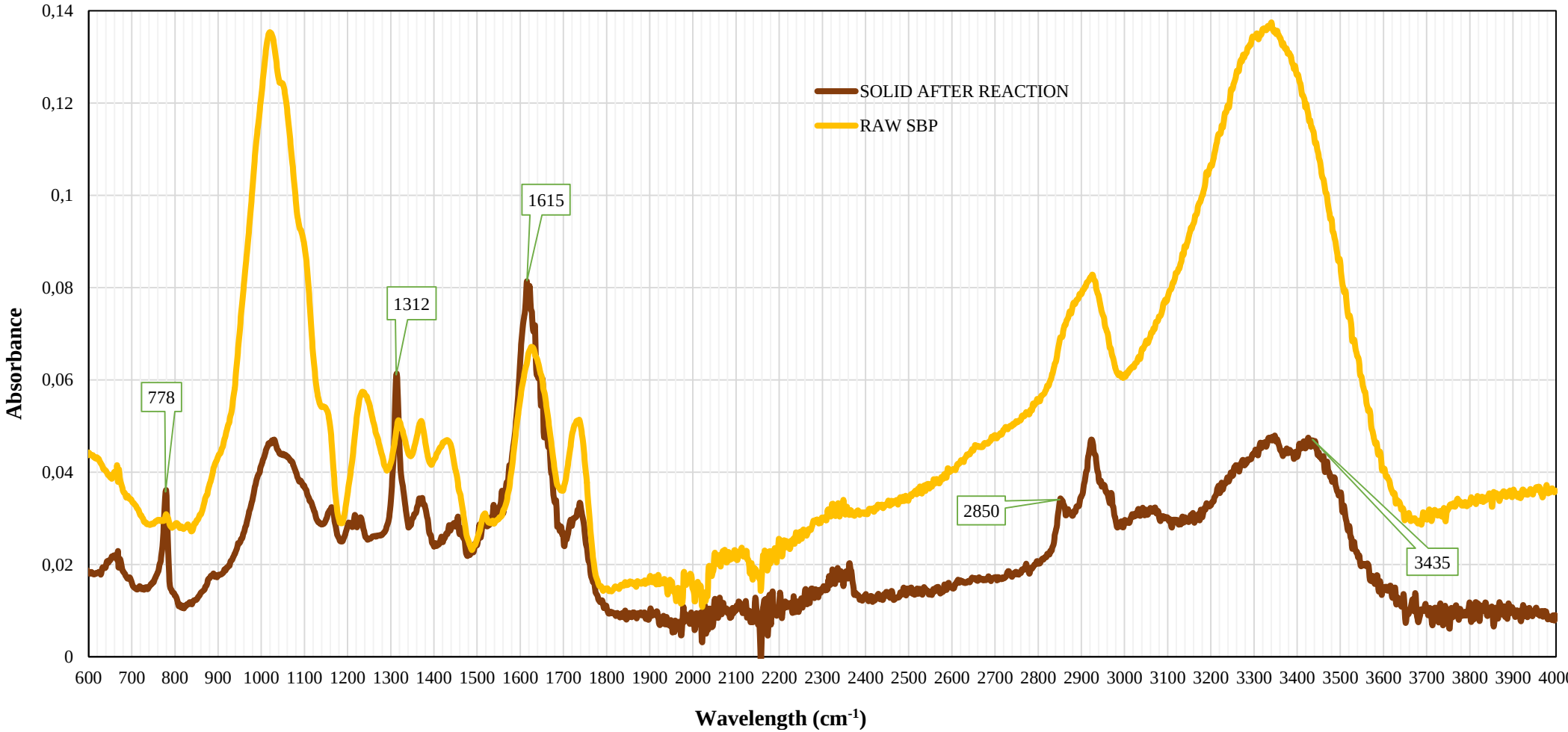
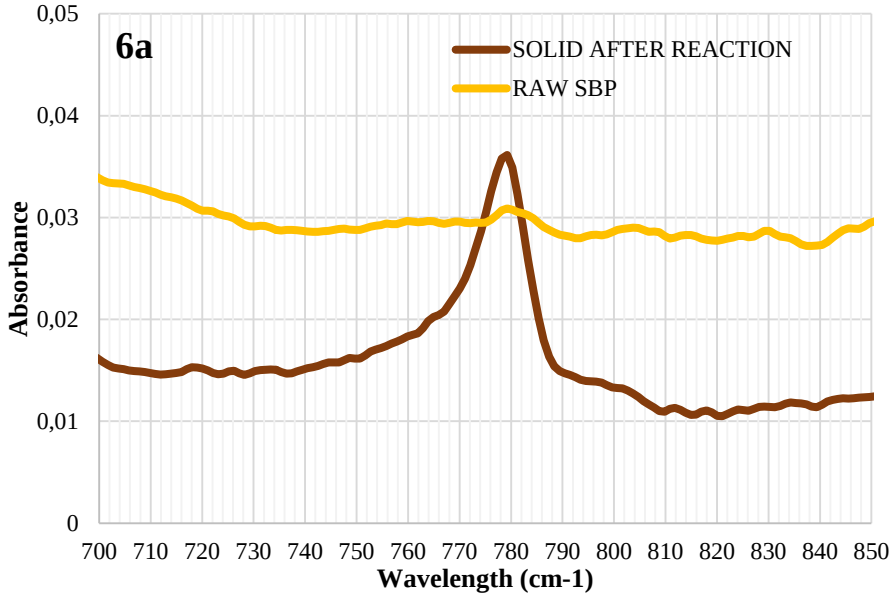
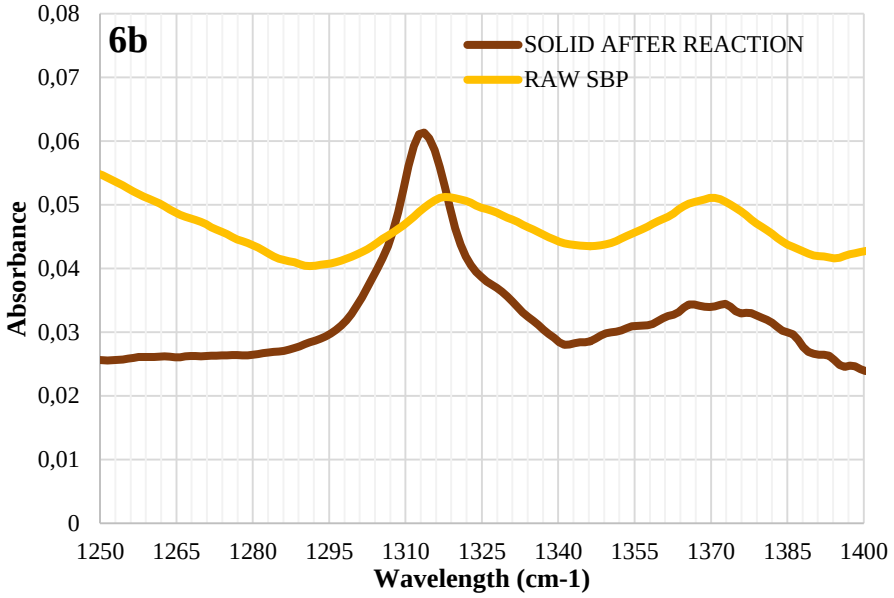
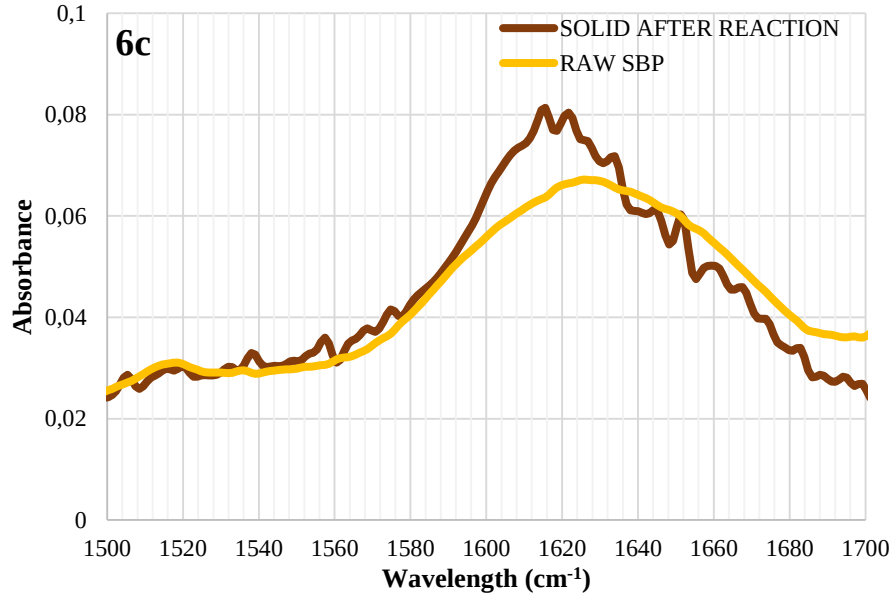
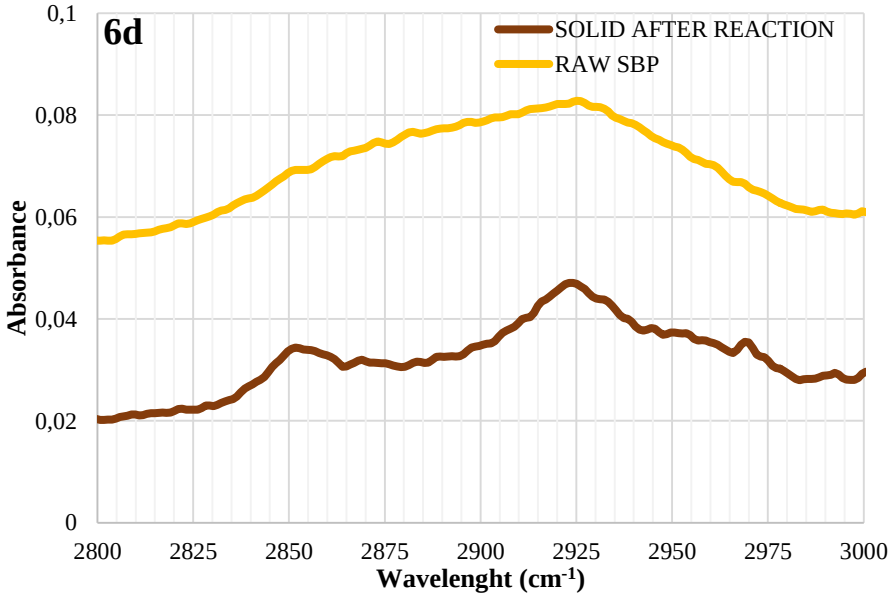


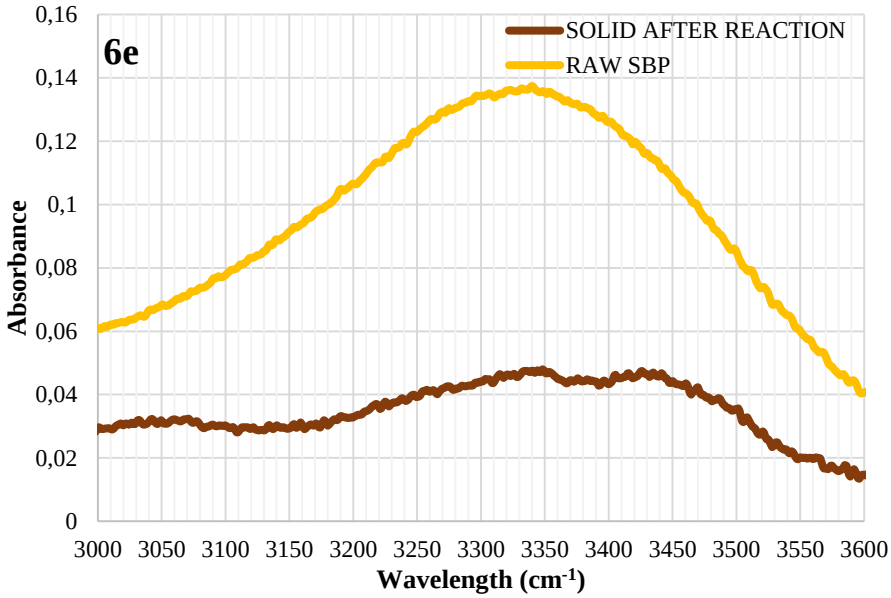
Figure 6. FTIR spectra for the raw sugar beet pulp and solid after reaction in the FASTSUGARS process at 390 °C, 25 MPa and 0.14 s. Lignin related peaks were tagged. Areas of interest were separately plotted in Fig. 6a, 6b, 6c, S6d and 6e.


6a
SOLID AFTER REACTION
RAW SBP
Absorbance
0,05
0,04
0,03
0,02
0,01
0
700 710 720 730 740 750 760 770 780 790 800 810 820 830 840 850
Wavelength (cm-1)


6b
SOLID AFTER REACTION
RAW SBP
Absorbance
0,08
0,07
0,06
0,05
0,04
0,03
0,02
0,01
0
1250 1265 1280 1295 1310 1325 1340 1355 1370 1385 1400
Wavelength (cm-1)


6c
SOLID AFTER REACTION
RAW SBP
Absorbance
0,1
0,08
0,06
0,04
0,02
0
1500 1520 1540 1560 1580 1600 1620 1640 1660 1680 1700
Wavelength (cm-1)


6d
SOLID AFTER REACTION
RAW SBP
Absorbance
0,1
0,08
0,06
0,04
0,02
0
2800 2825 2850 2875 2900 2925 2950 2975 3000
Wavelenght (cm-1)



4. Conclusions

Sugar beet pulp was hydrolyzed for the first time in supercritical water for sugars recovery. The FASTSUGARS process allowed the selective and simultaneous recovery of both cellulose and hemicellulose fractions as C-6 and C-5 sugars, which was not possible through enzymatic hydrolysis. Apart from testing a new biomass, the reaction temperature for this study was dropped from previous studies at 400 ºC to 390 ºC to evaluate the ability of the system to still produce high sugars' selectivity while cutting the energy demand. In this way, a liquid effluent suitable for further conversion into ethylene glycol and sorbitol was obtained. On the other hand, a solid product was obtained which could be used as additive for composites production. Moreover, the FASTSUGARS process would allow the energetic integration into the current sugar production industry. All in all, the FASTSUGARS process demonstrated being an effective method to perform the supercritical water hydrolysis of sugar beet pulp, operating at 390 ºC, 25 MPa and reaction time of 0.11 s, yielding 66 % of total sugars.

Acknowledgments

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Chapter 3. Scaling up the production of sugars from agricultural biomass by ultrafast hydrolysis in supercritical water [c]

Abstract

The FASTSUGARS process for sugars' recovery from agricultural biomass was scaled up from laboratory to pilot plant scale. System performance was evaluated by comparing the results obtained from sugar beet pulp and wheat bran in laboratory and pilot plants. Similar trends were found for each biomass in both plant: as reaction time increased, selectivity to sugars decreased and conversion and degradation rate increased. Then, to bring the FASTSUGARS process closer to industrial applications, the particle size of the biomass was increased in the pilot plant. It was found that the particle size acted as a mass transfer resistance, slowing down the hydrolysis of biomass, providing lower conversion and therefore reducing sugars' degradation (degradation yield was lower than 15 % in the pilot plant). In that way, higher selectivity to sugars was obtained, reaching values around 90 % for both sugar beet pulp and wheat bran in the pilot plant.

[c] C. M. Martínez, T. Adamovic, D.A. Cantero and M. J. Cocero. Scaling up the production of sugars from agricultural biomass by ultrafast hydrolysis in supercritical water. *The Journal of Supercritical Fluids*, 2019. 143: pp 242-250.

1. Introduction

During the last years, countless studies have focused on the use of biomass as feedstock for the production of fuels, platform chemicals, materials and energy as a step towards biorefineries. Indeed, by 2030 the bio-based economy is expected to have grown substantially [1] and biorefineries would be playing an essential role in the future industries. A functional biorefinery should be able to use a wide variety of raw materials, making profit out of each biomass fraction with the lowest energy cost and environmental impact.

The majority of the literature reports on acid or enzymatic hydrolysis of biomass to obtain valuable compounds [2, 3]. However, those methodologies have important drawbacks: acid hydrolysis easily leads to the production of degradation products, reducing the selectivity towards sugars and enzymatic hydrolysis demands high costs and reaction times [4]. During the last years, supercritical water (SCW, meaning water above its critical point: 374 ºC, 22 MPa) has been gaining increasing interest as a suitable reaction medium for biomass transformations, since the reactions and separations in SCW have several advantages over conventional methods [5, 6]. It shows very different properties from those of liquid water, since the values of density, dielectric constant and ionic product decrease drastically and therefore, SCW shows properties of non-polar solvents with high diffusivity and excellent transport properties [7]. In fact, under SCW conditions, certain biomass fractions face reactions that occur too rapidly to be controlled by conventional methods [8]. That is why the High Pressure Processes Group (HPPG) developed a novel technology to selectively hydrolyze cellulose and biomass into sugars, called as FASTSUGARS process [9].

Along with the FASTSUGARS process, several technologies involving SCW hydrolysis have been developed in the last years to recover sugars from lignocellulosic biomass at laboratory scale [10, 11]. However, the available information about the process at pilot and industrial scale is still limited [12, 13]. To add some valuable knowledge in this area, in this work the FASTSUGARS process was scaled up from laboratory to pilot scale plant.

Therefore, the aim of this work was to prove that it was possible to selectively produce sugars from biomass by SCW hydrolysis in a new pilot scale plant, facing new challenges but demonstrating at the same time the versatility and potential of the FASTSUGARS process as a key step towards functional biorefineries.

2. Materials and Methods

2.1.Materials

After completion of the pilot plant construction and commissioning, the unit was tested with two biomass: sugar beet pulp and wheat bran. A local sugar industry (ACOR) provided the sugar beet pulp used in the experiments. Wheat bran was supplied also from a local supplier (Emilio Esteban). Deionized water was used as the hydrolysis medium for the experiments. The High Performance Liquid Chromatography (HPLC) standards were purchased from Sigma-Aldrich, being: cellobiose, glucose, xylose, fructose, arabinose, glyceraldehyde, pyruvaldehyde, glycolaldehyde dimer, lactic acid, formic acid, acetic acid, 5-hydroxymethylfurfural (5-HMF) and furfural. Milli-Q water and sulfuric acid were used as the mobile phase in the HPLC analysis.

2.2.Methods

2.2.1. Compositional analysis of biomass

The sugar beet pulp for this work (SBP) was provided as pellets, so the particle size was first reduced using a cutting mill Retsch SM100 and then with a ball mill Retsch PM100 for 1 hour to obtain a final particle size (PS) of 250 µm. On the other hand, the wheat bran, with a smaller initial PS was milled just using the ball mill for 1 hour to obtain an average PS of also 250 µm. The PS was measured using a Dynamic Light Scattering (DLS) Mastersizer 2000.

To determine the composition of the raw material, several standardized procedures were followed. First, a Laboratory Analytical Procedure from NREL was used to determine the structural carbohydrates and lignin content in the biomass [14]. That protocol was described in detail in previous works [9, 15]. Proteins were determined through Kjeldahl nitrogen analysis as presented in a previous work [15]. The factor to convert Kjendahl nitrogen into proteins was 6.25 for SBP and 5.7 for wheat bran.

Finally, the pectin content in SBP was determined using a method based on precipitation of calcium pectate [16]. Briefly, the pectins were firstly extracted from SBP by using water with HCl to pH 2, so that 10 g of SBP were added to 400 mL of acidic water at 90 ºC for 30 minutes. The liquid was collected for the calcium pectate precipitation. 50 mL of NaOH (0.25 N) were added to a liquid aliquot of 50 mL and stirred for 25 min. Then, 50 mL acetic acid (2N) were added together with 50 mL calcium chloride (1M), stirring for 15 min. After centrifugation, the precipitate was collected and weighted allowing to determine the pectin content of the initial sample.

2.2.2. Products analysis

The composition of the liquid product was determined by HPLC analysis, using a Shodex SH-1011 as it was previously described elsewhere [15]. Directly analyzing the liquid samples by HPLC it was possible to determine the concentration of acids, aldehydes, furfural and 5-HMF. The concentration of soluble oligosaccharides in the liquid was determined via acid hydrolysis and HPLC determination, so that the oligosaccharides from cellulose were hydrolyzed to glucose and the oligosaccharides from hemicellulose were converted to arabinose and xylose. After acid hydrolysis, total soluble sugars derived from cellulose (meaning cellobiose, glucose, fructose and oligosaccharides transformed into glucose) were called as C-6 sugars and those derived from hemicellulose (xylose, arabinose and oligosaccharides transformed into xylose and arabinose) were called as C-5 sugars. The carbon content in the liquid product was determined by total organic carbon (TOC) analysis with Shimadzu TOC-VCSH equipment.

On the other hand, two solid fractions were recovered from the SCW hydrolysis of biomass in the FASTSUGARS pilot plant. As it happened in the laboratory scale plant, the liquid sample contained suspended solids that were separated by centrifugation, dried at 105 ºC for 24 h and then weighted. In the pilot plant two filters were added to make easier the recovery of solids, so after reaction another solid fraction was recovered from the filters, dried and weighted. Then, its composition was determined following the same NREL procedure used for lignin

determination in the raw material [14]. The carbon content of the solid fractions was determined by elemental analysis using an elemental analyzer Flash 200 analyzer.

2.2.3. Experimental set up: from laboratory to pilot scale

As mentioned before, the aim of this work was presenting for the first time the scaled up plant for the FASTSUGARS process, moving from a laboratory scale to a pilot scale. The laboratory scale set up was thoroughly described in previous works [9, 15, 17, 18]. The main parameters to compare both plants were summarized in Table 1. The new continuous pilot plant was designed to operate at reactor temperatures up to 400 ºC and reactor pressures up to 30 MPa, and it is schematically represented in Fig. 1. The process can be divided into 5 stages as follows:

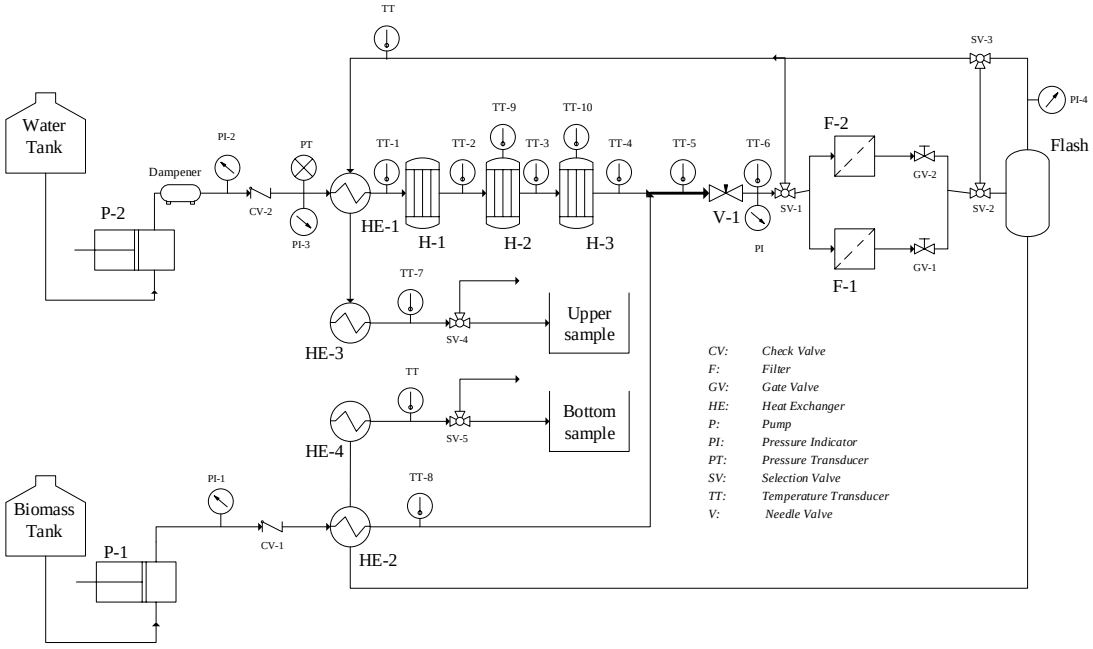


Figure 1. FASTSUGARS pilot plant used to carry out the hydrolysis of biomass in supercritical water.

1) Pressurization. A Milton Roy MC61 piston pump was used to pump water up to 20 kg/h of water (P – 2) and a Lewa LDD1 piston pump (P – 1) was used to pump up to 15 % w/w biomass suspensions up to 10 kg/h. The maximum biomass particle size allowed by this pump was 500 µm. Both pumps were

pressurizing water and biomass suspensions to operation pressure (25 MPa) and the flows ratio was manipulated so that inlet biomass concentration to the reactor was between 1 and 5 % w/w.

2) Heating. The pilot plant heating system was designed in three separated steps (H – 1, H – 2 and H – 3) being the total power 33 kW (11 kW/heater). Water was preheated (HE – 1) and biomass suspension could be preheated when using the flash (HE – 2). Then, biomass and SCW were mixed in a tee junction, where biomass was instantaneously heated up to the reaction temperature (up to 400 ºC) and simultaneously starting the reaction. To avoid heat losses and keep a constant temperature in the reactor, all the hot elements of the equipment were thermally insulated using rock wool.

3) Reaction. Once the reaction conditions were achieved (380 – 400 ºC, 25 MPa), the key factor in the FASTSUGARS process was the accurate control of the reaction time, meaning the time that biomass and SCW spent together between the mixing point (starting the reaction) and the needle valve (end of reaction). Reaction time, *'t_R'* in seconds, were calculated as the ratio of reactor volume and volumetric flow in the reactor, as shown in Eq. 1. The reactor volume, *'V'* in m^3, was calculated using the dimensions of the reactor (the reactors were made out of ¼" tubing, so that the diameter *'D'* was always the same and the length of the pipe *'L'* could be varied). Since the reactor was thermally isolated and the heating and cooling methods were instantaneous, it could be considered that the reaction was isothermal. Therefore, the density was considered constant through the reactor. Using the ratio *'ρ_h/ρ_0'*, it was possible to transform the flow measured at ambient conditions, *'$F_{v,0}$'* in m^3/s, into *'F_v'*.

$$t_R = \frac{V}{F_v} = \frac{\pi D^2}{4} L \frac{\rho_h}{F_{v,0}\rho_0} \quad (1)$$

4) Depressurization. Sudden depressurization through a needle valve allowed an instantaneous cooling based on Joule – Thomson effect and therefore stopping the reactions. The sudden depressurization was carried out through a needle valve, V-1. This instantaneously cooling method allowed decreasing

temperature from 400 to 150 ºC, avoiding in that way uncontrolled reactions. The manual needle valve used was 60VM4882-HT from Autoclave Engineers.

5) Sampling. Two high temperature filter housings (Classic Filters SS235.221H) were installed with a mesh able to retain particles with diameters bigger than 20 µm (Classic Filters 25-178-S20H). So that, after leaving the valve, the effluent could go through the filters (SV-1 should be opened to the filters, F – 1 and F – 2). When leaving the filters, since the biggest solid particles were removed from the effluent, it could go then to the flash separator (SV – 2 and SV – 3 being opened), where the liquid – vapor mixture would be separated into a vapor condensed phase (named as upper phase) mainly composed of water and a liquid phase (bottom phase) with a higher concentration of sugars. After these new stages, two heat exchangers were used to cool down the liquid and condensed vapor samples (HE – 3 and HE – 4, respectively).

   The pilot plant was designed as a versatile facility, so that the sampling could be done following different configurations, meaning neither using the filters nor the flash (just closing the SV – 2 and SV – 3 valves and changing the position of the SV – 1 valve) or allowing to use the filters but skipping the flash separation.

Table 1. Comparison between the FASTSUGARS laboratory scale plant and pilot scale plant presented in this work.

	LABORATORY PLANT	**PILOT PLANT**
Pressurization	Flow up to 8 kg/h (3 BM + 5 SCW) 5 % biomass suspension pressurized PS ≤ 150 µm	Flow up to 30 kg/h (10 BM + 20 SCW) 5% biomass suspension no pressurized PS ≤ 500 µm
Heating	1 step → 10 kW	3 steps (11 kW/step) → 33 kW Biomass preheating (HE – 2)
Reaction	2 reactors (selecting short or long t_R) Min t_R→0.06 s (min reactor & max flow) Reaction conditions: 390 – 400 ºC, 25 MPa Inlet concentration: 0.5 – 2 % w/w.	1 reactor Min t_R→0.05 s (min reactor & 25 kg/h) Reaction conditions: 380 – 400 ºC, 25 MPa Inlet concentration: 1 – 5 % w/w
Depressurization	AE 30VRMM4812-GY	AE 60VM4882-HT
Sampling	1 sample containing liquid + suspended solids	Filters & flash → 3 samples: concentrated liquid with suspended solids + condensed vapor + solids retained in the filters

3. Results and Discussion

The first objective in this work was to scale up the FASTSUGARS process. To evaluate this scaling up sugar beet pulp (SBP) and wheat bran (WB) were hydrolyzed in the FASTSUGARS pilot plant and results were compared to previous ones obtained in the laboratory scale plant [9, 15].

First of all, the characterization of each biomass was presented together with relevant experimental data used to close the carbon balance and calculate the main hydrolysis parameters for each biomass in the pilot plant (i.e. sugars yield, conversion, selectivity and degradation yield). Then, to validate these results, the results from sugar beet pulp hydrolysis in the laboratory plant (labelled as sbp, from [15]) and those from wheat bran (wb, from [9]) were used for comparison between laboratory and pilot scale plants.

3.1.Biomass characterization and calculations

The compositional analysis for both sugar beet pulp and wheat bran is shown in Table 2 and it was carried out with the raw material as it would be entering the plant, meaning including extractives. As it can be seen, one of the main differences between both biomass is the presence of pectin, which were found in SBP but not in WB and then starch that was found just in WB.

Table 2. Compositional analysis for sugar beet pulp ('SBP' used in the pilot plant and 'sbp' used in the laboratory scale plant) and wheat bran ('WB' used in the pilot plant and 'wb' used in the laboratory scale plant) as they entered to the plant (dry basis).

	IL[a]	Ash	C – 6	C – 5	Proteins	Pectin/Starch[b]	Others [c]	PS (µm)
SBP	4	1	29	21	12	22	10	250
sbp	4	1	19	22	10	28	18	60
WB/wb	2	0	23	28	12	15	20	250 / 125

[a]IL = Insoluble lignin content

[b]Starch (just for wheat bran) was subtracted from cellulose before and after soxhlet extraction

[c]Others were calculated as difference to 100 %.

For the carbon balance, the outlet carbon was divided to the carbon entering the plant. The *'carbon in'* was calculated as shown in Eq. 2, being *'Cin'* (% w/w) the concentration of dry biomass at the inlet of the reactor converted into ppm of carbon (ppmC) by multiplying by 10000 and then by *'CFbiomass'* that was the carbon

factor of the raw material measured by elemental analysis for each biomass. Then, *'carbon out'* was the sum of the carbon due to the liquid (directly measured by TOC in ppmC) and the carbon due to the solids products, being in this case both solids from filters (*'carbon filters'*) and suspended solids (*'carbon susp'*). In order to calculate *'carbon outlet'*, Eq. 3 was used. Average carbon balance results were shown in Table 3.

$$carbon\ in\ = Cin \cdot 10000 \cdot CFbiomass \tag{2}$$

$$carbon\ out = carbon\ liq + carbon\ filters + carbon\ susp\ = \\ TOC + carbon\ \ filters\ + \%susp \cdot 10000 \cdot CFsusp \tag{3}$$

Table 3. Experimental data and carbon balance calculations for sugar beet pulp (SBP) and wheat bran (WB) hydrolyzed in the FASTSUGARS pilot plant.

EXPERIMENT	**SBP – 1**	**SBP – 2**	**SBP – 3**	**WB – 1**	**WB – 2**
t_R (s)	0.07 ± 0.03	0.11 ± 0.03	0.17 ± 0.04	0.12 ± 0.02	0.17 ± 0.02
T (ºC)	387 ± 5	399 ± 7	389 ± 4	382 ± 6	379 ± 4
P (bar)	257 ± 2	266 ± 4	273 ± 1	262 ± 5	258 ± 5
Cin (%)	1.14 ± 0.09	0.90 ± 0.12	0.87 ± 0.38	1.40 ± 0.09	1.45 ± 0.14
FCbiomass	0.40			0.43	
% susp	0.08 ± 0.03	0.05 ± 0.02	0.13 ± 0.09	0.50 ± 0.06	0.45 ± 0.03
FC suspended	0.50	0.49	0.41	0.52	0.52
Carbon susp (ppmC)	380 ± 127	236 ± 78	531 ± 137	2448 ± 307	2262 ± 219
Carbon filters (ppmC)	1507 ± 122	1810 ± 440	994 ± 243	373 ± 54	887 ± 85
Carbon liquid, TOC (ppmC)	2506 ± 301	2177 ± 55	2039 ± 726	3438 ± 61	3467 ± 86
CARBON IN (ppmC)	5049 ± 379	4223 ± 361	3564 ± 1209	6260 ± 130	6617 ± 364
CARBON OUT (ppmC)	4392 ± 285	3756 ± 638	3506 ± 1518	6062 ± 368	6284 ± 589
CARBON BALANCE (%)	87 ± 2	89 ± 7	97 ± 17	97 ± 4	95 ± 14

The experiments carried out for both biomass were presented in Table 3 and the concentrations profile were shown in Table 4. Each experimental point was the result of three repetitions of the selected conditions. Then, to calculate the main parameters of hydrolysis, namely sugars and degradation yield, conversion and selectivity, first thing to define was the calculation basis for the liquid effluent. Data and results for the calculation of these parameters were shown in Table 5. Several facts should be taken into account to determine this calculation basis. First, biomass is composed not only of cellulose, hemicellulose and lignin but also proteins, pectin and/or starch. The hydrolysis of each fraction would be yielding different products: cellulose hydrolysis would be yielding C-6 sugars (cellobiose, glucose and fructose); hemicellulose hydrolysis would release arabinoxylans (also called as C-5 sugars); lignin hydrolysis would produce polyphenolic compounds; pectin would mainly yield galacturonic acid; starch would be also producing glucose and proteins would release amino-acids. Within this wide variety of products, sugars were selected as target products and thus a HPLC column able to separate sugars and their degradation products (being acids, aldehydes and furfural-like compounds) was selected for analysis. Then, within all the biomass compounds, just cellulose, hemicellulose, pectin (in the case of SBP) and starch (for WB) were considered for calculating the *'total hydrolysable basis'* as shown in Eq. 4. However, an important clarification should be done regarding pectin and starch hydrolysis, since even though they were also yielding some products detectable by the HPLC column, under SCW hydrolysis conditions they were so rapidly degraded that it was considered that they were not a source for sugars but just for degradation products. So that, another basis for calculation was defined and called as *'sugars basis'*, considering just cellulose and hemicellulose for sugars-related calculations and calculated as shown in Eq. 5. The HPLC results in carbon basis for each experiment were shown in Table 4.

$$total\ hydrolysable\ basis = carbon\ in \cdot [\%C-6 + \%C-5 + \%pectin \mid starch] \quad (4)$$

$$sugars\ basis = carbon\ in \cdot [\%C-6 + \%C-5] \quad (5)$$

Table 4. Concentration profile for sugar beet pulp (SBP) and wheat bran (WB) experiments in the FASTSUGARS pilot plant (carbon concentrations in ppmC).

EXP.	SBP – 1	SBP – 2	SBP – 3	WB – 1	WB – 2
C – 6 sugars	824 ± 84	634 ± 24	559 ± 34	1117 ± 46	1097 ± 30
C – 5 sugars	593 ± 92	462 ± 15	387 ±105	813 ± 44	874 ± 43
Glyceraldehyde	25 ± 6	37 ± 29	16 ± 11	16 ± 3	26 ± 6
Pyruvaldehyde	-	40 ± 1	39 ± 12	94 ± 17	140 ± 17
Glycolaldehyde	87 ± 15	87 ± 17	117 ± 1	118 ± 21	168 ± 24
Lactic acid	16 ± 6	61 ± 17	70 ± 42	75 ± 9	90 ± 11
Formic acid	89 ± 14	118 ± 21	96 ± 32	24 ± 5	34 ± 11
Acetic acid	79 ± 13	66 ± 24	74 ± 7	14 ± 0	15 ± 1
5 – HMF	10 ± 3	5 ± 1	4 ± 1	4 ± 0	7 ± 0
Furfural	9 ± 4	4 ± 0	5 ± 0	3 ± 0	3 ± 0

The *'sugars yield'* was calculated as shown in Eq. 6, where the sum of both C-6 and C-5 sugars in the liquid effluent (*'sugars liq'*) was divided to the *'sugar basis'*. Next, the conversion of polysaccharides into soluble sugars, simply called as *'conversion'* was calculated in Eq. 7, by subtracting the sugars that remained in the solids, *'sugars solids'* to the *'sugars basis'* and then dividing to the *'sugars basis'*. The sugars that remained in the solids were calculated by multiplying the percentage of remaining sugars in the solid (*'% sugars solids'*) to the carbon from both filters and suspended solids. Finally, selectivity towards sugars (*'selectivity'*) was calculated by dividing the *'sugars yield'* by *'conversion'*.

$$sugars\ yield = \frac{sugars\ liq}{sugars\ basis} \tag{6}$$

$$conversion = \frac{sugars\ basis - sugars\ solids}{sugars\ basis} \tag{7}$$

On the other hand, the *'degradation yield'* was calculated as shown in Eq. 8 by dividing the sum of the degradation products (*'degradation liq'*, being: glyceraldehyde, pyruvaldehyde, glycolaldehyde, lactic acid, formic acid, acetic acid, galacturonic acid, furfural and 5-HMF) by the *'total hydrolysable basis'*, since

not just cellulose and hemicellulose would be producing degradation products, but also pectin and starch that were rapidly degraded under SCW conditions.

$$\deg radation\ yield = \frac{\deg radation\ liq}{total\ hydrolysable\ basis} \quad (8)$$

Table 5. Main hydrolysis parameters calculated for sugar beet pulp (SBP) and wheat bran (WB) experiments in the FASTSUGARS pilot plant.

EXP.	SBP – 1	SBP – 2	SBP – 3	WB – 1	WB – 2
tr (s)	0.07	0.11	0.17	0.12	0.17
% Hydrolysable	**73 %** (29 % C – 6 + 21 % C – 5 + 22 % pectins)			**66 %** (23 % C – 6 + 28 % C – 5 + 15 % starch)	
Total hydrolysable basis (ppmC)	3687 ± 277	3049 ± 202	2776 ± 327	4121 ± 86	4512 ± 100
% Sugars	**51 %** (29 % C – 6 + 21 % C – 5)			**51 %** (23 % C – 6 + 28 % C – 5)	
Sugars basis (ppmC)	2561 ± 192	2117 ± 141	1928 ± 227	3205 ± 67	4121 ± 86
Sugars liq (ppmC)	1417 ± 175	1096 ± 35	946 ± 140	1930 ± 22	1971 ±55
Sugars in solid (ppmC)	915 ± 65	810 ± 148	560 ± 120	1252 ± 31	1203 ± 109
Degradation liq (ppmC)	315 ± 59	406 ± 48	407 ± 28	347 ± 46	482 ± 64
Sugars yield (%)	55 ± 4	52 ± 5	48 ± 3	60 ± 1	56 ± 2
Conversion (%)	62 ± 3	62 ± 4	70 ± 6	61 ± 0	66 ± 3
Selectivity (%)	89 ± 8	84 ± 3	69 ± 5	99 ± 1	86 ± 7
Degradation yield (%)	9 ± 1	13 ± 1	16 ± 4	8 ± 1	11 ± 1
SOLID COMPOSITION (from filters)					
Sugars (%)	51	40	37	44	38
AIF (%)	35	53	54	41	41
Others (%)	9	3	4	2	6
Ash (%)	5	5	5	13	15

In Fig. 2 and Table 6 a typical temperature and pressure profile for a whole experiment is shown (specifically from SBP – 3). It can be seen in Table 3 that for this experiment the operating conditions were 389 ºC and 273 bar. Pressure and subsequent temperature variations visible in Fig. 2 were due to deposition of solids inside the needle valve, behavior that was already reported in previous works [9]. To obtain those reactor conditions, the water was gradually heated up from the heat exchanger to the outlet of the three electrical heaters, leaving last heater at 460 ºC. Then biomass, which entered to the plant at 22 ºC, was mixed with the SCW stream in the reactor, so that the average temperature during reaction was 389 ºC ± 4 ºC. As it happened in the laboratory scale plant, installing a heat exchanger to pre-heat the SCW stream allowed reducing the heat requirements by 13%. After depressurization the temperature was around 190 ºC, which was slightly higher compared to the laboratory scale plant (160 ºC) [9], probably due to the pressure drop produced as consequence of filters' installation in the scaled up plant. Then, the sample went through the filters and then to the heat exchangers HE – 1 and HE – 3, cooling down the effluent and allowing to collect the liquid sample at 20 ºC.

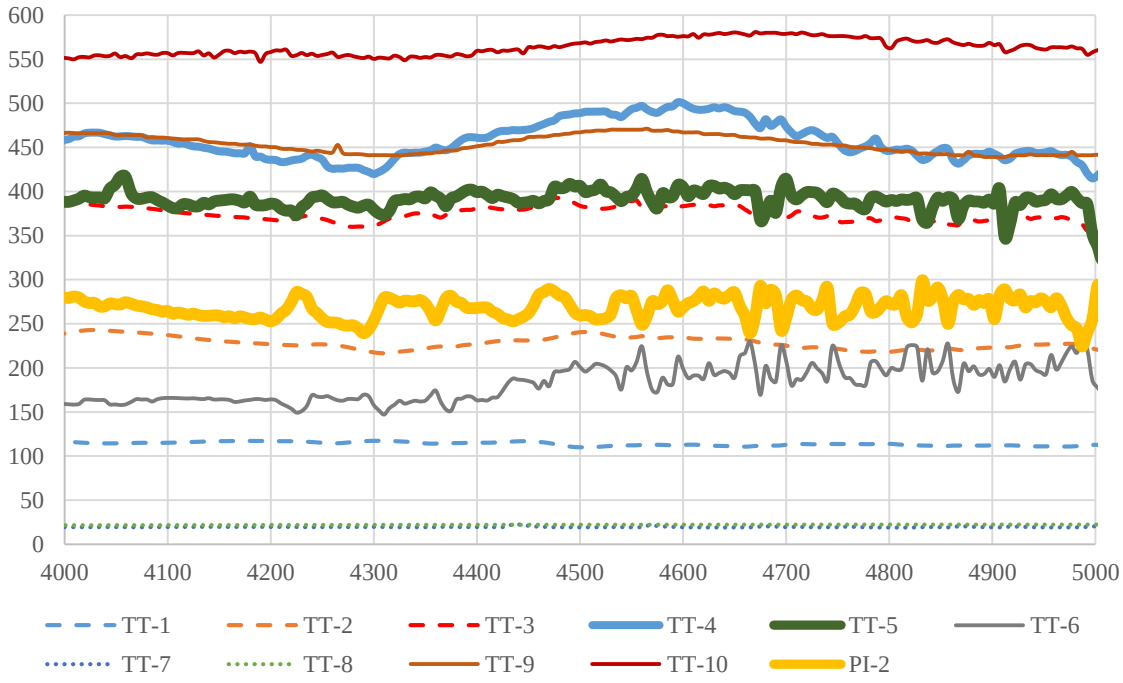


Figure 2. Temperature and pressure profile for the operation at FASTSUGARS pilot plant. Data from experiment SBP – 3.

Table 6. Average temperatura and pressure data from experiment SBP – 3.

TT – 1	HE – 1 to H -1	113 ± 2 ºC
TT – 2	H – 1 to H – 2	227 ± 6 ºC
TT – 3	H – 2 to H – 3	375 ± 8 ºC
TT – 4	SCW to reactor	463 ± 22 ºC
TT – 5	REACTOR	389 ± 4 ºC
TT – 6	Reactor oultet	192 ± 18 ºC
TT – 7	Upper sample	20 ± 1 ºC
TT - 8	Biomass to reactor	22 ± 0 ºC
TT – 9	H – 2	453 ± 11 ºC
TT – 10	H – 3	568 ± 8 ºC
PI – 2	PRESSURE	273 ± 13 bar

For the hydrolysis at the laboratory scale plant, the reactor temperature was set to 390 – 400 ºC, so that SCW temperature was around 500 ºC. For the new pilot plant, reactor temperature was also set to 390 ºC (max) to reduce energy demand and the heating elements were distributed in three heaters, in which the enthalpy difference between each stage was the same (ΔH for each stage was 1000 kJ/kg). In that way, the new heating system in 3 steps, improved the heaters' performance by distributing the energy demand and therefore avoiding overheating problems.

Regarding the reactor dimensions, same geometry was used compared to the laboratory scale plant. A tee-piece (4TTT316 HOKE®) was used as mixer, where biomass was introduced at a right angle to the SCW flow (in a horizontal plane) as shown in Fig. 3. Then, the combination of higher flow rates and lower reactor temperature in the pilot plant, produced a Reynolds number around $3 \cdot 10^4$. Meanwhile, in the laboratory plant, the Reynolds number was around $1.7 \cdot 10^4$, which was doubled compared to the pilot plant, but still in the same order of magnitude. Thus, the flow through the reactor can be considered as turbulent (Re>4000) in both cases, ensuring the mixing of both streams.

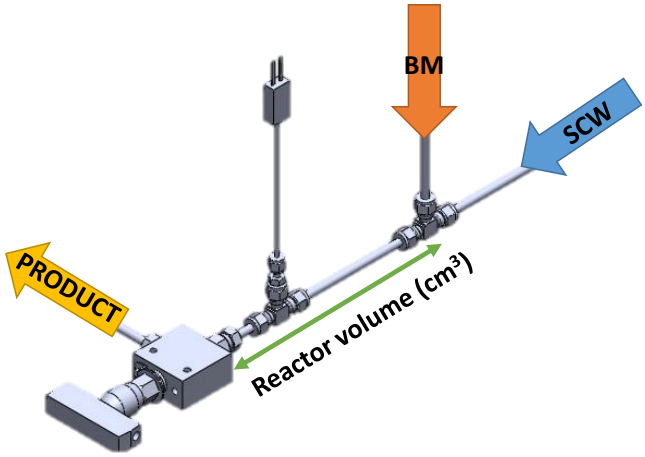


Figure 3. Detailed scheme of the ultrafast reactors for the FASTSUGARS process.

3.2.Pilot plant performance: sugar beet pulp (SBP) vs wheat bran (WB)

3.2.1. Liquid product results

The results for the main hydrolysis parameters were presented in Fig. 4 (see page 132) and numerical results were shown in Table 5. In Fig. 4 it can be seen that same trends were found for both biomass since as reaction time increased, the conversion increased and as a consequence, the degradation yield increased and on the contrary, sugars yield and selectivity decreased. Conversion should be understood as a measurement of the reaction extent or hydrolysis severity. It is important understanding that conversion is not only determined by reaction time, but also reaction conditions (temperature, pressure). This is one of the main reason for the difference between the conversion rates of WB and SBP, since the experiments were carried out with very similar reaction times (0.11 and 0.17 s for SBP vs 0.12 and 0.17 s for WB) but not same temperatures (temperatures around 390 ºC for SBP and around 380 ºC for WB). Then, even though reaction times were almost the same, as it can be seen in Fig. 4b the conversion for WB experiments was slightly lower compared to SBP. That was due the lower temperature used for WB that reduced the severity of the reaction and therefore the conversion. Visualizing the hydrolysis of a single biomass particle, first step would be SCW dissolving the hydrolysable fractions (namely cellulose, hemicellulose, pectin and starch) and then hydrolyzing them to sugars and/or degradation products (depending on reaction

extent, i.e. conversion). Supposing that the dissolution rate was constant, as reaction time increased, the produced sugars would expend more time exposed to the SCW hydrolysis and therefore a higher degradation rate would be produced. That fact explained the behavior observed, since as reaction time increased, conversion in Fig. 4b increased and therefore sugars yield (Fig. 4a) and selectivity (Fig. 4c) decreased and at the same time degradation yield increased (see Fig. 4d). As it happened in previous works, it was found that optimal reaction time was the shortest one, since the lowest conversion led to the highest sugars yield with the lowest degradation production. Then, in this case, optimal reaction time for SBP was 0.07 s, when 55 % of the initial cellulose and hemicellulose were recovered as sugars. On the other hand, the optimal reaction time for WB was found to be 0.12 s, achieving a sugars yield of 60 %.

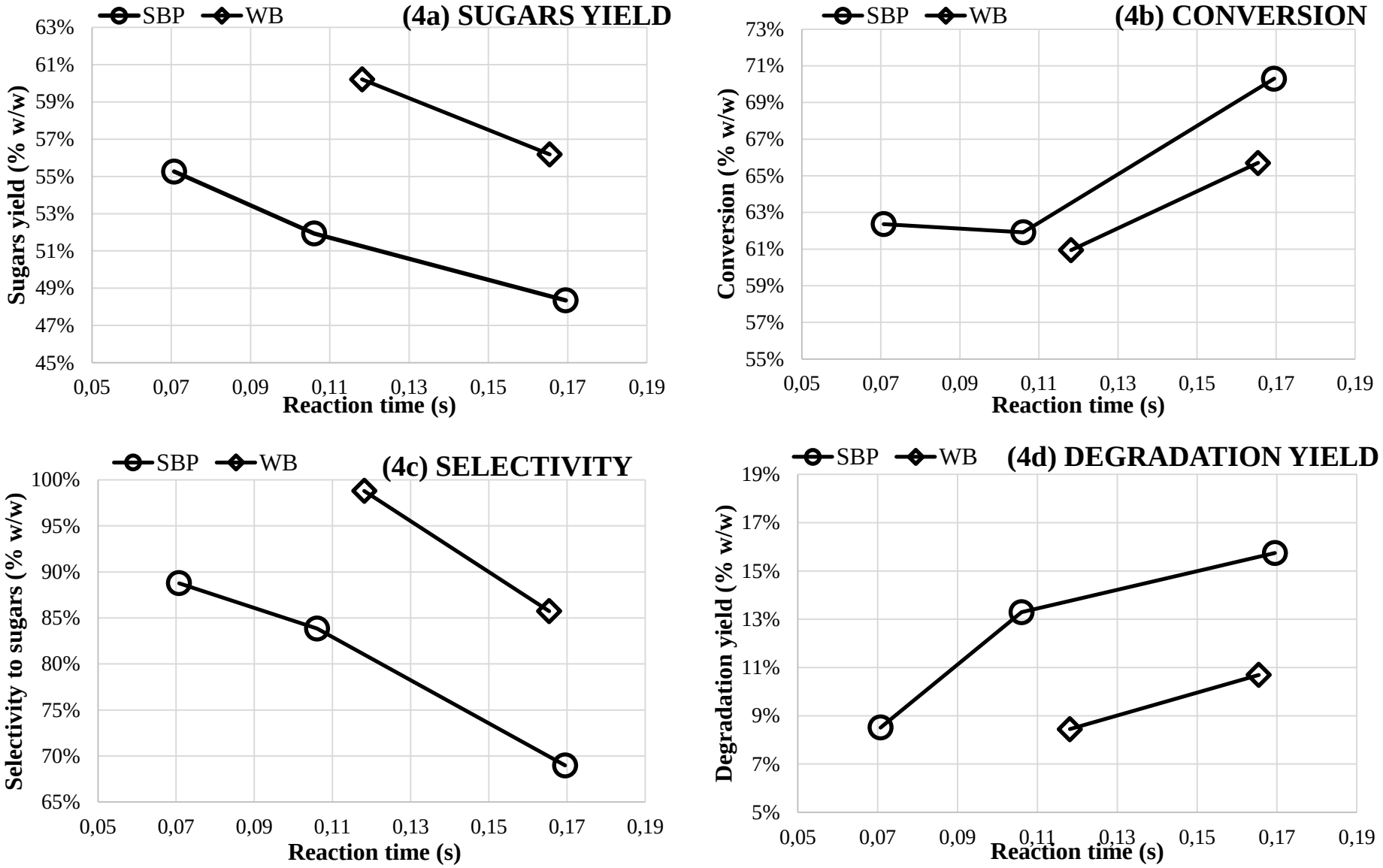


Figure 4. Average hydrolysis parameters for both sugar beet pulp (SBP) and wheat bran (WB) in the pilot plant at different reaction times. 4a) Sugars yield, 4b) conversion, 4c) selectivity and 4d) degradation yield.

3.2.2. Solid product results

To corroborate that behavior, Fig. 5 represented the composition of the solids from the filters for each biomass and reaction time. For each experiment, solids were obtained as suspended solids together with the liquid and also as an agglomerate in the filters. Those solid fractions were obtained for each experiment, meaning that it was not possible to achieve total liquefaction of the biomass. The solid from the filters were hydrolyzed with acid to get some insights about its composition (same protocol followed for the raw material characterization). As a result, it was found that the main portion of the solid product was insoluble in acid. That acid-insoluble fraction that would be related to insoluble lignin (called as AIF from now on) was visibly increasing with reaction time in the case of SBP. On the contrary, the fraction corresponding to the trapped sugars decreased with reaction time. As explained above, as reaction time increased, the attack of SCW on biomass was more severe and each particle was hollowed out to a higher extent, leaving behind the most recalcitrant fractions of biomass, i.e. ash and AIF. When comparing SBP to WB, it can be seen that under similar reaction times, SBP was producing a solid with a higher content in AIF. Again, taking into account the lower conversion of WB due to lower temperatures, it makes sense that lower conversion to soluble sugars led to higher amount of sugars trapped in the solids and as a consequence, lower concentration of AIF in the remaining solid.

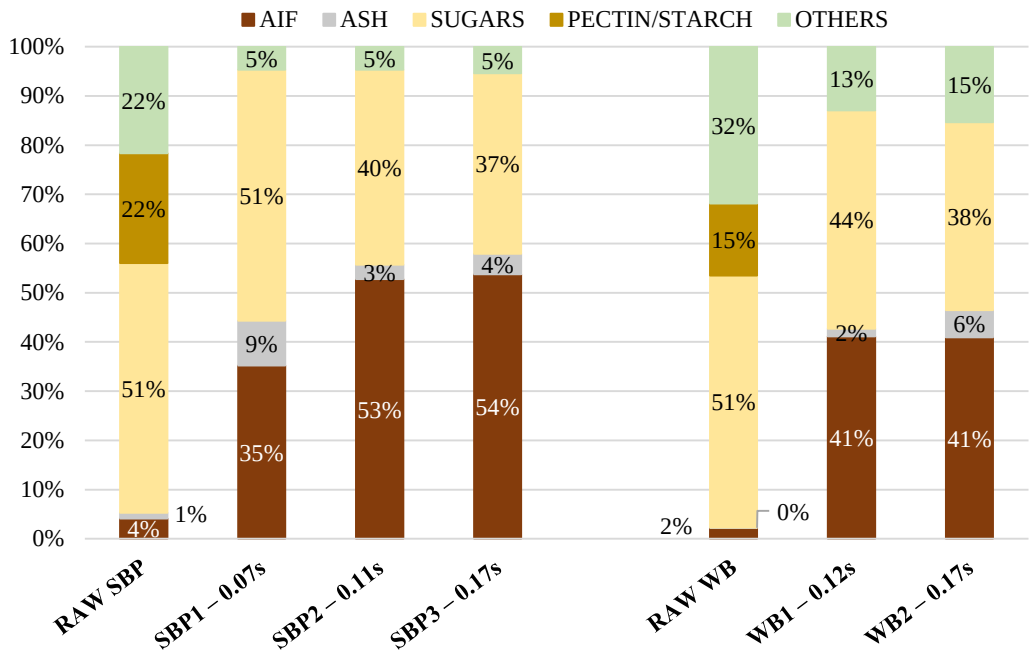


Figure 5. Composition of the solid product obtained after SCW hydrolysis of both sugar beet pulp (SBP) and wheat bran (WB) at the pilot plant at different reaction times. AIF = acid-insoluble fraction. See Table 5 for detailed composition.

3.2.3. Discussion

To summarize, focusing the liquid analysis in the conversion (Fig. 4), main difference between SBP and WB was the temperature of reaction, since for SBP it was always around 390 ºC but for WB temperature was around 380 ºC. That lower temperature led to lower conversion that provided higher sugars yield and lower degradation yield. For each biomass, it could be seen that as reaction time increased, the severity of the reaction increased and therefore the conversion increased, reducing the sugars yield and increasing the degradation rate. For the remaining solids from the filters (Fig. 5), a similar trend was found for each biomass, since as reaction time increased, the amount of trapped sugars decreased and the AIF increased. That was related to an increase in conversion that enhanced the removal of labile fractions leaving behind the most recalcitrant fractions. All in all, conversion was found to be the governing parameter for the SCW hydrolysis performance, since it helped understanding the products yields for both liquid and solid products.

To compare the results obtained from the FASTSUGARS pilot plant to similar studies, scarce literature was found. To the best of our knowledge, just a continuous pilot scale system using acid catalyst to hydrolyze woody biomass at 380 ºC, 230 bar and reaction times below 1 second was found [13]. In that work, it was possible to recover up to 50 % w/w of the inlet cellulose and hemicellulose as sugars when adding 0.05 % H_2SO_4. In the current work, the maximum sugar recovery for SBP was 55 % and 60 % w/w for WB. So that, even using acid as catalyst, the recovery of sugars in that work was lower compared to the current work. Apart from the differences between biomass, another thing to take into account when comparing both studies was the vicinity to the vapor state in the case of the woody biomass experiments. Regarding temperature effect, those results from woody biomass should be comparable to the current ones from WB, since temperature was 380 ºC in both cases. In that work, operating at 380 ± 5 ºC and 230 ± 5 bar, would mean that at some point the reaction could have been performed at 375 ºC and 225 ºC, just 4 bars away from the critical point of water. On the other hand, for the current study, the lowest operating conditions were those for WB – 2, being 379 ± 4 ºC and 258 ± 5 bar. So that, worst case scenario, the reaction would have been carried out at 375 ºC and 253 bar, still 32 bars away from the critical point. Then, it could be concluded that the FASTSUGARS pilot plant, apart from avoiding the addition of acids, was still providing high sugars recovery by reliably operating above the critical point of water.

3.3. Pilot plant performance compared to laboratory plant performance: SBP vs sbp and WB vs wb

The objective in this section was to compare the results previously obtained in the laboratory scale plant for both sugar beet pulp, sbp [15] and wheat bran, wb [9] to the ones presented in this work. First important difference to mention was the biomass used for each set of experiments. In the case of sugar beet pulp, even though both of them were supplied for the same local company (ACOR), they resulted to be different in terms of composition. The composition for each biomass was presented in Table 2. Also, the milling for each biomass was different, resulting in a different particle size. For SBP it was used the cutting mill and then the ball

mill for 1 hour to obtain a final particle size (PS) of 250 µm, meanwhile the sbp was milled with the ball mill but for 4 hours to reduce the PS to 60 µm. Wheat bran was milled just with the ball mill in both cases, for 1 hour in the case of WB to obtain a final PS of 250 µm and during 4 hours in the case of wb to obtain a PS of 125 µm.

Table 7. Experimental data and carbon balance calculations for sugar beet pulp (sbp) hydrolyzed in the FASTSUGARS laboratory plant. Data was collected from previous work [15].

EXP.	**sbp – 1**	**sbp – 2**	**sbp – 3**	**sbp – 4**	**sbp – 5**
t_R (s)	0.11 ± 0	0.14 ± 0.02	0.19 ± 0.01	0.23 ± 0.02	1.15 ± 0.05
T (ºC)	392 ± 2	392 ± 1	395 ± 1	393 ± 2	393 ± 2
P (bar)	250 ± 6	251 ± 6	249 ± 1	256 ± 6	251 ± 3
Cin (%)	1.90 ± 0	1.68 ± 0.14	1.64 ± 0.06	1.72 ± 0.02	1.73 ± 0.02
FCbiomass			0.33		
% susp	0.15 ± 0.04	0.13 ± 0.06	0.06 ± 0.05	0.12 ± 0.02	0.03 ± 0.01
FC suspended			0.39		
Carbon susp (ppmC)	588 ± 158	526 ± 236	221 ± 197	459 ± 79	111 ± 39
Carbon liquid, TOC (ppmC)	5883 ± 391	5093 ± 656	5189 ± 184	5092 ± 479	5386 ± 258
Carbon inlet (ppmC)	6264	5546	5428	5690	5713
Carbon outlet (ppmC)	6471	5619	5411	5551	5497
Carbon balance (%)	103	101	100	98	96

Table 8. Experimental data and carbon balance calculations for wheat bran (wb) hydrolyzed in the FASTSUGARS laboratory plant. Data was collected from previous work [9].

EXP.	wb – 1	wb– 2	wb – 3	wb – 4
t_R (s)	0.19 ± 0	0.22 ± 0.01	0.30 ± 0.03	0.69 ± 0
T (ºC)	398 ± 0	405 ± 4	401 ± 0	399 ± 0
P (bar)	267 ± 0	261 ± 6	262 ± 9	265 ± 0
Cin (%)	1.32 ± 0	0.79 ± 0	0.64 ± 0	0.53 ± 0
FCbiomass	0.43			
% susp	0.17 ± 0.07	0.07 ± 0.02	-	-
FC suspended	0.52			
Carbon susp (ppmC)	874 ± 364	371 ± 104	-	-
Carbon liquid, TOC (ppmC)	4857 ± 271	3242 ± 405	2789 ± 86	2275 ± 47
Carbon inlet (ppmC)	*5731*	3418	*2789*	*2275*
Carbon outlet (ppmC)	5731	3612	2789	2275
Carbon balance (%)	100	106	100	100

The input data for each biomass from the laboratory scale plant is shown in Table 7 (sbp) and 8 (wb) and the results obtained after applying same equations previously applied to the pilot plant were shown in Table 9 (sbp) and 10 (wb). As it happened for the pilot plant, each experimental point was the results of at least three replicates. First remarkable difference was the reaction time range selected for each plant. One of the advantages of the pilot scale plant was the possibility of reducing the reaction time, so shorter reaction times were selected to see if, as it would be expected, the results improved by reducing the reaction time. Then, another difference was the inexistence of filters for the laboratory plant, so that all the solids were collected as suspended solids. In Tables 9 and 10 it can be seen how the conversion for the laboratory scale experiments was very close to 100 % meanwhile for the pilot plant it was around 65 %. It was already mentioned that both reaction time and reaction temperature would affect conversion. In the case of sugar beet pulp experiments,

two experiments with the same reaction time could be compared (0.11 s). The conversion achieved for each experiment was 62 % for SBP and 94 % for sbp. Being both experiments carried out with a temperature around 395 ºC (399 ºC for SBP and 392 ºC for sbp), neither reaction time nor temperature could be the reason for such a different conversion. At this point it becomes important to evaluate the particle size of the different feedstock. For both biomass, the particle size in the pilot plant was 250 µm, meanwhile in the laboratory scale plant it was 60 µm for sbp and 125 µm for wb.

Table 9. Main hydrolysis parameters calculated for sugar beet pulp (sbp) experiments in the FASTSUGARS laboratory plant. Data was collected from previous work [15]

EXP.	**sbp – 1**	**sbp – 2**	**sbp – 3**	**sbp – 4**	**sbp – 5**
t_R (s)	0.11	0.14	0.19	0.23	1.15
% Hydrolysable	**68 %** (19 % C – 6 + 22 % C – 5 + 28 % pectins)				
Total hydrolysable basis (ppmC)	4289	3797	3716	3896	3911
% Sugars	**41 %** (19 % C – 6 + 22 % C – 5)				
Sugars basis (ppmC)	2564	2270	2222	2329	2338
Sugars liq (ppmC)	1703	1357	1115	903	305
Sugars in solid (ppmC)	146	110	28	21	2
Degradation liq (ppmC)	1903	1827	1958	1835	1898
Sugars yield (%)	66	60	50	39	13
Conversion (%)	94	95	99	99	100
Selectivity (%)	70	63	51	39	13
Degradation yield (%)	44	48	53	47	49
SOLID COMPOSITION (suspended)					
Sugars (%)	25	21	13	5	1
AIF (%)	55	62	65	81	88
Others (%)	12	13	19	11	1
Ash (%)	8	4	4	3	10

Table 10. Main hydrolysis parameters calculated for sugar wheat bran (wb) experiments in the FASTSUGARS laboratory plant. Data was collected from previous work [9]

EXP.	wb – 1	wb – 2	wb – 3	wb – 4
t_R (s)	**0.19**	**0.22**	**0.30**	**0.69**
% Hydrolysable	**66 %** (23 % C – 6 + 28 % C – 5 + 15 % starch)			
Total hydrolysable basis (ppmC)	3773	2250	1836	1498
% Sugars	**51 %** (23 % C – 6 + 28 % C – 5)			
Sugars basis (ppmC)	2935	1750	1428	1165
Sugars liq (ppmC)	1452	1173	643	562
Sugars in solid (ppmC)	195	71	-	-
Degradation liq (ppmC)	1085	881	737	813
Sugars yield (%)	49	67	45	48
Conversion (%)	93	96	100	100
Selectivity (%)	53	70	45	48
Degradation yield (%)	29	39	40	54
SOLID COMPOSITION (suspended)				
Sugars (%)	22	19	5	5
AIF (%)	68	77	80	81
Others (%)	8	5	5	4
Ash (%)	0	1	3	3

Several studies focused on cellulose hydrolysis as first step to better understand biomass hydrolysis in SCW. In a previous work [18] it was observed that cellulose hydrolysis in SCW could occur in a heterogeneous media if inlet concentration was sufficiently high. In that case, cellulose behaved as if it had been hydrolyzed at subcritical conditions. Under those conditions, the process was governed by the cellulose dissolution velocity, which resulted to be lower than the hydrolysis rate [19]. As a consequence, the cellulose was not totally dissolved and mass transfer resistances were affecting the reaction rate.

Moving from pure cellulose to biomass hydrolysis in the FASTSUGARS process, working with reaction times below 1 second, the dissolution of biomass was also incomplete (due to the recalcitrant and intricate nature of biomass matrix compared to pure cellulose). Then, as it happened for highly-concentrated cellulose, when hydrolysis starts, there is still a fraction of undissolved cellulose in solid state [19]. In that heterogeneous reaction media, the accessibility to hydrolysable fractions would be determining the dissolution rate and therefore the conversion to soluble products. The accessibility to biomass matrix is closely related to particle size since it was already proved that bigger particles, having lower surface area per unit of volume, would hinder the accessibility to cellulose and hemicellulose fractions [20]. All in all, considering these limitations, it makes sense that a bigger particle needs more severity (meaning higher reaction time or more severe reaction conditions) to get hydrolyzed to the same extent than a particle half its size. Therefore, following the same reasoning already observed when comparing sbp to wb results [15], initial particle size was acting as a mass transfer resistance, so that under same reaction time and operating conditions, bigger particle size produced lower conversion.

3.3.1. Liquid product results

In terms of liquid performance, sugars yield, conversion, selectivity and degradation yield were plotted in Fig. 6 (see page 142) for both pilot and laboratory scale. The longest reaction times for sbp (1.15 s) and wb (0.69 s) were discarded from the plots in order not to distort the scale of the plots. In both biomass it can be seen that the trends already mentioned for SBP and WB were also found here, since as increasing reaction time for each set of experiments, the conversion (Fig. 6b) increased and as a consequence, the sugars yield (Fig. 6a) and selectivity (Fig. 6c) decreased. On the contrary, the degradation yield (Fig. 6d) increased with reaction time. It was previously mentioned that the lower conversion would produce higher sugars yield, since the produced sugars would be less exposed to degradation. Then, when carrying out the experiments in the pilot plant for both biomass, as the conversion was lower, a higher sugars yield would have been expected compared to the laboratory scale plant. However, as it was clearly visible for sugar beet pulp at 0.11 s, the sugars yield for SBP was lower than the one for sbp, being 55 % and

66 %, respectively. If having the same particle size, the sugars yield for SBP should have been higher, but since particle size was acting as a mass transfer limitation, a higher severity would have been needed to get same yields. For wheat bran that difference was not so remarkable since the difference between the particle size for pilot and laboratory plants was not so large (125 vs 250 μm) as it was for sugar beet pulp (60 vs 250 μm). Another important difference between both plants was the degradation yield that was much higher for the laboratory scale experiments. Again, as conversion was higher for sbp and wb, the produced sugars were exposed to a higher severity that favored their degradation.

Since the aim of this work was the selective transformation of biomass into sugars, when comparing the differences in the scaling up, selectivity towards sugars became the key parameter for comparison. Then, just considering selectivity and degradation yield to evaluate the scaling up it could be seen that the pilot plant provided better results, since higher sugars selectivity was obtained with a lower degradation rate. In the previous section it was concluded that conversion was the determining parameter to understand the SCW hydrolysis performance and it was also proved that it was affected not only by reaction time but also temperature. In the current section, when comparing the performance of same biomass in different plants, it was demonstrated that the conversion was also affected by the particle size of biomass. Indeed, in the pilot plant, as the initial particle size was bigger, the hydrolysis of biomass was slowed down, producing a lower conversion and therefore enhancing sugars selectivity by reducing the degradation rate.

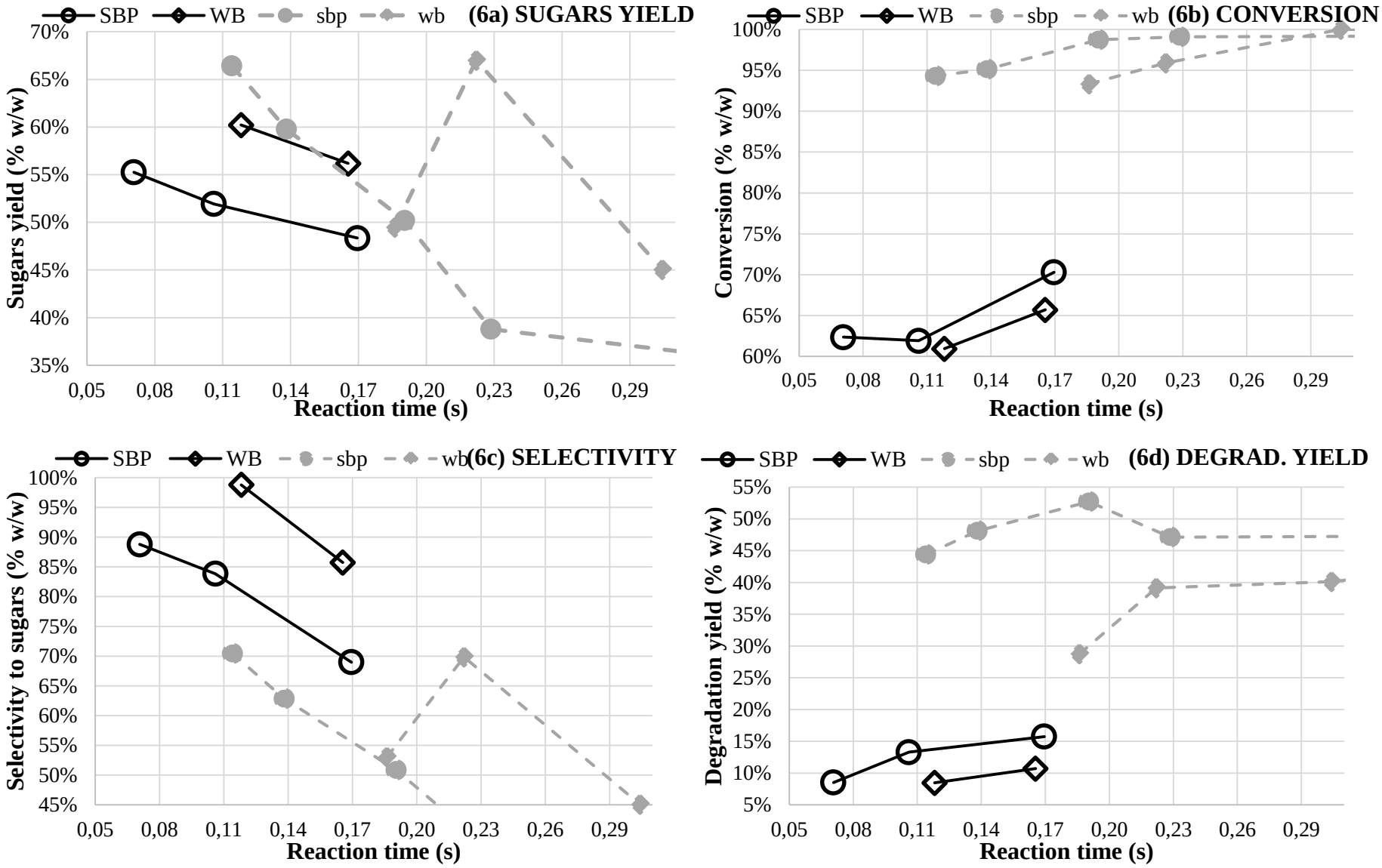
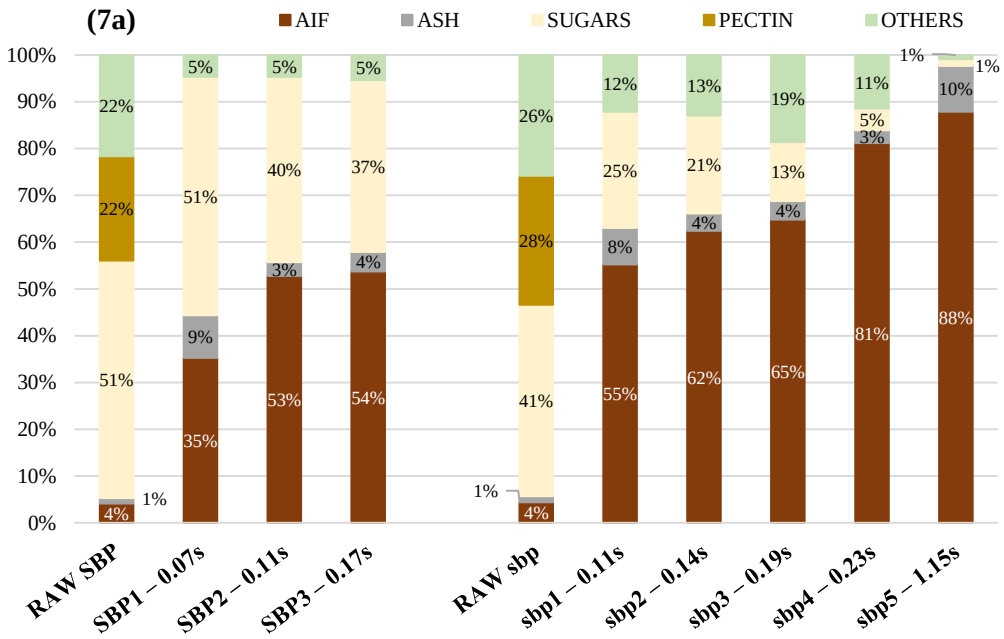


Figure 6. Hydrolysis parameters for both pilot (SBP and WB, continuous lines) and laboratory (sbp and wb, dotted lines) scale plants at different reaction times, representing: 6a) Sugars yield, 6b) conversion, 6c) selectivity and 6d) degradation yield.

3.3.2. Solid product results

Similar trends were found for the remaining solid composition presented in Fig. 7. For sugar beet pulp (Fig. 7a) it can be seen that for SBP the AIF content was always lower and the trapped sugars were higher compared to the laboratory scale plant. Same trend was observed for wheat bran (Fig. 7b). These facts would be related to the conversion or severity of the reaction medium, as in the pilot plant the conversions were lower, a weaker hydrolysis of biomass was carried out, leaving behind a higher amount of sugars in the remaining solids and therefore a lower AIF content. Taking again sugar beet pulp at 0.11 s as a reference, it could be seen how the AIF was slightly lower in the case of SBP and at the same time, the sugars content was almost double compared to sbp. The reason for these differences was again the particle size that acted as a mass transfer resistance and provided a lower conversion for the experiments in the pilot plant.



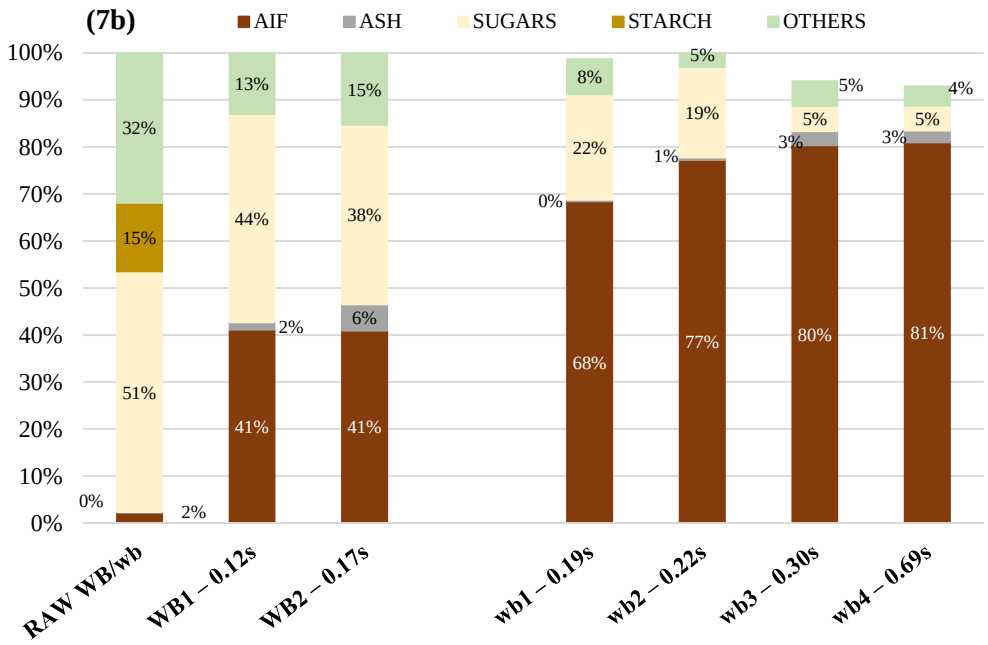


Figure 7. Composition of the solid product obtained after SCW hydrolysis of sugar beet pulp (7a) and wheat bran (7b) in both laboratory scale plant (lower case letters) and pilot plant (capital letters) at different reaction times. AIF = Acid-insoluble fraction.

3.3.3. Discussion

Then, when comparing the performance of the SCW hydrolysis of both sugar beet pulp and wheat bran in the pilot plant and the laboratory scale plant, some valuable conclusions were drawn. First conclusion was that the particle size was acting as a mass transfer resistance in the FASTSUGARS process. For the experiments in the pilot plant, even though the reaction time was reduced the results were not significantly improved in terms of sugars yield, due to the lower conversion achieved. Conversion was lower due to the bigger particle size used in the pilot plant that slowed down the hydrolysis of the biomass. This slowing down effect in the pilot plant resulted to be positive, since having a lower conversion allowed producing more sugars instead of degradation products. Then, focusing the discussion on the selectivity towards sugars, the pilot plant process provided much higher selectivity compared to the laboratory plant and at the same time, lower degradation rates were produced as a consequence.

4. Conclusions

The FASTSUGARS process for the hydrolysis of biomass in supercritical water was scaled up from laboratory to pilot plant scale. Sugar beet pulp and wheat bran were used to validate the scaling up. When performing the hydrolysis of these biomass in the pilot plant, similar trends were obtained, as sugars yield and selectivity decreased with reaction time and then, conversion and degradation yield increased with reaction time. Differences between the results obtained for each biomass were due to composition and reactor conditions. On the other hand, when comparing the results from the pilot plant to those from the laboratory scale plant, it was found that main difference was due to the initial particle size of biomass. To bring the FASTSUGARS process closer to industrial applications, a bigger particle size (PS) was used in the pilot plant (250 μm) compared to the laboratory scale plant (PS ≤ 150 μm). It was observed that increasing the particle size slowed down the hydrolysis reaction and as a consequence the conversion was decreased. This slowing down effect in the pilot plant resulted to be positive, since selectivity was increased and at the same time, the degradation production was remarkably reduced.

Acknowledgments

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Chapter 4. Ultrafast hydrolysis of inulin in supercritical water: Fructooligosaccharides reaction pathway and Jerusalem artichoke valorization [d]

Abstract

In a biorefinery approach, inulin and inulin-rich biomass as Jerusalem artichoke (JA) could be transformed into platform chemicals such as fructose and/or pyruvaldehyde. To do so, the FASTSUGARS pilot plant proved to be a promising alternative for the selective conversion of biomass. In this work, inulin and JA were hydrolyzed in supercritical water (SCW) for the first time. Commercial inulin was selected as a model for fructooligosaccharides (FOS) and its reaction pathway in SCW was elucidated. It was found that fructose was the primary product from FOS hydrolysis in SCW, which was then selectively transformed into pyruvaldehyde as reaction time increased. Operating with extremely low reaction times (0.12 s) the sugars selectivity of JA was as high as 76 % w/w. Finally, comparing JA results to those from lignocellulosic biomass it was found that higher conversion was achieved in the case of JA due to its inulin-based composition.

[d] C. M. Martínez, T. Adamovic, D.A. Cantero and M. J. Cocero. Ultrafast hydrolysis of inulin in supercritical water: Fructooligosaccharides reaction pathway and Jerusalem artichoke valorization. Submitted to *Carbohydrate Polymers*.

1. Introduction

Inulin is a linear polysaccharide containing D-fructose units linked together by β(2→1) bonds terminated by a D-glucose molecule [1, 2]. When isolating inulin, smaller oligosaccharides and monomers are commonly separated, so that the mean polymerization degree (DP) of commercial inulin is usually between 12 and 25 [3]. Therefore molecules with DP < 10 are identified as fructooligosaccharides (FOS) [4]. Inulin and FOS are natural polymers that can be found in around 15 % of all flowering plants, being the most common sources for their industrial production the chicory (*Cichorium intybus*) and Jerusalem artichoke (*Helianthus tuberosus*) [3].

Once the inulin from biomass is isolated, a hydrolysis process should be carried out to produce the FOS and monomeric fructose. Inulin could be hydrolyzed by acid under mild conditions [5]. However, as fructose is easily degraded at low pH values, acid hydrolysis would lead to degradation products instead of fructose-rich effluents [6]. On the industrial scale, fructose and FOS are produced either from sucrose by transfructosylation or from inulin by controlled enzymatic hydrolysis [7, 8]. For the first one, the main drawback is the strong thermodynamic limitation due to the glucose and fructose equilibrium, which is close to 50 % [6, 8]. The challenge for the second method involving inulin is still the growing of such specific microorganisms [7, 9].

Supercritical water (SCW, meaning water above its critical point, 374 ºC and 221 bar) has been previously used as hydrolysis medium for pure cellulose [10], fructose [11] and agricultural biomass [12] in the so-called FASTSUGARS process. As one of the challenges for biomass refining is the fundamental knowledge of biomass structure and composition, the success of the FASTSUGARS process would be deeply understanding the performance of model polymers such as inulin and FOS. FOS are a promising food additive, which showed to stimulate the immune systems in the body [13], to help controlling diabetes [14] and reducing triglycerides and fatty acids content in blood serum [15] and also showed to have anti-cancer activity [16].

Then, the first objective of this work was to study for the first time the hydrolysis of inulin in SCW. Commercial inulin with a DP close to 10 was selected as FOS model, which allowed proposing a degradation profile for FOS in SCW. The effects of reaction time and inlet concentration were studied, being the production of fructose and/or pyruvaldehyde the main targets. Once the hydrolysis of FOS was evaluated, Jerusalem artichoke (*Helianthus tuberosus*) was selected as inulin-rich biomass to study its hydrolysis in SCW. Jerusalem artichoke (JA) results were compared to the results from pure inulin and other biomass hydrolyzed in the FASTSUGARS process.

2. Materials and Methods

2.1.Materials

Inulin was supplied by Beneo (Orafti® GR), as granulated powder extracted from chicory root (*Cichorium intybus*). Frozen Jerusalem artichoke tubers (*Helianthus tuberosus*) were provided by a local supplier. Deionized water was used as the hydrolysis medium for the experiments. The High Performance Liquid Chromatography (HPLC) standards were purchased from Sigma-Aldrich, being: glucose, fructose, glyceraldehyde, pyruvaldehyde, glycolaldehyde dimer, lactic acid, formic acid, acetic acid, 5-hydroxymethylfurfural (5-HMF) and furfural. MilliQ® water and sulfuric acid (0.01 N) were used as the mobile phase in the HPLC analysis. Sodium nitrate ($NaNO_3$ 0.1 M) and sodium azide (NaN_3 0.02%) in MilliQ® water were used as the mobile phase in the HPLC-SEC analysis. Pululans purchased from Shodex were used as standards (STANDARD P-82).

2.2.Methods

2.2.1. Inulin experiments

The carbon content in the inulin powder was determined by elemental analysis using an EA Flash 200 analyzer. The composition of the effluent from SCW hydrolysis was analyzed by HPLC, using a Shodex SH-1011 column as described in previous works [10]. Carbon content in the liquid samples was determined by total organic carbon (TOC) analysis by using a Shimadzu TOC-VCSH. Average

molecular weight (MW) of inulin feed and products was determined by Size Exclusion Chromatography (HPLC-SEC), using a Shodex OHpak SB-803 HQ column as described elsewhere [17].

2.2.2. Jerusalem artichoke (JA) experiments

To characterize biomass, JA tubers were defrosted, chopped and dried at 65 ºC. To determine the lignin and ash content, an acid hydrolysis was performed following a NREL protocol [18]. Proteins were determined via Kjeldahl nitrogen analysis as shown in previous works [19], using a proteins factor of 6.25 [20]. The free sugars and inulin contents were determined through an extraction procedure [20], where 0.1 g of dried material was weighted into 100 mL of water at room temperature and stirred for 15 min. Then, the remaining liquid was analyzed by HPLC to determine the fructose and glucose due to free sugars. In order to obtain the total fructose and glucose content, 0.1 g of dry material was weighted into 100 mL of 0.2% H_2SO_4 and hydrolyzed at 105 ºC for 60 min in an autoclave. After hydrolysis, the liquid was analyzed by HPLC to determine the total fructose and glucose concentrations.

The average degree of polymerization (DP) in a complex matrix was defined by Eq. 1, where *'F_i'* and *'G_i'* are the fructose and glucose due to inulin, which can be calculated by Eq. 2 and 3.

$$DP = \frac{\%Fi}{\%Gi} + 1 \quad (1)$$

$$\%Fi = \%Ft - \%Ffs \quad (2)$$

$$\%Gi = \%Gt - \%Gfs \quad (3)$$

'F_t' and *'G_t'* are the total fructose and glucose obtained from acid hydrolysis and *'F_{fs}'* and *'G_{fs}'* are the fructose and glucose obtained from free sugars determination. Next, once the DP was calculated, to calculate the concentration of polymeric sugars from the concentration of corresponding monomeric sugars a conversion factor *'k'* was calculated by Eq. 4. Then, to determine the total inulin content, Eq. 5 was used. Additionally, the hydrolysable fraction of JA was calculated as the addition of both inulin and free sugars.

$$k = \frac{180 + 162(DP - 1)}{180 \cdot DP} \quad (4)$$

$$\%INULIN = k(\%Fi + \%Gi) \quad (5)$$

Once the JA experiments were performed, liquid and solid products were obtained. The liquid was directly analyzed by HPLC analysis to determine the concentration of acids, aldehydes, furfural and 5-HMF. Then, the concentration of soluble oligosaccharides in the liquid effluent was determined via acid hydrolysis, as it was done in previous works [12]. TOC analysis was also performed to the liquid samples obtained from JA. The solid product was analyzed by elemental analyzer to know their carbon content. Then, it was hydrolyzed following same protocol followed for the raw material. In this case, after acid hydrolysis an acid-insoluble fraction (AIF) was obtained as remaining solid. The liquid aliquot was used to determine the amount of trapped/unconverted sugars by HPLC analysis.

2.2.3. Experimental set up

The experiments were performed in the continuous pilot plant of the so-called FASTSUGARS process shown in Fig. 1. This FASTSUGARS pilot plant was designed and built in a previous work, which operating procedure was thoroughly described there [21]. The key parameter in the FASTSUGARS process was the method to accurately control the reaction time. In the so-called ultrafast reactors, the reaction started when biomass (room temperature) and SCW (450 ºC) were mixed together in a tee junction, so that biomass was instantaneously heated up to reaction temperature (around 390 ºC). Then, the effluent was suddenly decompressed through a needle valve, stopping the reaction due to the cooling produced as consequence of Joule-Thomson effect.

The reaction time was referred to the time that biomass and SCW spent together between the mixing point and the valve and it was calculated as shown in Eq. 6, where it can be seen it was a function of reactor volume and flow. The reactor volume, *'V'* in m^3, was calculated using the dimensions of the reactor. The volumetric flow in the reactor, *'F_v'* in m^3/s, was calculated as a function of the density of the reaction medium at ambient conditions *'ρ_0'* and reaction conditions

'ρ_r', both in kg/m^3 and considering the fluid as pure water. Using the ratio *'ρ_r/ρ_0'*, it was possible to transform the flow measured at ambient conditions, *'$F_{v,0}$'* in m^3/s, into *'F_v'*. Therefore, in order to change the reaction time for the different experiments, either reactor's length, total flow or both were varied.

$$t_R = \frac{V}{F_v} = \frac{\pi \cdot L \cdot D^2}{4} \frac{\rho_r}{F_{v,0} \cdot \rho_0} \tag{6}$$

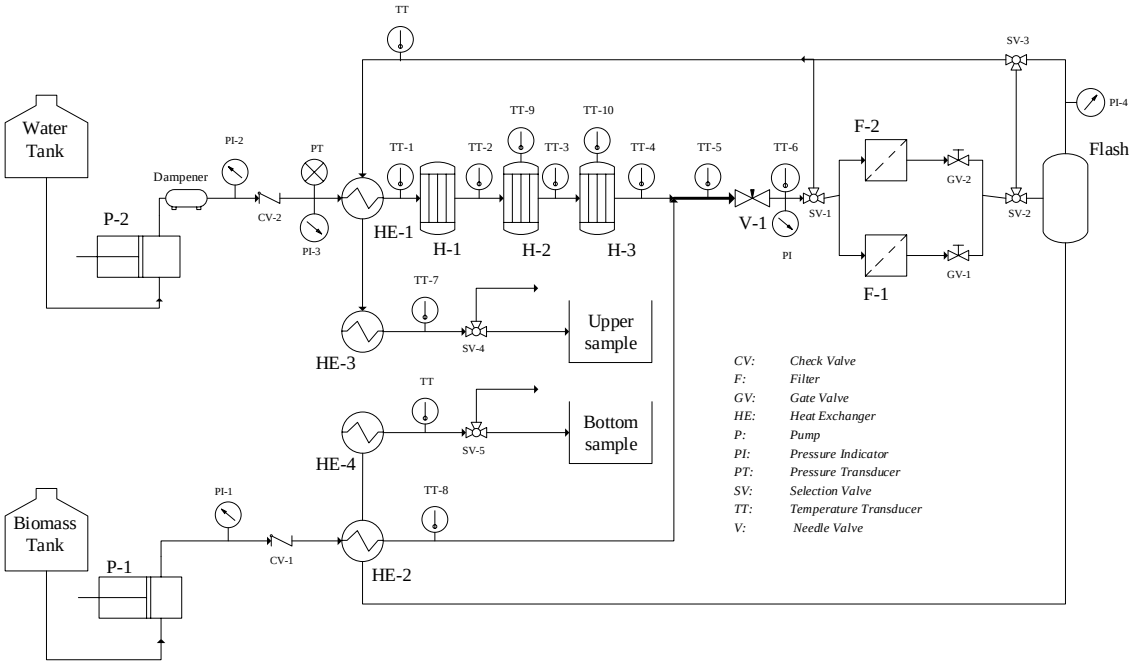

Figure 1. FASTSUGARS pilot plant used to carry out the hydrolysis of inulin and Jerusalem artichoke in supercritical water.

3. Results and Discussion

3.1. Inulin hydrolysis in supercritical water (SCW)

Using the pilot plant showed in Fig. 1, the hydrolysis of inulin solutions was carried out at 385±7 ºC and 250±7 bar, with reaction times between 0.12 and 0.74 seconds. The concentration of the solutions varied from 5 to 30 % w/w, which corresponded to inlet concentrations to the reactor between 1 and 9 % w/w. The experimental data is shown in Table 1, where each experimental point is the average of at least 5 samples.

Table 1. Experimental data from inulin experiments in the FASTSUGARS pilot plant.

	Reactor (cm^3)	T (ºC)	P (bar)	tr (s)	Cin (%)	CARBON IN (ppmC)
EXP 1 – 5%	2.27	388	253	0.16	0.7	2914
EXP 2 – 10%	2.27	386	254	0.17	2.0	8290
EXP 3 – 20%	2.27	379	256	0.17	5.0	21075
EXP 4 – 30%	2.27	379	255	0.17	9.2	38600
EXP 5 – 20%	2.78	383	257	0.21	4.9	20794
EXP 6 – 20%	1.49	383	257	0.12	5.8	24489
EXP 7 – 20%	9.96	384	258	0.74	5.7	23798
EXP 8 – 20%	5.04	386	257	0.33	5.1	21419

The carbon content of inulin was found to be 0.42 g carbon/g inulin through elemental analysis. Using that factor it was possible to calculate the inlet concentration in terms of carbon as shown in Eq. 7 and Table 1. The HPLC results were translated into carbon units, and then specific yields were calculated as shown in Eq. 8 and collected in Table 2.

$$CARBON\ IN\ (ppmC) = Cin\ (\%) \cdot 10000 \cdot 0.42 \tag{7}$$

$$YIELD\ (\%) = \frac{HPLC\ concentration\ (ppmC)}{CARBON\ IN\ (ppmC)} \tag{8}$$

Table 2. Yields for each individual component detected by HPLC for inulin hydrolysis in SCW in the FASTSUGARS pilot plant. Continue in the next page.

	Oligomers	Glucose	Fructose	Glycerald.	Pyruvald.	Lactic acid
EXP 1 – 5% – 0.16 s	16 %	4 %	25 %	6 %	28 %	5 %
EXP 2 – 10% – 0.17 s	15 %	5 %	32 %	7 %	24 %	2 %
EXP 3 – 20% – 0.17 s	14 %	7 %	38 %	7 %	18 %	3 %
EXP 4 – 30% – 0.17 s	12 %	9 %	43 %	7 %	12 %	6 %
EXP 5 – 20% – 0. 21 s	13 %	8 %	35 %	8 %	15 %	7 %
EXP 6 – 20% – 0.12 s	13 %	7 %	35 %	8 %	15 %	4 %
EXP 7 – 20% – 0. 74 s	4 %	6 %	28 %	10 %	23 %	9 %
EXP 8 – 20% – 0.33 s	8 %	6 %	31 %	9 %	20 %	8 %

	Formic acid	Acetic acid	Levulinic acid	5 – HMF	Furfural
EXP 1 – 5% – 0.16 s	17 %	0 %	2 %	0 %	0 %
EXP 2 – 10% – 0.17 s	13 %	0 %	1 %	0 %	0 %
EXP 3 – 20% – 0.17 s	11 %	0 %	1 %	1 %	1 %
EXP 4 – 30% – 0.17 s	8 %	0 %	1 %	2 %	1 %
EXP 5 – 20% – 0. 21 s	11 %	0 %	1 %	1 %	1 %
EXP 6 – 20% – 0.12 s	12 %	0 %	1 %	1 %	1 %
EXP 7 – 20% – 0. 74 s	13 %	4 %	2 %	3 %	2 %
EXP 8 – 20% – 0.33 s	10 %	3 %	1 %	2 %	1 %

3.1.1. Reaction pathway for FOS hydrolysis in SCW

To simplify the discussion about reaction mechanisms, they were grouped as shown in the reaction scheme in Fig. 2 and Table 3. As it can be seen in Fig. 2, four different reaction mechanisms were studied here. The reaction pathway started from fructooligosaccharides (FOS) to understand the hydrolysis reaction of the FOS produced from inulin hydrolysis. Molecular weight (MW) of the procured inulin was measured by HPLC-SEC analysis, obtaining an average MW of 1676 Da. As inulin chemical formula is $C_{6n}H_{10n+2}O_{5n+1}$, its polymerization degree ('*n*' from the formula) was found to be 10. Then, as FOS were defined as those with a DP<10 [4], the assumption made in this work to use that procured inulin as a representing model of FOS was validated. Moreover, through HPLC analysis it was determined that the fructose to glucose ratio (F/G) in the procured inulin was 8.

Reaction pathway for FOS hydrolysis in SCW was proposed based on related literature about fructose hydrolysis in near-critical water [11, 22] and it was presented in Fig. 2. First step would be its depolymerization mostly yielding monomeric fructose (R1). As inulin also contains glucose molecules in its structure, it could also be directly hydrolyzed into glucose (R2). Both glucose and fructose could isomerize to each other via ring opening and keto-enol tautomerism (R3) [23]. However, it was already demonstrated that under SCW conditions the glucose to fructose transformation was preferred over the opposite one [24], so that glucose production via isomerization would be minimal. The sum of fructose, glucose and unconverted oligomers would be named as *'TOTAL SUGARS'* from now on.

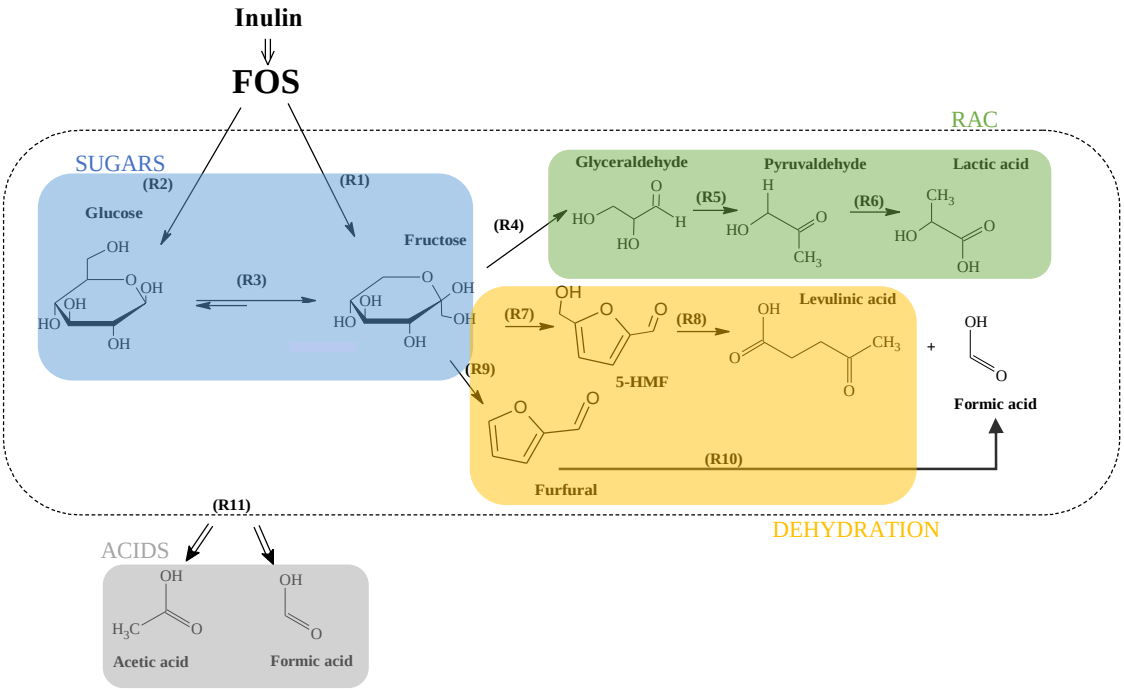

Figure 2. Reaction pathway proposed for the degradation of FOS from inulin in SCW hydrolysis.

Table 3. Yields grouped by reaction mechanism as shown in Fig. 2 for inulin hydrolysis in the FASTSUGARS pilot plant.

	tr (s)	Glycer+Pyruv +Lactic	Furfural+5-HMF+Levulinic	Formic + Acetic acids	Monomers+ Oligomers
		RAC	**DEHYDRATION**	**ACIDS**	**TOTAL SUGARS**
EXP 1 – 5%	0.16	39 %	3 %	17 %	46 %
EXP 2 – 10%	0.17	33 %	2 %	13 %	52 %
EXP 3 – 20%	0.17	28 %	2 %	11 %	59 %
EXP 4 – 30%	0.17	25 %	5 %	8 %	64 %
EXP 5 – 20%	0.21	30 %	3 %	11 %	56 %
EXP 6 – 20%	0.12	37 %	3 %	12 %	55 %
EXP 7 – 20%	0.74	42 %	7 %	16 %	39 %
EXP 8 – 20%	0.33	37 %	4 %	13 %	45 %

The released sugars would be available for further conversion into different products via several mechanisms, being: dehydration, retro-aldol condensation (RAC) and/or degradation into acids. Fructose could suffer dehydration, yielding 5- HMF (R7) and/or furfural (R9) [22]. Then, levulinic acid (R8) could be produced from 5-HMF via hydration, also releasing formic acid [25]. Furfural could be also degraded into formic acid (R10) [26]. The addition of 5-HMF, furfural and levulinic would be identified as *'DEHYDRATION'* from now on. Another important mechanism would be the RAC that would yield aldehydes from fructose. Specifically, fructose would be converted to glyceraldehyde (R4) and subsequently it would be transformed into pyruvaldehyde (R5). Then, under favorable conditions, pyruvaldehyde would be converted into lactic acid (R6) [11]. The sum of glyceraldehyde, pyruvaldehyde and lactic acid was called as *'RAC'*. Apart from these two mechanisms, the released sugars could be degraded into acids (R11), namely formic and acetic acid [22], labelled as *'ACIDS'*. The yield for each pathway is shown in Table 3.

To validate the proposed reaction mechanisms, main products from FOS hydrolysis (fructose, pyruvaldehyde and formic) were selected to follow the kinetics. Individual yields were plotted against fructose, pyruvaldehyde and formic yields as shown in Fig. 3. The first plot (3a), representing the fructose yield in the X axis, would be providing an idea of the fructose conversion towards other products. The fructose could be converted via 4 reactions, being: R3 to produce glucose, R4 to produce glyceraldehyde, R7 to produce 5-HMF and/or R9 to produce furfural. Fructose to glucose isomerization (R3) should be minimal under SCW conditions and it can be corroborated from Fig. 3a that they were produced in parallel, not from each other. If isomerization would be occurring, fructose and glucose would be following opposite trends instead of proportional ones as shown in Fig. 3a. Moreover, with a ratio fructose/glucose of 8F/1G, the maximum yield of glucose obtained from direct depolymerization of inulin would be 11 %, being the remaining 89 % w/w related to fructose-derived products. Then, assuming that the fructose to glucose isomerization could happen under the selected conditions in this work, the yield of glucose should be greater than 11 % w/w. Nevertheless, the

maximum glucose yield was 8 % w/w (0.21 s), suggesting that isomerization of fructose to glucose was minimum.

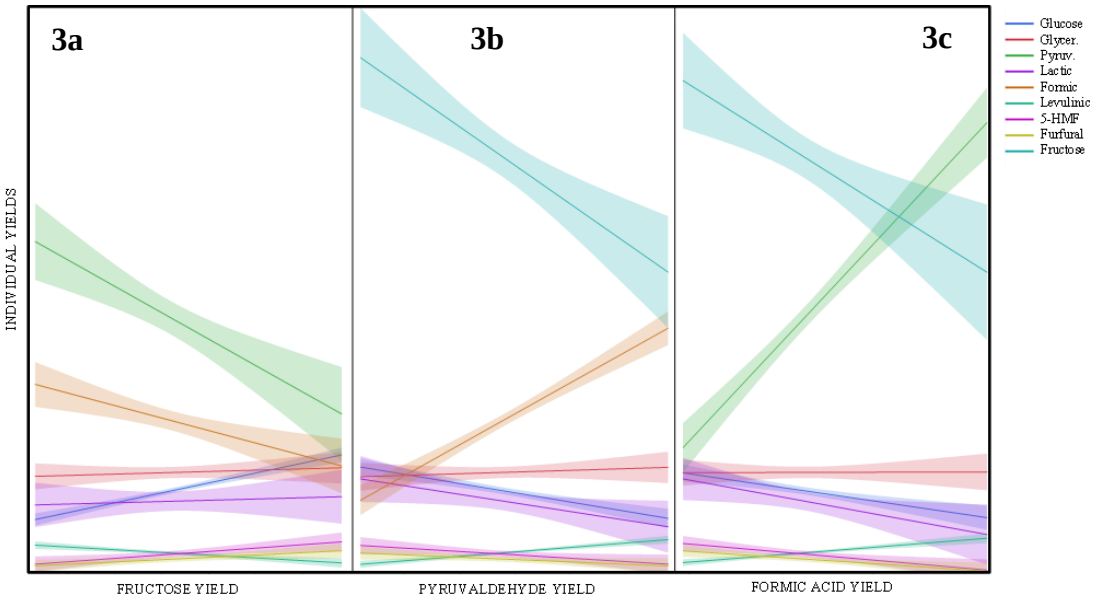

Figure 3. Kinetic analysis for inulin hydrolysis in SCW. Individual yields (% w/w) were plotted against fructose (3a), pyruvaldehyde(3b) and formic acid yields (3c).

On the other hand, the glyceraldehyde (R4) did not show any clear trend related to fructose yield. However, the pyruvaldehyde production (R5), was clearly increased when fructose yield decreased. Previous studies proved that the reaction of glyceraldehyde to produce pyruvaldehyde (R5) was faster than the glyceraldehyde production from fructose (R4), which resulted in low yields of glyceraldehyde [11]. In Fig. 3b it can be seen how fructose yield was decreasing as pyruvaldehyde yield increased, corroborating that the conversion of glyceraldehyde to pyruvaldehyde was very fast, providing high pyruvaldehyde yields and low glyceraldehyde yields. Then, once the pyruvaldehyde was produced, it could be converted into lactic acid under favorable conditions. Indeed, this conversion was occurring, since lactic acid yield was inversely proportional to pyruvaldehyde yield.

Focusing on formic acid as target product, in Fig. 3c it can be seen how as formic yield increased, the yield of fructose and glucose decreased. As mentioned above, both formic and acetic acid would be obtained as final products from sugars degradation (R11). However, the whole formic production was not only due to

direct sugars degradation, but also consequence of the degradation of 5-HMF (R8) and furfural (R10). As it can be seen in Fig. S3c, the 5-HMF and furfural yields were inversely proportional to formic acid yield, corroborating that the formic acid was produced from the degradation of those compounds. At the same time, the levulinic acid yield was following same trend as formic acid, meaning that they were produced in parallel and therefore validating reaction R8.

Through a simple kinetics analysis, the reaction pathway for the FOS degradation from inulin hydrolysis in SCW was validated. It was demonstrated that the production of primary products such as glyceraldehyde (R4), 5-HMF (R7) and furfural (R9) was slower compared to the degradation of these compounds. The reactions producing pyruvaldehyde (R5) and formic acid (via R8, R10 and R11) were enhanced compared to the previous ones and therefore they were the main degradation products from inulin hydrolysis in SCW.

3.1.2. FOS hydrolysis in SCW: effect of reaction time

This section is focused on experiments 3, 5, 6, 7 and 8, carried out with 20 % w/w FOS solutions and reaction times between 0.12 and 0.74 s. In Fig. 4, the yields for those experiments were presented. Sugars were the main product obtained at low reaction times, reaching values around 60 % w/w between 0.12 and 0.21 s and then continuously decreasing with reaction time. The opposite trend was found for the retro-aldol condensation (RAC) products, since they increased with reaction time, becoming the major products (42 % w/w) at 0.74 s. Combining these two trends it was clear that at short reaction times, the governing mechanism was the hydrolysis of FOS to sugars and then as reaction proceeded they were converted into RAC products, mainly yielding pyruvaldehyde.

In a previous work, the hydrolysis of pure fructose in SCW was evaluated under different reaction conditions [11]. Operating at 400 ºC, 230 bar and 0.67 s, the major product was pyruvaldehyde (80 % w/w). When comparing those results to the ones obtained from FOS hydrolysis in this work at 385 ºC, 255 bar and 0.74 s, it can be seen that the pyruvaldehyde yield was much lower (23 % w/w). With different starting material (pure fructose is a monomer and the procured inulin (FOS) is a

polymer with a DP = 10) but under similar reaction conditions, the hydrolysis of FOS compared to its constituent monomer occurs to a shorter extent. It was discussed before that the production of glyceraldehyde from fructose (R4) was a limiting step, which restrained the production of pyruvaldehyde as consequence. This limitation was especially important at short reaction times (between 0.12 and 0.21 s), where high fructose yield was obtained compared to the relatively low yield of pyruvaldehyde (38 % fructose vs 18 % w/w pyruvaldehyde). Indeed, working with much higher reaction times (3s) hydrolyzing inulin in same previous work [11], pyruvaldehyde was the main product (30 % w/w) but still some fructose was found in the liquid product. That fact showed that the complete conversion of inulin still requires more severe reaction conditions to obtain higher yields of pyruvaldehyde comparable to those from pure fructose.

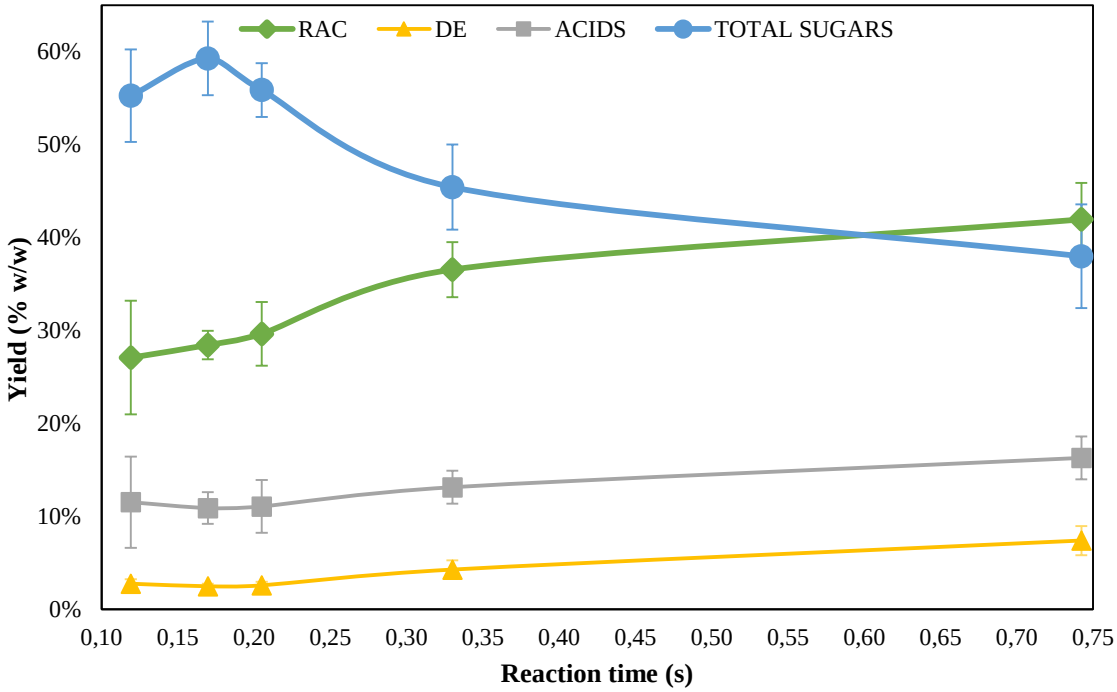

Figure 4. Yield of the different reaction pathways for SCW hydrolysis of FOS in the FASTSUGARS plant at 385 ºC, 250 bar and different reaction times. RAC=retro-aldol condensation; DE=dehydration.

The degradation of fructose into other products was increased with reaction time, increasing the production of acids from 12 % w/w at 0.12 s to 16 % w/w at 0.74 s. On the other hand, the total dehydration yield was always lower than 7 % w/w and

it was slightly increased with reaction time, from 3 % w/w at 0.12 s to 7 % w/w at 0.74 s. With such low values, the production of dehydration products was considered negligible.

All in all, the different reaction mechanisms for the FOS hydrolysis in SCW were studied. It was corroborated that isomerization, dehydration and hydration reactions were highly dependent on the protons availability of the medium as reported before [23], so that working above the critical point of water, the ionic product was drastically reduced and therefore these reaction were disfavored. Moreover, when comparing FOS to fructose hydrolysis in SCW it was found that lower pyruvaldehyde yields were obtained in the case of FOS. Since FOS is an oligomer with a DP=10 and fructose a monomer, higher reaction times or temperatures were needed to achieve similar pyruvaldehyde yields from FOS. At short reaction times, low yields of pyruvaldehyde were obtained due to slow conversion of fructose into glyceraldehyde. However, as reaction time increased from 0.21 to 0.74 s, the reaction severity increased and the sugars yield drastically decreased, increasing the RAC yield.

3.1.3. FOS hydrolysis in SCW: effect of inlet concentration

Experiments carried out with the same reactor (2.27 cm^3) but different inlet concentrations (experiments 1, 2, 3 and 4) were selected to evaluate inlet concentration effect. For these experiments, the FOS concentrations were 5, 10, 20 and 30 % w/w, corresponding to inlet concentrations to the reactor of 1, 2, 5 and 9 % w/w, respectively. The influence of concentration was evaluated for the main reaction pathways found in the previous section, being sugars, RAC pathway and further degradation (referred to the addition of dehydration products and acids). In Fig. 5, the yields of each pathway were presented.

First remarkable difference visible in Fig. 5 was related to the sugars yield which increased with increasing inlet concentration. That fact should not be understood as an increment in sugars production, but a restraint in its further conversion to other products. It was concluded before that the conversion of fructose into further products started from reactions R4, R7 and R9 (see Fig. 2). It was also demonstrated

that those reactions producing glyceraldehyde, 5-HMF and furfural were slower compared to the production of pyruvaldehyde (R5) and/or formic acid (R8 and R10). It can be corroborated from Fig. 5 that those reactions were slowed down, providing lower amounts of derived products (RAC and degradation) when inlet concentration increased. That fact suggested that inlet concentration could act as a mass transfer limitation for the conversion of FOS. Increasing the amount of FOS to be converted, lower conversion rate was obtained due to reduced accessibility for the same amount of SCW in a more concentrated FOS stream. Similar behavior was found for the hydrolysis of cellulose in SCW in a previous work [10], where the increment of cellulose concentration for a constant reaction time provided lower conversion rates.

In fact, the results obtained in this section could be compared to those of cellulose hydrolysis in SCW from that work [10], where cellulose was hydrolyzed at 400 ºC and 250 bar in the FASTSUGARS laboratory scale plant using different inlet concentrations. In that work, two trends regarding sugars production were found depending on inlet concentration. For low cellulose concentrations (up to 10 % w/w), total conversion (X=1) of cellulose was achieved with reaction times as low as 0.12 s. Under those conditions, the dissolution and hydrolysis of cellulose in SCW were produced simultaneously [27]. On the other hand, for highly-concentrated suspensions (20 % w/w) it was found that operating with reaction times below 1 s the hydrolysis was incomplete (X<1). Then, the limiting step was the dissolution of cellulose particles in SCW, which produced a heterogeneous media where the mass transfer resistances limited the reaction rate [10]. In the case of FOS, as inulin is soluble in water at ambient conditions, dissolution is not playing a role in FOS hydrolysis. Then, the hypothesis here was that increasing the amount of FOS to be converted, lower conversion rate was obtained due to reduced accessibility for the same amount of SCW in a more concentrated FOS stream.

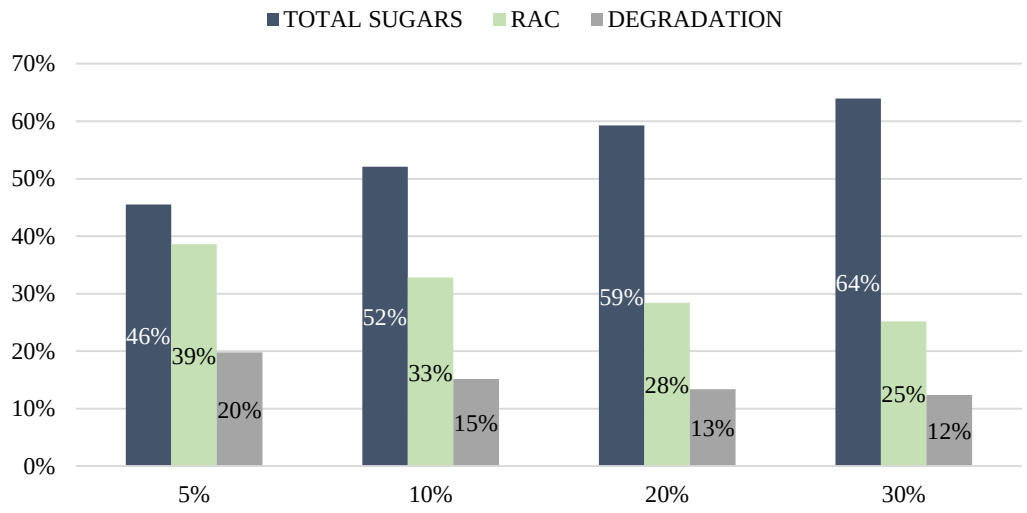

Figure 5. Yield of main compounds obtained from FOS hydrolysis operating at 385 ºC, 250 bar and 0.17 s and different inlet concentrations.

Therefore, inlet concentration may act as a selective factor that will modify the conditions depending on desired products. So that if sugars are the target, higher inlet concentration would provide higher yield of sugars. On the other hand, if RAC products are the target, more sever conditions (time and temperature) should be used, as the conversion rate would be slower and fructose would take more time to be transformed into other products.

3.2.Jerusalem artichoke (JA) hydrolysis in SCW

The hydrolysis of Jerusalem artichoke (JA), which main component is inulin (see composition in Table 4), was carried out to compare the SCW hydrolysis of a model compound to a real biomass. The compositional analysis provided results similar to those obtained by other authors [20], with a total hydrolysable content of 78 % w/w, calculated as the addition of inulin and free sugars.

Table 4. Compositional analysis for Jerusalem artichoke (dry basis).

Ash	Proteins	Insoluble lignin	Free sugars	Inulin	Others	TOTAL HYDROLYSABLE
2 %	8 %	6 %	6 %	72 %	6 %	78 %

Using the same reactor, which volume was 1.36 cm^3, two experiments were carried out, obtaining 12 experimental points that were shown in Table 5, where it can be

seen that the average operating conditions were 375±4 ºC, 253±5 bar. The carbon factor of dried JA was obtained by elemental analysis and it was 0.34 g carbon/g biomass. With that data, it was possible to calculate the carbon inlet to the reactor, as shown in Eq. 7, substituting the carbon factor of inulin (0.42) by the carbon factor of JA (0.34). Once the hydrolysis was carried out, two fractions were obtained for each sample: a liquid fraction which carbon content was measured by TOC analysis and a solid fraction that could be obtained from the filters (exp 1) or directly as suspended solids (exp 2). Then, carbon outlet was calculated as shown in Eq. 9. For experiment 1, just carbon from filters was taken into account and for experiment 2 just suspended solids were considered (being its carbon factor *'CFsusp'* equal to 0.43 g carbon/g suspended solids). The average carbon balance obtained for JA by dividing the carbon outlet to the carbon inlet was 97 % ± 5 %. Results from carbon balance were collected in Table 5.

$$carbon\,out = carbon\,liq + carbon\,filters + carbon\,susp = TOC + carbon\ filters + \%susp \cdot 10000 \cdot CFsusp \quad (9)$$

Table 5. Experimental and carbon balance data from Jerusalem artichoke experiments in the FASTSUGARS pilot plant.

		T (ºC)	P (bar)	tr (s)	Cin (%)	CARBON IN (ppmC)	Carbon liquid=TOC (ppmC)	Carbon solids (ppmC)	CARBON OUT (ppmC)
EXP 1	**JA-01**	374	252	0.12	0.66	2253	1795	460 (from filters)	2255
	JA-02	347	251	0.13	0.73	2467	2007		2467
	JA-03	367	251	0.13	0.74	2526	2066		2526
	JA-04	372	252	0.12	0.81	2740	1919		2379
	JA-05	384	263	0.10	0.74	2518	1920		2380
	JA-06	373	249	0.12	0.79	2676	2216		2676
EXP 2	**JA-07**	379	249	0.11	0.85	2903	2433	467 (from suspended solids)	2903
	JA-08	374	252	0.12	0.86	2911	2442		2911
	JA-09	378	243	0.10	0.97	3292	2545		3059
	JA-10	376	251	0.12	0.93	3158	2645		3158
	JA-11	369	255	0.13	0.85	2890	2372		2653
	JA-12	375	262	0.13	0.88	3004	2447		3004
	AV.	**375±4**	**253±5**	**0.12±0.01**	**0.82±0.09**	**2778±305**	**2234±283**	**466±89**	**2700±303**

Once the carbon balance was closed, it is worth mentioning that the treatment of the liquid sample for JA was different compared to the inulin liquid samples. After each inulin experiment, the samples were just filtered and analyzed by HPLC, obtaining in that way the concentrations of each compound that were then grouped

in four reaction mechanisms (see Section 3.1.1). However, as JA is not a polymer but a complex biomass, the HPLC analysis was done in two steps. Firstly, the sample (as it was obtained after SCW hydrolysis) was analyzed by HPLC, obtaining the amount of '*monomeric glucose'* (MG) and '*monomeric fructose'* (MF) together with the degradation products concentration. Then, that same sample was hydrolyzed with acid, neutralized and then analyzed by HPLC. After acid hydrolysis, the oligomers were totally broken into monomers, obtaining in that way *'total glucose'* (TG) and *'total fructose'* (TF) concentrations, which addition provided *'total sugars'* content for JA. So that, by subtracting the monomeric sugars that were obtained as consequence of SCW hydrolysis (meaning MG and MF) to the '*total sugars'* obtained after acid hydrolysis, the amount of fructooligosaccharides (FOS) was obtained. The concentrations obtained from HPLC analysis were translated into carbon units and shown in Table 6. Once the concentrations of each compound were obtained in Table 6, yields should be calculated by referring those concentrations to the inulin entering the reactor. To do so, Eq. 10 was used, where the carbon inlet shown in Table 5 was multiplied by the amount of inulin of the raw material. As shown in Table 4, 78 % of the raw JA was inulin, so that the *'carbon in'* would be multiplied by 0.78 to obtain the calculation basis for the yields calculations, being the average inulin inlet concentration 2167 ppmC. The yield calculated for each reaction pathway was also shown in Table 7.

$$YIELD\,(\%) = \frac{HPLC\ concentration\,(ppmC)}{CARBON\ IN\,(ppmC) \cdot 0.78} \qquad (10)$$

Table 6. Carbon concentrations (in ppmC) for each individual component detected by HPLC for Jerusalem artichoke hydrolysis in SCW in the FASTSUGARS pilot plant.

	Acid hydrolysis			Untreated sample			TOTAL – MONOMERIC
	Total Glucose (TG)	Total Fructose (TF)	TOTAL SUGARS	Monomeric glucose (MG)	Monomeric fructose (MF)	MONOMERIC SUGARS	FOS
JA-01	381	852	1233	80	707	788	445
JA-02	384	1140	1523	130	850	980	544
JA-03	448	1038	1485	91	860	951	535
JA-04	351	1054	1405	72	823	895	510
JA-05	358	958	1316	98	732	830	486
JA-06	380	1128	1508	137	900	1037	471
JA-07	437	1066	1503	28	936	964	539
JA-08	346	1142	1488	98	932	1029	458
JA-09	345	1193	1538	135	937	1072	466
JA-10	432	1088	1520	131	946	1077	443
JA-11	379	1066	1444	104	909	1014	431
JA-12	389	1155	1545	93	941	1033	511
AV.	**386±36**	**1073±94**	**1459±96**	**100 ± 31**	**873 ± 82**	**972 ± 93**	**487 ± 40**

	Untreated sample						
	Pyruvald.	Lactic acid	Formic acid	Acetic acid	Levulinic acid	5-HMF	Furfural
JA-01	73	141	61	137	60	2	2
JA-02	36	113	109	114	51	2	2
JA-03	70	176	41	144	55	2	1
JA-04	75	149	74	124	63	0	0
JA-05	87	161	57	116	61	3	3
JA-06	76	201	59	167	98	5	2
JA-07	126	176	66	83	55	2	1
JA-08	78	120	136	76	62	2	2
JA-09	91	163	103	119	76	5	2
JA-10	121	186	85	90	66	3	2
JA-11	95	187	39	87	49	2	1
JA-12	109	191	43	85	76	5	1
AV.	**86±25**	**164±28**	**73±30**	**112±28**	**64±14**	**3±2**	**1±1**

Table 7. Yields grouped by reaction mechanism as shown in Fig. 2 for Jerusalem artichoke hydrolysis in the FASTSUGARS pilot plant..

CALCULATION BASIS (ppmC)	**2167 ± 238**
Monomeric sugars	45%
FOS	23%
SUGARS YIELD	**68 %**
Glyceraldehyde	2 %
Pyruvaldehyde	6 %
Lactic acid	4 %
RETRO-ALDOL YIELD	**11 %**
Formic acid	4 %
Acetic acid	3 %
ACIDS YIELD	**7 %**
5-HMF	0 %
Furfural	0 %
Levulinic acid	3 %
DEHYDRATION YIELD	**3 %**
DEGRADATION YIELD (acids+dehydration)	**10 %**

3.2.1. Jerusalem artichoke (JA) vs FOS hydrolysis in SCW

For FOS hydrolysis, different inlet concentrations were tested under same reaction time and presented in Fig. 6, together with the ones obtained from JA. In terms of inlet concentration, the results from JA should be comparable to those of FOS 5 %, since for JA the inulin concentration entering the reactor was 2167 ppmC and for FOS 5% it was 2914 ppmC. However, higher sugars yield and lower RAC products yield were obtained for JA compared to FOS 5%. In fact, the results of JA were more similar to those of FOS 30 % even though the inlet concentrations were quite different. In Section 3.1.2 it was concluded that starting from a polymer instead from a monomer, slowed down the hydrolysis reaction due to the addition of a depolymerization step. In this case, JA has an average DP of about 27 – 29 [8], which is almost 3 times higher than the DP from FOS. With much longer polymeric chains, the fructose conversion would be slowed down for JA compared to FOS, as it happened for FOS compared to fructose. As a consequence, the amount of unconverted sugars in JA was higher compared to FOS 5% and at the same time, the yield of degradation products was lower. In Section 3.1.3. it was also concluded that the inlet concentration of FOS acted as a mass transfer resistance, restraining fructose conversion into further products. Therefore, the hydrolysis of JA at low

concentration was similar to that of FOS at high concentration since in both cases the conversion of inulin was restrained by mass transfer limitations.

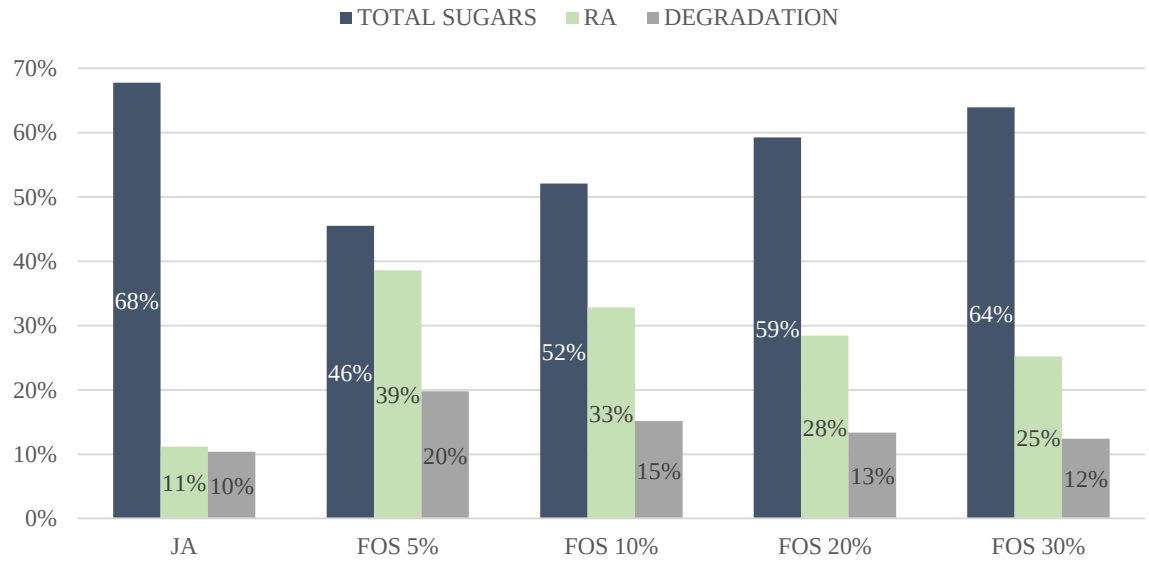

Figure 6. Yield of main compounds obtained from FOS hydrolysis (operating at 385 ºC, 250 bar and 0.17 s.) compared to yields obtained from JA hydrolysis operating at 375 ºC, 250 bar and 0.12 s.

Fig. 7 showed the MW profiles for pure fructose, FOS and the products obtained after FOS and JA hydrolysis in SCW. It can be seen that the product from FOS hydrolysis in SCW (experiment 3) showed almost same profile as fructose, meaning that fructose was the major product. That was something expected, as starting from FOS with a DP=10, high monomeric sugars yield was obtained from the very beginning (35 % w/w fructose at 0.12 s). On the other hand, the product from JA hydrolysis in SCW had an average MW of 1266 Da, which corresponded to an average DP of around 8. It can be seen in Fig. 7 that the JA profile was closer to FOS than fructose. So that, lower conversion (understood as DP reduction) was obtained in the case of JA because initial DP was higher and first set of reactions was mainly the production of lower DP oligomers.

For JA, it was found that both RAC and degradation products took similar values (11 % RAC vs 10 % for degradation). This suggests, either that the RAC was not the preferred pathway in the case of JA or that the free monomers or others fraction are converted into degradation products.

Degradation yield accounts for furfural, 5-HMF and levulinic acid and also formic and acetic acids. In Table 7 it can be seen that the yield of levulinic acid from JA hydrolysis was 3 %, meanwhile the yield of 5-HMF was zero. This would suggest that all the 5-HMF produced from the inulin fraction of JA was rapidly converted to levulinic acid or; levulinic acid is produced from the others fraction in a different reaction pathway. Moreover, acetic acid was produced at a similar rate to formic acid, which was not observed for pure inulin, which supports a different degradation route. That new route would be related to the free sugars in JA. The free sugars are monomeric sugars, which are more easily converted into acids and furfurals than inulin (which requires pre hydrolysis steps to produce monomers) and therefore they were completely degraded at 0.12 s, increasing the amount of degradation products in JA effluent as consequence.

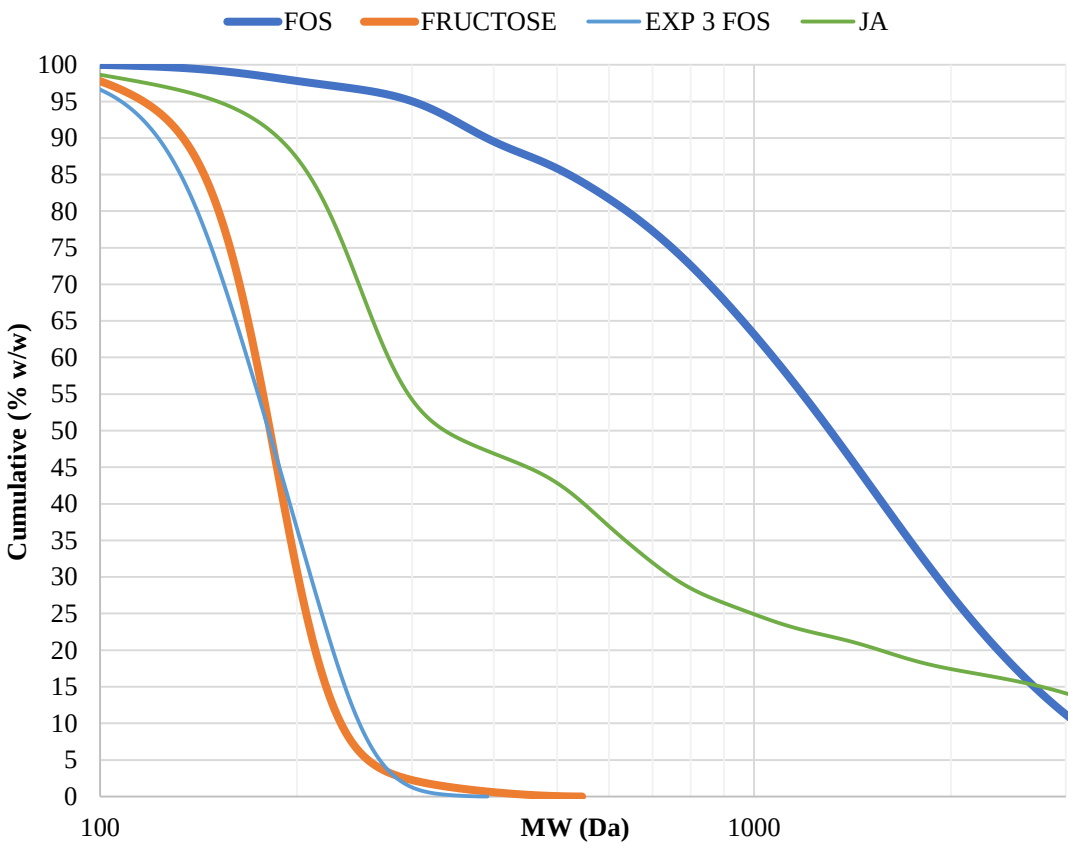

Figure 7. Molecular weight (MW) profile for commercial inulin, pure fructose and reaction products from inulin and Jerusalem artichoke (JA).

3.2.2. Jerusalem artichoke (JA) vs lignocellulosic biomass hydrolysis in SCW

The performance of JA hydrolysis in SCW was analyzed in terms of its resemblance to FOS in the previous section. In the current section, the authors conducted a comparison with other biomass. The compositional analysis of the remaining solid obtained after hydrolysis was presented in Table 8. Several parameters were calculated according to the calculations done in previous works where the hydrolysis of different biomasses was studied [19].

Table 8. Compositional analysis of the remaining solid obtained after SCW hydrolysis of Jerusalem artichoke in the FASTSUGARS pilot plant. Hydrolysis parameters were calculated according to equations 11 to 13, from the calculations shown below.

Sugars	AIF	Others	Ash	Sugars in solid	**TOTAL SUGARS YIELD**	**BIOMASS CONVERSION**	**SELECTIVITY**	**DEGRAD YIELD**
59 %	27 %	13 %	1 %	276 ppmC	**67 %**	**87 %**	**77 %**	**22 %**

The parameter *'sugars in solid'* was calculated by multiplying the average carbon in the solids (466 ppmC in Table 5) by the amount of trapped sugars in the remaining solid (59 %). The *'total sugars yield'* was referred to the amount of total sugars (glucose + fructose after acid hydrolysis, see average value in Table 6) and it was calculated as shown in Eq. 11, by dividing to *'calculation basis'*, which corresponded to the inulin entering the reactor, shown in Table 7 (2167 ppmC).

$$TOTAL\ SUGARS\ YIELD\ (\%) = \frac{TOTAL\ SUGARS\ (ppmC)}{CALCULATION\ BASIS\ (ppmC)} \qquad (11)$$

The *'biomass conversion'* was calculated as shown in Eq. 12 and it should be understood as the amount of biomass that was converted to soluble products. Then, selectivity was calculated by dividing the *'total sugars yield'* to the *'biomass conversion'*.

$$BIOMASS\ CONVERSION\ (\%) = \frac{CALCULATION\ BASIS\ (ppmC) - SUGARS\ IN\ SOLIDS\ (ppmC)}{CALCULATION\ BASIS\ (ppmC)} \qquad (12)$$

Finally, the *'degradation yield'* was calculated as shown in Eq. 13. It was the sum of degradation products in the liquid effluent, meaning those apart from sugars (pyruvaldehyde, acetic, formic, lactic and levulinic acids, 5-HMF and furfural) that were analyzed by HPLC (see Table 6).

$$DEGRAD\,YIELD\,(\%) = \frac{\sum DEGRADATION\ PRODS\,(ppmC)}{CALCULATION\ BASIS\,(ppmC)} \qquad (13)$$

The results from JA were compared to the optimal results for sugar beet pulp (SBP) and wheat bran (WB) obtained in previous works [21] and they were presented in Fig. 8. In previous works, when comparing the performance of each biomass and experimental set up, it was proved that having a bigger particle size, the hydrolysis reaction was carried to a shorter extent and therefore it could be said that it was acting as a mass transfer limitation. For SBP and WB, the particle size was selected according to the pumping difficulties of each biomass. However, in the case of JA, which was provided as wet frozen matter instead of dried solids, that pumping limitation was much lower due to the stability and homogeneity of the prepared suspension compared to those from SBP and WB. Another difference between biomasses would be their composition, since both SBP and WB were lignocellulosic biomass, mainly composed of cellulose, hemicellulose and lignin. On the other hand, JA was mostly composed of inulin.

Looking at Fig. 8, it could be seen that even using the same pilot plant, different results were obtained for each biomass. Starting from an inulin-based biomass instead of a lignocellulosic biomass, seemed to facilitate biomass conversion due to the solubility of its constituent polymer. The degradation yield's behavior would be also supporting this theory, since the yield of degradation products for SBP and WB was remarkably lower compared to JA. As it was already discussed in previous works, the biomass conversion was related to the severity of the reaction, so that having higher conversion would mean that the hydrolysis reaction was more severe and therefore, higher degradation was produced, reducing selectivity towards sugars. All in all, as particle size was not a limitation for the hydrolysis of JA, a

higher conversion was obtained compared to lignocellulosic biomass. As a consequence of that enhanced hydrolysis, the produced sugars were more rapidly degraded, increasing the degradation yield.

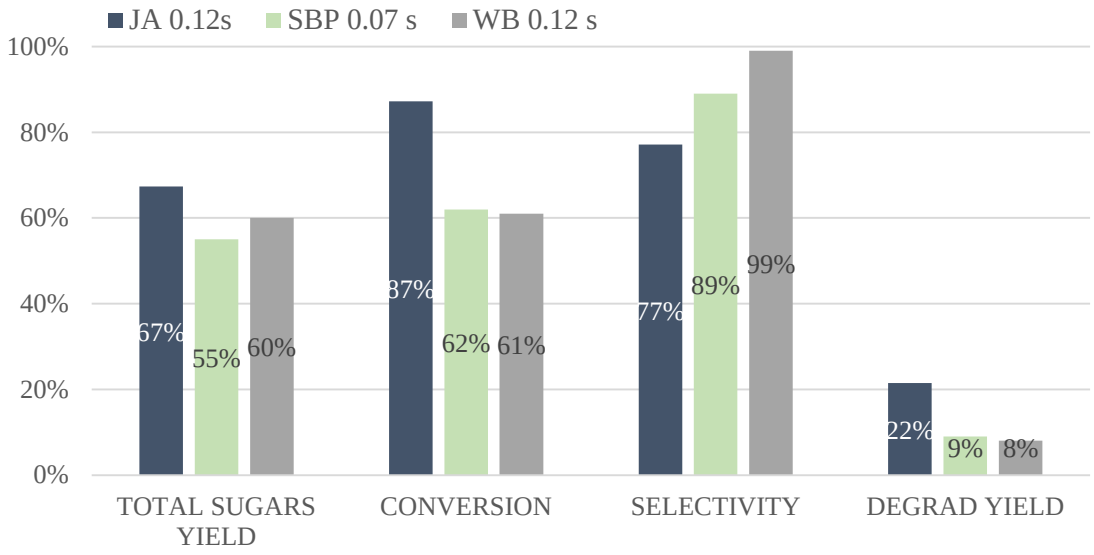

Figure 8. Sugars yield, conversion, selectivity and degradation yield for Jerusalem artichoke (JA), sugar beet pulp (SBP) and wheat bran (WB) at the FASTSUGARS pilot plant.

4. Conclusions

In this work the hydrolysis of commercial inulin with a polymerization degree comparable to fructooligosaccharides (DP=10) was hydrolyzed in SCW to evaluate the reaction mechanisms. It was observed that the conversion of fructose to glyceraldehyde, 5-HMF and furfural was slower than the subsequent production of pyruvaldehyde and formic acid. It was also found that reaction time affects selectivity and it was demonstrated that increasing the inlet concentration, the conversion of inulin was reduced.

Jerusalem artichoke (JA) was selected as an inulin-based biomass for the production of sugars via SCW hydrolysis. It was observed that the hydrolysis of JA was similar to that of FOS at high concentration, producing up to 68 % w/w of sugars. The results from JA were also compared to those from lignocellulosic biomass (specifically sugar beet pulp and wheat bran). For JA, the main constituent was inulin, which was much more easily converted than cellulose in SCW and therefore higher degradation yield was produced in the case of JA. Anyway, the sugars

selectivity of JA hydrolysis reached 77 % w/w, demonstrating the efficiency of the FASTSUGARS process to selectively produce highly valuable compounds from biomass.

Acknowledgments

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Conclusions

Demonstration of a selective process to transform biomass into sugars by ultrafast hydrolysis in supercritical water

In this PhD thesis, the hydrolysis of different biomass-related substrates (cellulose, sugar beet pulp, wheat bran, inulin and Jerusalem artichoke) in supercritical water was thoroughly studied and developed, using ultrafast reactors and operating with reaction times below 1 second. The investigation was carried out firstly in a laboratory scale plant and then, with the design, construction and operation of a pilot plant scale. The new continuous pilot plant was designed to operate at reactor temperatures up to 400 ºC, pressures up to 30 MPa and with total flows up to 35 kg/h. In this work, it was concluded that it is possible to selectively produce sugars from biomass by supercritical water hydrolysis in a pilot plant scale, facing new challenges but demonstrating at the same time the versatility and potential of the FASTSUGARS process as a key step towards functional biorefineries. The overall conclusions of this PhD thesis are presented below.

So far, existing models describing the conversion rate of cellulose in near critical water assumed that the hydrolysis of cellulose particles takes place at their surface. Therefore, particle size was considered the only key parameter for the conversion rate. Nevertheless, it was proved that biomass reactor concentration also affects conversion rate of cellulose in supercritical water. Indeed, cellulose in water at 4 % w/w was found to be the boundary concentration between homogeneous reaction media and heterogeneous media for cellulose hydrolysis in supercritical water. Increasing the inlet concentration up to 4 % w/w at reactor inlet, the cellulose behaved as if it was hydrolyzed at subcritical conditions, being partially dissolved (heterogeneous media) and therefore reducing the conversion rate.

The FASTSUGARS process allowed the valorization of sugar beet pulp, producing a liquid effluent rich in sugars suitable for further conversion into marketable products such as sorbitol and ethylene glycol. Maximum sugars yield (66 % w/w) was obtained operating at 390 ºC, 25 MPa and 0.11 s. Moreover, the solid fraction obtained after hydrolysis was analyzed by acid hydrolysis, TGA and FTIR analysis to prove its lignin-like nature. The solid obtained after reaction was mainly composed of an acid-insoluble fraction (up to 88 % w/w), which was a mixture of insoluble lignin and char. That solid showed a resistance to thermal degradation comparable to that from pure kraft lignin.

Then, the FASTSUGARS process was scaled up from laboratory to pilot plant scale in order to overcome technical difficulties found in the laboratory setup. The new pilot plant was able to operate with up to 35 kg/h, 400 ºC and 30 MPa with reaction times below 1 second. Maximum total flow was increased from 8 kg/h in the laboratory scale to 30 kg/h in the pilot plant and the allowed particle size for biomass suspensions was increased up to 300 μm. Additionally, two filters were installed to improve solids collection. Thanks to that improvements and in order to bring the FASTSUGARS process closer to industrial applications, a bigger particle size was used in the pilot plant (250 μm) compared to the laboratory scale plant (≤ 150 μm). It was found that the particle size acted as a mass transfer resistance, slowing down the hydrolysis of biomass, which resulted to be positive, since selectivity was increased and at the same time, the degradation was remarkably reduced.

Finally, inulin and Jerusalem artichoke were hydrolyzed for the first time in supercritical water to obtain fructose and pyruvaldehyde. Commercial inulin was selected as a model for fructooligosaccharides (FOS), so that its degradation pathway in supercritical water was defined. It was observed that the conversion of fructose to glyceraldehyde, 5-HMF and furfural was slower than the subsequent production of pyruvaldehyde and formic acid. On the other hand, the hydrolysis of Jerusalem artichoke was similar to that of FOS at high concentration, yielding up to 68 % w/w of fructose-derived sugars. When comparing Jerusalem artichoke results to those from lignocellulosic biomass in Chapter 3, it was found that higher conversion was achieved in the case of Jerusalem artichoke due to its composition, being inulin much more easily converted than cellulose.

Future work

Considering the complexity and diversity of biomass compositions, each raw material represents a technological challenge that should be studied separately. In that sense, it would be interesting to compare the behavior of biomasses with different lignin contents. In that way, lignocellulosic feeds with different cellulose/lignin ratios would produce solid products with different compositions

that could be used as composites. Then, mechanical and structural properties should be deeply studied to look for specific applications.

Regarding the operation at the pilot plant, some changes can be implemented to improve its performance. To facilitate the feeding of highly concentrated suspensions, a pressurized and stirred tank should be installed. Installing a flowmeter in the water stream would improve the precision of flow measurements. Improvements on the heating system are needed to allow increasing the capacity of the plant. So far, the automatic valve was used just for solid-free effluents, but if different materials are implemented, it should be also used for biomass effluents.

Finally, separation and downstream processes are required to improve the quality and applicability of the products obtained in the current work. Isolation and/or purification of target products such as sugars could be done by membranes and ultrafiltration technologies. On the other hand, pretreatments should be also taken into account in order to improve the performance of the supercritical water hydrolysis step.

Resumen

Demostración de un proceso selectivo para la transformación de biomasa en azúcares mediante hidrólisis ultrarrápida en agua supercrítica

El pilar fundamental de la industria química es la conversión de materias primas en combustibles, productos químicos, materiales y energía. Tradicionalmente, el sector químico se ha basado en la explotación de recursos no renovables, como el petróleo, para la fabricación de un amplio abanico de productos. Sin embargo, en los últimos años el agotamiento de estos recursos fósiles, la dependencia energética exterior unida a la inestabilidad de precios y el cambio climático hacen que Europa se replantee radicalmente las bases de la industria química tradicional. Surge así la necesidad de explotar nuevas materias primas renovables a través de procesos más sostenibles en las llamadas biorefinerías. El término biorefinería se refiere a la conversión de biomasa en productos comercializables a través de la integración de tecnologías y procesos respetuosos con el medio ambiente. A pesar de los avances tecnológicos de los últimos años, las biorefinerías aún tienen que mejorar su eficiencia para poder sacar partido de cada una de las complejas fracciones de la biomasa. Por ejemplo, los procesos hidrotermales se presentan como una alternativa muy prometedora, ya que simplemente usando agua se puede fraccionar y convertir biomasa en productos de interés. Sin embargo, esta tecnología todavía tiene que desarrollarse para conseguir reducir costes produciendo a la vez altos rendimientos y selectividades en los productos deseados.

Objetivos

Para superar estas limitaciones, el objetivo de esta Tesis doctoral (dentro del marco del proyecto nacional FASTSUGARS) es desarrollar una tecnología selectiva para transformar biomasa agrícola en azúcares a través de la hidrólisis ultrarrápida en agua supercrítica, escalando el proceso desde escala laboratorio a escala planta piloto. Para alcanzar este objetivo, se proponen los siguientes objetivos concretos:

- Estudio del efecto de la concentración de entrada de biomasa sobre los rendimientos y cinética de la hidrólisis ultrarrápida de celulosa en agua supercrítica en la planta laboratorio.
- Estudio de la hidrólisis ultrarrápida en agua supercrítica de una biomasa local en la planta laboratorio: Valorización de pulpa de remolacha.
- Escalado del proceso FASTSUGARS desde escala laboratorio a escala planta piloto para la hidrólisis de biomasa agrícola en agua supercrítica:

Diseño, construcción y operación de una planta piloto para hidrolizar biomasa lignocelulósica en agua supercrítica, siendo capaz de operar con hasta 35 kg/h a 400 ºC, 30 MPa y tiempos de reacción menores a 1 segundo.

- Estudio de la hidrólisis en agua supercrítica de inulina y una biomasa rica en inulina (pataca) en la planta piloto para producir fructosa y piruvaldehído: Estudio del efecto del tiempo de reacción y concentración de biomasa a la entrada del reactor sobre la hidrólisis de inulina. Determinación del mecanismo de reacción para la hidrólisis de inulina en agua supercrítica.

Para cumplir con los objetivos marcados en esta Tesis doctoral, el presente trabajo se ha estructurado en cuatro capítulos. En cada uno de los capítulos se han presentado los objetivos específicos así como una revisión bibliográfica sobre el tema en cuestión.

Para llevar a cabo el trabajo experimental vinculado a esta Tesis doctoral, se han empleado tanto la planta escala laboratorio como la planta piloto. La planta escala laboratorio, desarrollada en la Tesis del Dr. Danilo Cantero, permite operar el reactor ultrarrápido con temperaturas de hasta 400 ºC y presiones de hasta 30 MPa. Los tiempos de reacción se pueden variar entre 0.004 y 5 segundos, con un flujo total de hasta 8 kg/h (máximo 3 kg/h de biomasa). Usando esta instalación, se han desarrollado los capítulos 1 y 2. Como principal objetivo de esta Tesis, se ha llevado a cabo el escalado de esta planta para pasar así a escala planta piloto. La nueva planta piloto permite operar el reactor ultrarrápido con temperaturas de hasta 400 ºC y presiones de hasta 30 MPa y tiempos de reacción desde 0.07 s. La planta piloto se ha diseñado para operar con hasta 35 kg/h de flujo total y hasta 10 kg/h de biomasa. Los capítulos 3 y 4 se han llevado a cabo con esta nueva instalación. Los principales logros alcanzados en cada capítulo se detallan a continuación.

En el Capítulo 1, *"Hydrolysis of cellulose in supercritical water: reagent concentration as a selectivity factor"*, se analiza el efecto de la concentración de entrada sobre la hidrólisis de celulosa en agua supercrítica a escala laboratorio. Para ello, se combinó la utilización de un medio de reacción selectivo (400 ºC, 25 MPa) con diferentes concentraciones de entrada entre 5 y 20 % w/w (correspondientes a concentraciones entre 1.5 – 6 % w/w a la entrada del reactor), operando con tiempos

de reacción entre 0.07 y 1.57 segundos. El efecto del tiempo de reacción se evaluó con respecto a los rendimientos de los principales productos de hidrólisis (azúcares y glicolaldehído). Se observó que es necesario aumentar el tiempo de reacción para conseguir conversión total de celulosa altamente concentrada. Sin embargo, el aumento del tiempo de reacción conlleva la degradación de glucosa y por tanto la disminución del rendimiento de azúcares. Así, se demostró que la hidrólisis selectiva de celulosa en agua supercrítica para obtener altos rendimientos de azúcares se debe llevar a cabo con tiempos de reacción extremadamente cortos y bajas concentraciones de biomasa. Específicamente, operando a 0.07 s con una concentración de entrada de 1.5 % w/w fue posible obtener 79 % w/w de azúcares como producto final. Por otra parte, cuando el producto deseado es glicolaldehído, se deben seleccionar tiempos de reacción mayores y concentraciones de entrada altas. De hecho, el óptimo para la producción de glicolaldehído (42 % w/w) se obtuvo operando con un tiempo de reacción de 1.57 s y 6 % w/w de celulosa a la entrada del reactor. Además se estudió el efecto de la concentración de entrada sobre la cinética de hidrólisis de celulosa en agua supercrítica. Se pudo así demostrar que aumentando la concentración de entrada de celulosa al reactor hasta 4 % w/w, la solubilidad de celulosa en agua supercrítica disminuye y por tanto la reacción ocurre en un medio heterogéneo. En estas condiciones, las limitaciones a la transferencia de materia cobran importancia y como consecuencia la conversión de celulosa en agua supercrítica disminuye.

En la Figura 1 se puede ver la cinética de celulosa en agua supercrítica ajustada a una cinética de primer orden. Para los puntos experimentales a 1.5, 4.5 y 6 % w/w se puede ver como la pendiente (constante cinética) va disminuyendo conforme aumenta la concentración de entrada. Además, 4 % w/w es la concentración límite a partir de la cual el medio de reacción se asemeja más a agua subcrítica que supercrítica, ya que la celulosa no se disolvió completamente.

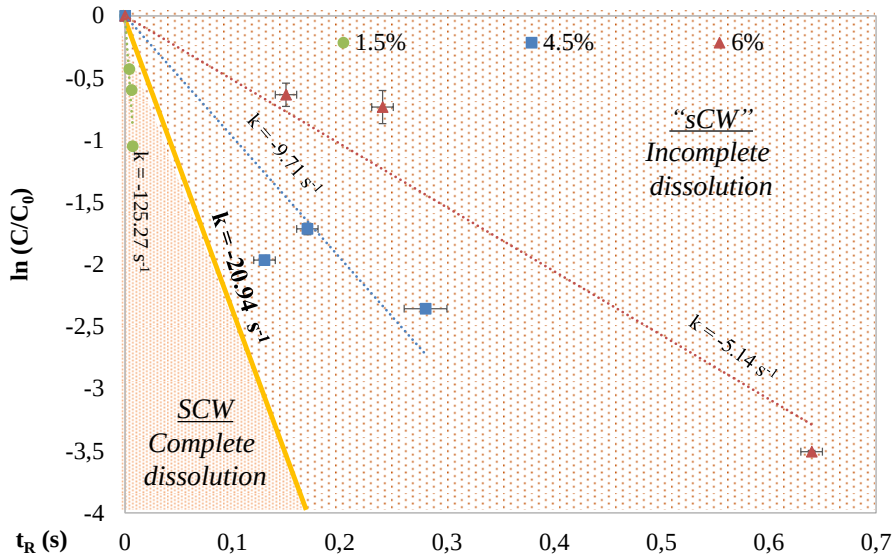

Figura 1. Análisis cinético para 5, 15 y 20 % w/w de celulosa, correspondiendo a 1.5, 4.5 y 6 % w/w a la entrada del reactor.

En el Capítulo 2, "*Production of saccharides from sugar beet pulp by ultrafast hydrolysis in supercritical water*", se estudia por primera vez la hidrólisis de pulpa de remolacha en agua supercrítica para la obtención de azúcares a escala laboratorio. Para ello, se operó a 390 ºC, 25 MPa y tiempos de reacción entre 0.11 y 1.15 segundos. La pulpa de remolacha es un subproducto de la industria azucarera, que debido a su alta disponibilidad en Castilla y León y su bajo valor comercial, representa una oportunidad para su valorización a través del proceso FASTSUGARS. La efectividad del proceso se evaluó en función de los rendimientos de azúcares C – 6 (producidos por la hidrólisis de celulosa) y azúcares C – 5 (producidos a partir de hemicelulosa). Como primer paso para la valorización de esta biomasa, se busca producir un efluente líquido rico en azúcares provenientes tanto de celulosa como hemicelulosa. Operando a 0.11 s, se consiguió recuperar un 61 % de la celulosa como azúcares C – 6 y se recuperó el 71 % de la hemicelulosa como azúcares C – 5. Por otra parte, el efluente producido a mayor tiempo de reacción (~0.2 s), que además de azúcares contenía altas concentraciones de glicolaldehído, fue empleado en otro proceso de hidrogenación para obtener sorbitol y etilenglicol. En cuanto al producto sólido obtenido tras hidrólisis, compuesto mayormente por una fracción insoluble en ácido (AIF), se analizó a

través de TGA y FTIR. Gracias a estos análisis se confirmó que esta AIF es similar a la lignina insoluble, con una pequeña fracción añadida de "char" que se forma durante el proceso de hidrólisis. Se estudiaron además las propiedades térmicas de este sólido y resultó ser comparable a la lignina obtenida por el proceso Kraft, con una resistencia térmica similar. Debido a estas propiedades, puede considerarse su uso como aditivo para composites.

Para este trabajo se introdujo una modificación en la planta escala laboratorio que se muestra en la Figura 2, donde se puede ver que se instalaron simultáneamente dos reactores, uno pequeño que permitía operar con tiempos de reacción por debajo de 1 segundo y otro reactor significativamente mayor que se empleó para tiempos de reacción mayores a 1 segundo. Así, en un mismo experimento se pudo operar primero con uno de los reactores y luego con el otro, estudiando la hidrólisis de pulpa de remolacha en agua supercrítica con tiempos de reacción muy diferentes.

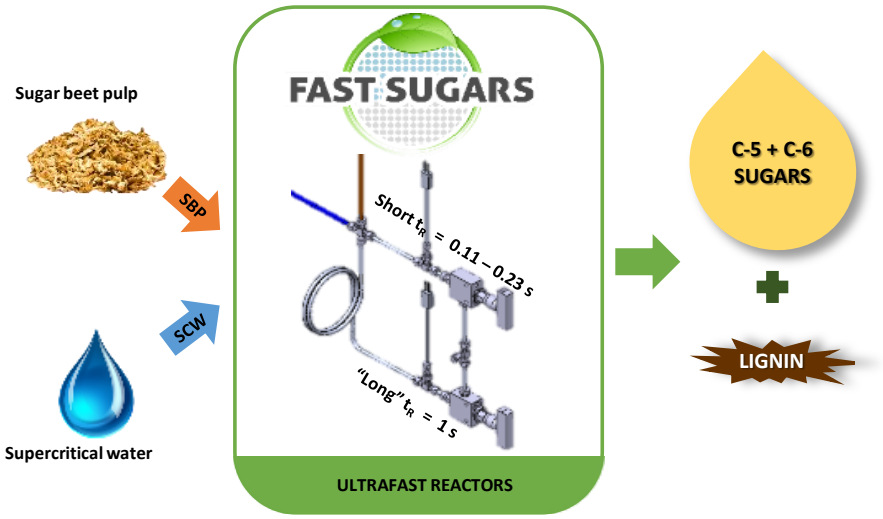

Figura 2. Representación esquemática del proceso de hidrólisis ultrarrápida de pulpa de remolacha en agua supercrítica.

En el Capítulo 3, *"Scaling up the production of sugars from agricultural biomass by ultrafast hydrolysis in supercritical water"*, se lleva a cabo el escalado del proceso FASTSUGARS, diseñando, construyendo y operando una nueva planta piloto. Esta nueva planta piloto, capaz de operar a 400 ºC, 30 MPa y tiempos de reacción desde 0.05 segundos ofrece una serie de ventajas con respecto a la escala

laboratorio, destacando: (a) el flujo total aumenta de 8 kg/h hasta 35 kg/h; (b) el aumento de capacidad conlleva la superación de ciertas limitaciones en el bombeo de biomasa, permitiendo que se pueda aumentar el tamaño de partícula bombeado, pasando de 125 µm en la planta laboratorio hasta un máximo de 300 µm en el nuevo diseño; (c) se instalan dos filtros de alta temperatura para facilitar la recolección de sólidos tras reacción y (d) se divide el calentamiento en tres etapas con tres calentadores eléctricos en los que se reparte el salto entálpico, por lo que se reducen los problemas de sobrecalentamiento existentes en la planta laboratorio. Para evaluar el desempeño de la nueva planta, se hidrolizaron dos biomasas que habían sido previamente hidrolizadas en la planta laboratorio, concretamente pulpa de remolacha y salvado de trigo. En general, el comportamiento de hidrólisis de ambas biomasas fue similar, tal que al aumentar el tiempo de reacción, la selectividad disminuyó y a la vez aumentó la conversión y degradación. Por otro lado, con el objetivo de aproximar el proceso FASTSUGARS a la realidad industrial, se aumentó el tamaño de partícula con respecto a la planta laboratorio, operando un tamaño de 250 µm. Se pudo demostrar así que el tamaño de partícula actúa como una limitación a la transferencia de materia, de tal manera que con la intrincada matriz de cualquier biomasa, el hecho de tener un tamaño de partícula mayor implica menor área superficial por unidad de volumen, reduciendo la accesibilidad a las fracciones hidrolizables (celulosa y hemicelulosa) y por tanto ralentizando la conversión. Este hecho, resultó ser positivo, porque al llevarse a cabo la hidrólisis de biomasa en menor extensión, se produjo menor degradación de los azúcares (≤15 % w/w) y a la vez la selectividad aumentó hasta el 90 % w/w.

En la Figura 3 se presenta de forma esquemática el escalado del proceso, como un paso más hacia la industrialización del proceso FASTSUGARS, en el que el aumento del tamaño de partícula tiene efectos positivos sobre el producto final.

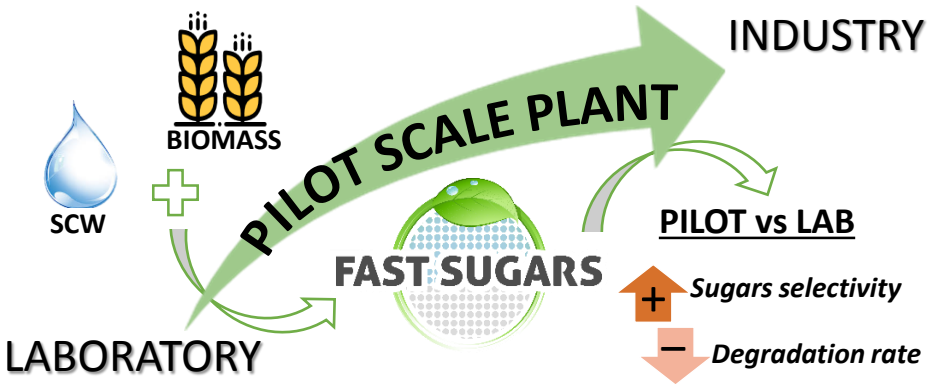

Figura 3. Representación esquemática del escalado del proceso FASTSUGARS, presentando las ventajas con respecto a la planta escala laboratorio.

En el Capítulo 4, *"Ultrafast hydrolysis of inulin in supercritical water: Fructooligosaccharides reaction pathway and Jerusalem artichoke valorization"*, se lleva a cabo la hidrólisis de inulina comercial y pataca en agua supercrítica, usando la planta piloto para ello. La inulina seleccionada tiene un grado de polimerización ~10, que se asemeja a los llamados fructooligosacáridos (FOS), por lo que se selecciona como modelo para estudiar la degradación de estos interesantes polímeros en agua supercrítica. La hidrólisis se llevó a cabo en la planta piloto operando a 385 ºC, 25 MPa y tiempos de reacción entre 0.12 y 0.74 segundos. En primer lugar, se intenta dilucidar el camino de reacción de los FOS en agua supercrítica. Así, se observó que la conversión de fructosa a gliceraldehído, 5 – HMF y furfural es lenta comparada con la producción de piruvaldehído y ácido fórmico. Como ya se comprobó con la celulosa, el tiempo de reacción afecta a la selectividad, promoviendo distintos productos según el tiempo de reacción seleccionado, de tal manera que cuando el tiempo de reacción aumenta, disminuye la cantidad de azúcares debido a su degradación en otros productos (privualdehído y ácido fórmico en el caso de inulina). Por otro lado, variando la concentración de entrada se observó que su aumento repercute en la conversión de inulina, que se ve reducida. De nuevo, esta reducción de la conversión resultó ser positiva pues se disminuyó la degradación de los azúcares. En la Figura 4 se puede ver el camino de reacción obtenido para la hidrólisis de FOS en agua supercrítica.

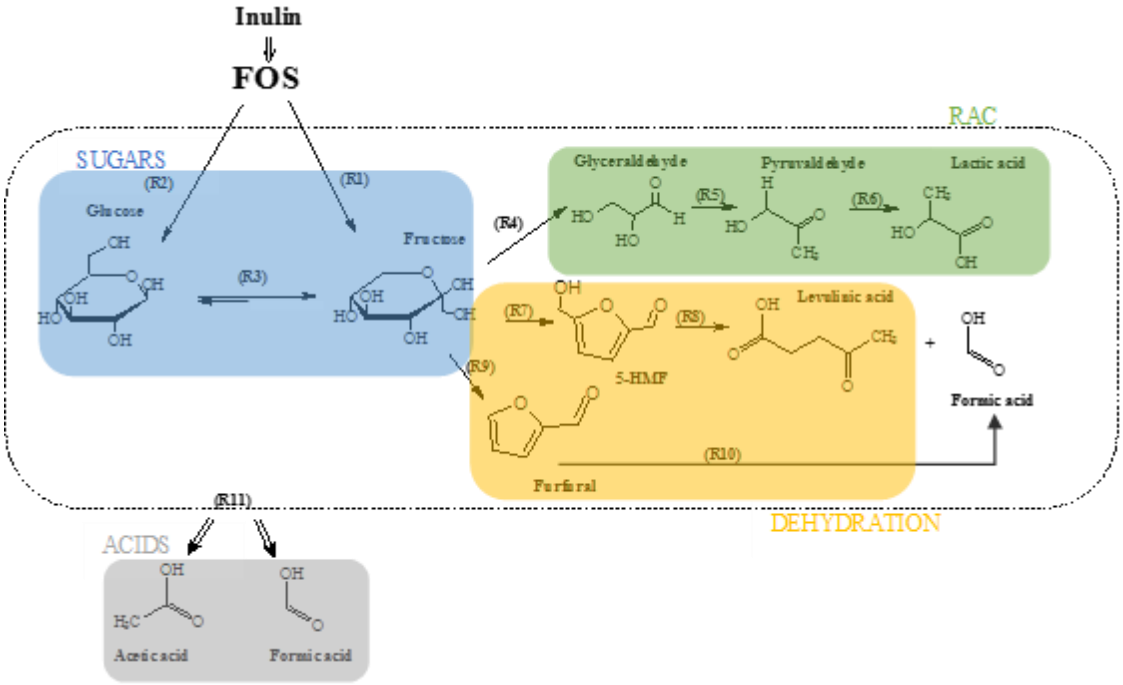

Figura 4. Mecanismo de reacción para la hidrólisis de fructoligosacáridos (FOS) en agua supercrítica.

La pataca (*Helianthus tuberosus*, "Jerusalem artichoke" en inglés) se seleccionó como biomasa rica en inulina (78 % w/w) para estudiar su hidrólisis en agua supercrítica. La hidrólisis de pataca fue similar a aquella de FOS a alta concentración (30 % w/w), produciendo hasta 68 % w/w de azúcares. En el caso de la pataca, el grado de polimerización fue superior a 25, casi tres veces más que la inulina comercial. Entonces, tiene sentido que la conversión de la pataca fuese menor que para la inulina, puesto que el mayor grado de polimerización ralentizó la hidrólisis. Además, los resultados de la pataca se compararon con los de pulpa de remolacha y salvado de trigo obtenidos en el capítulo anterior operando también con la planta piloto. En el caso de la pataca se obtuvo mayor conversión debido a su composición. Es decir, la pulpa de remolacha y salvado de trigo siendo biomasas de naturaleza ligonocelulósica, están mayormente compuestas de celulosa, hemicelulosa y lignina. Sin embargo, la pataca está mayormente compuesta por inulina, que es un polímero mucho más débil frente al ataque de agua supercrítica. Entonces, bajo condiciones similares, la pataca se convirtió más fácilmente que las biomasas lignocelulósicas.

En la Figura 5 se puede ver la comparación de pataca, pulpa de remolacha y salvado de trigo en términos de rendimiento de azúcares, conversión, selectividad y productos de degradación.

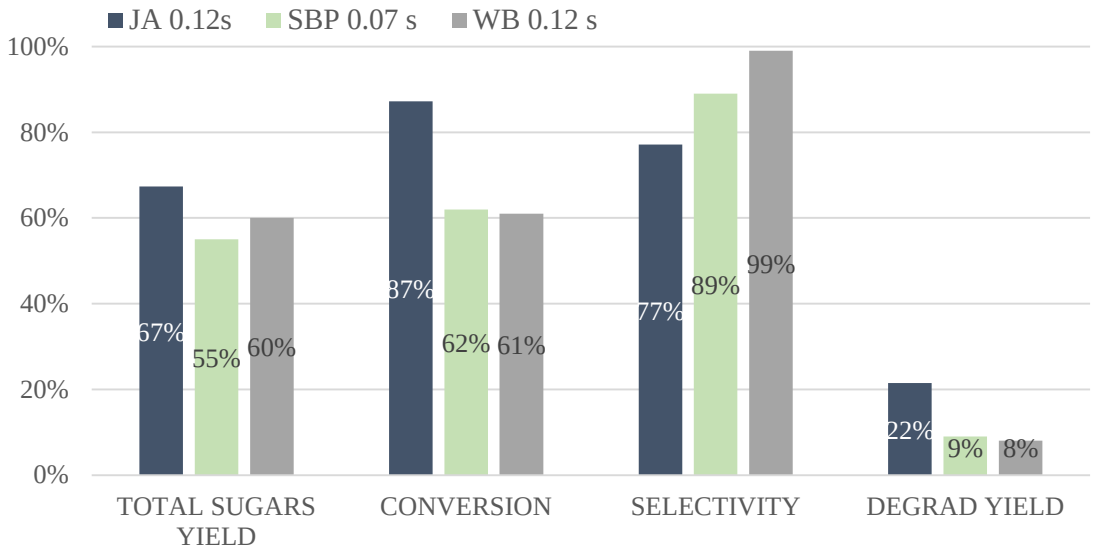

Figura 5. Comparación de los principales parámetros de hidrólisis para pataca, pulpa de remolacha y salvado de trigo, operando en los tiempos de reacción óptimos en la planta piloto.

Conclusiones

En la presente Tesis doctoral, se estudió la hidrólisis de diferentes biomasas (celulosa, pulpa de remolacha, salvado de trigo, inulina y pataca) en agua supercrítica, usando reactores ultrarrápidos y operando con tiempos de reacción menores a 1 segundo. La investigación se llevó a cabo primero en una planta escala laboratorio y luego se escaló el proceso a escala planta piloto. En este trabajo, se concluye que es posible producir azúcares de forma selectiva a partir de distintas biomasas, empleando agua supercrítica como medio de reacción y a escala planta piloto, lo que presentó nuevos desafíos pero a la vez demostró la versatilidad del proceso FASTSUGARS como una pieza clave en el desarrollo de biorefinerías. Las conclusiones generales de esta Tesis doctoral se presentan a continuación. Las conclusiones específicas de cada objetivo están presentes en las conclusiones de cada capítulo.

Hasta ahora, los modelos cinéticos para describir la conversión de celulosa en agua sub y supercrítica asumían que la hidrólisis de las partículas de celulosa ocurre en su superficie y por tanto el tamaño de partícula era considerado el parámetro clave para los cálculos cinéticos. Sin embargo, en el Capítulo 1 se probó que la concentración de entrada al reactor también afecta la cinética de conversión de celulosa en agua supercrítica. Específicamente, cuando la concentración de entrada de celulosa es superior al 4 % w/w, la celulosa se comporta como si fuese hidrolizada en condiciones subrcíticas, de tal manera que la disolución no fue completa (medio heterogéneo), ralentizando la conversión de celulosa.

El proceso FASTSUGARS permitió la valorización de la pulpa de remolacha, produciendo un efluente rico en azúcares que pudo ser luego convertido en productos de interés como sorbitol y etilenglicol. El máximo rendimiento de azúcares (66 % w/w) se alcanzó operando a 390 ºC, 25 MPa y 0.11 s de tiempo de reacción. Además, un producto sólido se obtuvo tras hidrólisis, que fue caracterizado vía hidrólisis ácida, TGA y FTIR para probar su semejanza a la lignina insoluble. Este sólido, compuesto mayoritariamente por una fracción insoluble en ácido (AIF) resultó ser una mezcla de lignina insoluble y char. Además, estudiando sus propiedades térmicas se concluyó que su resistencia a la degradación térmica era similar a la lignina Kraft.

Cumpliendo con el objetivo principal de esta tesis, se llevó a cabo el escalado del proceso FASTSUGARS desde escala laboratorio a planta piloto. La nueva planta piloto se diseñó para operar a 400 ºC, 30 MPa y flujos de hasta 35 kg/h. El incremento del flujo de trabajo permitió aumentar el tamaño de partícula de biomasa hasta 300 µm. Además, la instalación de filtros para la recolección de sólidos en línea y el reparto energético de la etapa de calentamiento en tres calentadores, mejoró significativamente la operación en la planta piloto con respecto a la escala laboratorio. Al comparar los resultados de pulpa de remolacha y salvado de trigo obtenidos en ambas plantas, se observó que el hecho de emplear un tamaño de partícula mayor en el caso de la planta piloto (250 µm), influyó positivamente en el producto final, ya que ralentizó la conversión y por tanto la degradación.

Finalmente, inulina y pataca fueron hidrolizadas en la planta piloto para obtener fructosa y piruvaldehído. La inulina comercial, debido a su grado de polimerización (~10), se empleó como modelo para el estudio de la hidrólisis de fructooligosacáridos (FOS) en agua supercrítica, definiendo así su mecanismo de reacción. Se observó que la conversión de fructosa a gliceraldehído, 5 – HMF y furfural fue más lenta que la posterior producción de piruvaldehído y ácido fórmico. Por otro lado, la hidrólisis de pataca resultó ser similar a la de FOS a alta concentración, produciendo hasta 68 % w/w de azúcares derivados de la fructosa. Comparando los resultados de pataca con los de pulpa de remolacha y salvado de trigo es observó que la pataca sufrió una mayor degradación debido a la presencia de inulina, que es más fácilmente hidrolizable que la celulosa.

Trabajo futuro

Teniendo en cuenta la complejidad y diversidad de biomasas existentes, cada materia prima representa un desafío tecnológico que debe ser resuelto por separado. En ese sentido, sería interesante comparar el comportamiento de biomasas con diferente relación celulosa/lignina. Así, se podría obtener productos sólidos con diferentes composiciones que podrían tener diferentes aplicaciones como composites. Vinculado a este objetivo, sería necesario profundizar en el estudio de las propiedades mecánicas y estructurales de estos sólidos.

En cuanto a la operación en la planta piloto, hay varias mejoras que podrían optimizar su funcionamiento. Para facilitar el bombeo de biomasas a mayor concentración, se debería instalar un tanque presurizado y agitado. Además, la instalación de un caudalímetro en la línea de agua mejoraría sustancialmente la operación y fiabilidad de las medidas. También se puede mejorar el sistema de calentamiento, probando otros fundamentos o diseños para poder aumentar la capacidad de la planta. Hasta la fecha, la válvula automática sólo se ha empleado con efluentes limpios como la inulina, pero vista su efectividad, podría emplearse para la hidrólisis de biomasa. Para ello, habría que evaluar la idoneidad de los materiales empleados en su construcción.

Finalmente, la implementación de procesos de separación y purificación es esencial para la comercialización de los productos obtenidos en este trabajo. La extracción o purificación de azúcares podría hacerse mediante membranas o ultrafiltración. Por otra parte, el uso de pretratamientos también debe tenerse en cuenta para mejorar los rendimientos en la etapa de hidrólisis supercrítica.

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Celia M. Martínez (Albacete – Spain, 1990) started the studies of Chemical Engineering at the University of Castilla – La Mancha in Ciudad Real (UCLM, Spain) in 2008 and graduated in 2013. Then, she joined the University of Valladolid (UVa, Spain) and specifically the High Pressure Processes Group (HPPG) in 2013 for a MSc. in Thermodynamic Engineering of Fluids and continued as a PhD student from October 2014. Her PhD is focused on the valorization of agricultural biomass by ultrafast hydrolysis in supercritical water, which is supervised by Prof. Dr. María José Cocero and Dr. Danilo A. Cantero. As part of her PhD, she developed a three months research stay at Cornell University (Ithaca, NY, USA) under the supervision of Prof. Dr. Jefferson W. Tester.

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Oral presentations

- **C. M. Martínez**, T. Adamovic, D.A. Cantero and M. J. Cocero. Valorization of agricultural biomass by supercritical water hydrolysis as a step towards biorefineries. *15th European Workshop on Lignocellulosics and Pulp (EWLP 2018)*, June 2018, Aveiro (Portugal).
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- **C. M. Martínez**, R. Posmanik, D.A. Cantero, B. Cantero-Tubilla, M.J. Cocero, J. W. Tester. Biomass Conversion into Low Oxygenated Hydrocarbons by Hydrothermal Liquefaction. *10th World Congress of Chemical Engineering (WCCE10)*, October 2017, Barcelona (Spain).
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- **C. Martínez**, D.A. Cantero, J. García-Serna and M. J. Cocero. Valorization of agricultural biomass wastes into valuable products via supercritical water hydrolysis. *2nd EuChemMS Congress on Green and Sustainable Chemistry*, Octubre 2015, Lisbon (Portugal)
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