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Simultaneous and Selective Recovery of Cellulose and Hemicellulose Fractions from Wheat Bran by Supercritical Water Hydrolysis

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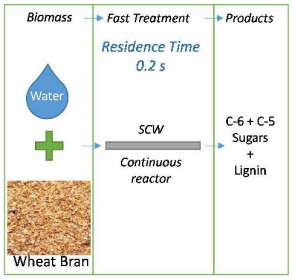
A contribution to biomass conversion into sugars and lignin by compact reactor easy to scale-up was developed. Wheat Bran was continuously fractionated under supercritical water conditions.



Abstract

Supercritical water (SCW) has demonstrated to be an excellent solvent and reaction medium to improve the cellulose hydrolysis selectivity by the control of the reaction time. In this study the conversion of wheat bran into soluble saccharides such as glucose, xylose and arabinose was analysed at 400ºC and 25 MPa with reaction times between 0.2 and 1 s. The process yield was evaluated for two different products: C-6 (glucose derived from cellulose) and C-5 sugars (saccharide derived from hemicellulose hydrolysis). The production of glycolaldehyde, furfural and 5-hydroxymethylfural (5-HMF) was analysed as by-products formation. The operation under supercritical conditions allows a biomass liquefaction of 84% $w \cdot w^{-1}$ at 0.3 s of residence time. The obtained solid after the hydrolysis was composed of mainly lignin (86% $w \cdot w^{-1}$). The highest recovery of cellulose (C-6) and hemicellulose (C-5) as soluble sugars (73% $w \cdot w^{-1}$) was achieved at 0.19 s of reaction time. An increase in the reaction time decreased the yield of C-6 and C-5. A total recovery of C-5 was achieved at 0.19 s. On the other hand, the highest yield (65% $w \cdot w^{-1}$) of C-6 was achieved at 0.22 s of reaction time. The main hydrolysis product of C-6 and C-5 was glycolaldehyde yielding the 20% $w \cdot w^{-1}$ at 0.22 s of reaction time. The furfural and 5-HMF production was highly inhibited in the experimented conditions obtaining yields lower than 0.5 % $w \cdot w^{-1}$. The hydrolysis reactions were performed in a continuous pilot plant at 400ºC, 25 MPa and residences times between 0.1 s and 0.7 s.

Keywords: Biorefinery, Glucose, Glycolaldehyde, Xylose

1. Introduction

The processes based on the conversion of biomass resources into fuels and chemicals have been intensively studied in the recent years due to the necessity of changing the current production philosophy based on oil to the bioeconomy[1, 2]. Plant biomass is a promising raw material for the production of chemicals and fuels because it is an abundant, renewable and world-wide distributed source of carbon[3]. The lignocellulosic biomass is generally composed of: 40 – 45 % cellulose, 25 – 35 % hemicellulose and 15 – 30 % lignin[4]. Even though plant biomass is one of the most abundant resources of carbon on the planet (primary production ≈ $1 \cdot 10^{14}$ tons C / year[5]), one of the main challenges of biomass usage is the efficient depolymerisation of cellulose and hemicellulose into its composing monomers[6].

Water as reaction medium presents advantages over other solvents because it is a non-expensive and environmentally friendly solvent. In addition, the medium identity can be tuned by changing temperature and pressure in order to favour the desired reactions without using catalyst. The use of sub and supercritical water has been proposed as a promising solvent to process biomass due to its special properties very promising to perform the hydrolysis reactions [1, 3, 7]. Supercritical water refers to the state of water at pressure and temperature conditions above its critical point. The critical point of water is 374ºC and 22.1 MPa. Near its critical point, a solvent experiments drastic changes in its physical properties by simply modifying pressure and temperature. This behaviour is a promising alternative to manage the selectivity in chemical reactions. The main variations in the properties of water can be summarized as follows: (1) in the surroundings of the critical point, the dielectric constant decreases by increasing temperature, increasing in this way the solubility of organic compounds and; (2) the ionic product of water varies from 10^{-10} to 10^{-22} when changing the temperature from 300ºC to 400ºC at 25 MPa, changing the benefited reaction mechanism from ionic to free-radical [8]. In addition, the hydrothermal processing presents the following advantages: (1) direct use of raw material regardless of its water content, which implies an important energy saving; (2) the same reaction

medium can be used for the transformation of different biomass fractions; (3) mass transfer limitations can be reduced or avoided, thus reaction rates are faster [7, 9-12].

The conversion of biomass components (cellulose, hemicellulose and lignin) into their constitutive monomers using supercritical water have been previously reported [13-15]. The challenge then is to apply this technology to complex biomass, in order to transform it in valuable products by using a clean, safe and environmentally benign technology. The fractionation and hydrolysis of vegetal biomass in a hydrothermal medium has been studied using different kinds of reactors: batch [16-22], semi continuous [23-28] and continuous reactors [29-31]. In the aforementioned works, the yields of cellulose recovery as soluble sugars are between 3% $w \cdot w^{-1}$ – 15% $w \cdot w^{-1}$; 16% $w \cdot w^{-1}$ – 22% $w \cdot w^{-1}$ and; 2% $w \cdot w^{-1}$ – 6% $w \cdot w^{-1}$ for batch, semi continuous and continuous reactors respectively. The hemicellulose yields recovery are between 17% $w \cdot w^{-1}$ – 97% $w \cdot w^{-1}$; 18% $w \cdot w^{-1}$ – 95% $w \cdot w^{-1}$ and 25% $w \cdot w^{-1}$ – 95% $w \cdot w^{-1}$ for batch, semi continuous and continuous reactors respectively. Finally, the lignin compositions of the solids in the reactor are between 45% $w \cdot w^{-1}$ – 53% $w \cdot w^{-1}$ and 20% $w \cdot w^{-1}$ – 50% $w \cdot w^{-1}$ for batch and semi continuous reactors respectively.

Wheat bran is a by-product of the milling of wheat to produce white flour. Bran fraction constitutes around 11% of the total milling by-products and only 10% of wheat bran available is used as fiber supplement in breakfast cereals and bakeries (human consumption) while the remaining 90% is used as animal feed [32]. Compositional analysis suggests that wheat bran contains approximately 30% $w \cdot w^{-1}$ – 40% $w \cdot w^{-1}$ of hemicellulose, 15% $w \cdot w^{-1}$ – 35% $w \cdot w^{-1}$ of cellulose and 5% $w \cdot w^{-1}$ – 25% $w \cdot w^{-1}$ of lignin, depending on growing conditions and varieties [33, 34].

Traditional hydrolysis processes using acid catalysts or enzymes have been improved in last years but they still present limitations in providing high yield in moderate residence times. The hydrolysis of wheat bran was carried out combining acid hydrolysis (0.2 % $w \cdot w^{-1}$ sulphuric acid, 160 ºC for 20 min) with enzymatic hydrolysis (2 % enzymes, 50 ºC for 72 h) obtaining a yield of 80% $w \cdot w^{-1}$ of sugars [35]. Wheat bran hydrolysis can also be achieved exclusively by acid

hydrolysis using sulphuric acid (1% $w \cdot w^{-1}$) as catalyst at temperatures between 110ºC and 180ºC for 40 min, obtaining around 80 % $w \cdot w^{-1}$ of sugars [36]. Other methods were proposed in order to reduce the concentration of degradation products, such as a combined method of milling, acid hydrolysis and two steps enzymatic hydrolysis. In that case sulphuric acid (0.3% $w \cdot w^{-1}$ 121 ºC for 30 min) was used and also two kinds of enzymes obtaining a yield of 63% $w \cdot w^{-1}$ in sugars and no degradation products [37].

In this work, a continuous micro-reactor was used to carry out the hydrolysis of wheat bran in supercritical water. This reactor has been previously used to hydrolyse pure cellulose in supercritical water, giving as a result a total conversion of cellulose in 0.02 s of residence time yielding a sugars production of 98% $w \cdot w^{-1}$[13]. The aim of this work was to test the capability of the aforementioned reactor to hydrolyse natural biomass.

2. Results and Discussion

The compositional analysis of the raw material is shown in Table 1. It was determined the percentage of moisture, extractive fraction, lignin, cellulose, hemicellulose and ash content. Lignin fraction was determined as the sum of 19.6 ± 0.3% $w \cdot w^{-1}$ due to soluble lignin and 2.7 ± 0.1% $w \cdot w^{-1}$ due to insoluble lignin.

Table 1. Chemical composition of wheat bran.

Component	Moisture	Extractives	Cellulose	Hemicellulose	Lignin	Ash	**TOTAL**
g / 100g wheat bran	8.2 ± 0.1	10.6 ± 0.8	31.4 ± 1.6	20.3 ± 1.0	22.3 ± 0.3	0.5 ± 0.1	**93.4 ± 1.6**

The wheat bran was continuously hydrolysed in supercritical water at 400ºC and 25 MPa at different reaction times. These conditions were previously chosen because they were optimized in a previous work for cellulose hydrolysis [13]. The reactions of cellulose hydrolysis under supercritical water conditions are fast. In fact, total conversion of cellulose can be achieved at reaction time as low as 0.02 s. Therefore, the reaction time was evaluated between 0 s and 1 s. Because of the geometry of micro reactor, flow rates and water properties, the Reynolds number developed in the reactor was around $1 \cdot 10^5$ [38]. Thus, the flow through the reactor can be considered turbulent. In fact, the mixing disposition in our reactor was set following the best

arrangements developed in literature [39, 40]. In those investigations about the mixing of supercritical water and room temperature water, the mixing time was calculated and represented as a function of the Richardson Number ($Ri=Gr/Re^2$). The mixing time between supercritical water and room temperature water would take values between 1 and 3 ms at $Ri=1\cdot10^{-2}$. The Richardson number developed in our reactor took a value around $1\cdot10^{-8}$ suggesting that the mixing time would be lower than 1 ms, thus, lower than 1% of the total time considered between the inlet and outlet of the reactor.

Each experimental point is a result of five repetitions of the analysed conditions. In Figure S1 in the Supporting Information it is shown a typical temperature and pressure profile for an experiment. The pressure variations along residence time would be produced due to a partial solid deposition near the depressurization valve. The reactor was maintained at 400 ± 5ºC and the pressure at 25 ± 1MPa. The temperature of the reactor outlet (after depressurization) was around 160ºC. This stream was fed to the HE-1 and leaves it at a temperature of 150ºC. So, a post cooling was needed to take the sample at 25ºC. On the other hand, the water stream pumped to the HE-1 was heated from 20ºC to 155ºC. That was the temperature, which the water stream entered to the heater to be further heated until 450ºC. The use of this heat exchanger allowed the reduction of the heat requirements in 20%.

The lignin composition of the solids (soluble lignin -SL- and insoluble lignin -IL-) obtained after hydrolysis is shown in Figure 1. The initial lignin composition was around 22% $w\cdot w^{-1}$. The lignin fraction was increased while reaction time was raised. At 0.3 s of reaction time, the lignin content reached a value of 85% $w\cdot w^{-1}$. Increasing the reaction time up to 0.69 s did not enhance this value.

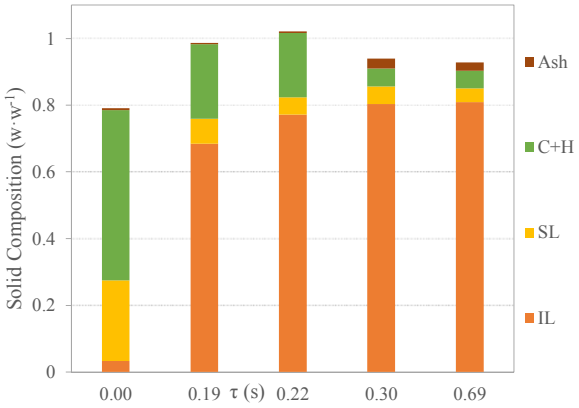


Figure 1. Composition of the solids products after hydrolysis in supercritical water. The residence time of 0 s refers to the composition of the raw material. *'C+H'* is the solid composition in cellulose and hemicellulose in % w·w^{-1}. *'SL'* is the solid composition in soluble lignin in % w·w^{-1}. *'IL'* is the solid composition in insoluble lignin in % w·w^{-1}.

It should be taken into account that most of the plant biopolymers (mainly cellulose, hemicellulose and lignin) are present in nature in an associated way. Cellulose micro/nanofibrils interact between them by hydrogen-bond interactions forming highly associated structures like cylinders. The structure of hemicellulose is more opened and random than cellulose one and they interact between them by van der Waals and H-bond interactions. The lignin fraction is a highly amorphous polymer formed basically by phenolic units. The structure of lignin is complex, like a network, due to the random polymerization reaction when it is produced in nature. The interaction between hemicellulose and lignin take place by covalent bonds [41]. It can be considered that some fractions of cellulose are less accessible in the inners structure of lignin or linked to lignin [42]. The ash content was increased from 0.5% w·w^{-1} in the raw material up to 3% w·w^{-1} after hydrolysis. Although the lignin composition of the solids was increased from 22 % to 85% w·w^{-1}, the soluble lignin fraction was decreased from 19% w·w^{-1} to 5% w·w^{-1}. This phenomenon would occur due to the lignin hydrolysis, which would occur firstly in the sites where lignin interacts with hemicellulose [42]. However, lignin remain in the solid as the main component after fractionation.

The cellulose and hemicellulose fractions in the solids were decreased as the reaction time was increased. However, these fractions remained constant at a value near to 5% $w \cdot w^{-1}$ when the reaction time was increased from 0.3 s to 0.7 s. In order to evaluate the solid characteristics after hydrolysis, SEM and FTIR analysis were carried out of them. In Figure S.2 in the Supporting Information, it is observed the spectrum comparison between the raw material (wheat bran) and the solid product after hydrolysis. The bands at 1135 cm^{-1} are indicative of the aromatic C–H in-plane deformation for syringyl type [43]. This suggests that syringyl type lignin was in the raw material as well as in the solid product after hydrolysis. However, aromatic C–H out of bending exhibits at 844 cm^{-1} [43]. This band was observed in the raw material but not in the solid product, suggesting that a fraction of lignin is decomposed after supercritical water hydrolysis. This agree with the results presented in Figure 2, in which the soluble lignin content is decreased after the process. Aromatic skeleton vibrations occurred at 1510 cm^{-1} and 1460 cm^{-1} [43]. The absorbance for these bands appeared at both, raw material and product, suggesting that the aromatic properties remained in the solid products after the hydrolysis. The band at 1720 cm^{-1} is originated from the carbonyl group, including unconjugated ketone and carbonyl group stretching [43]. This band was observed in the raw material but not in the product, so this suggests that typical cellulose bonds were broken or they were not present in the solid. In addition, the disappearance of the band at 1747 cm^{-1} would indicated the rupture of the ester link of acetyl, feruloyl and p-coumaroyl between hemicellulose and lignin [44]. It can be also observed that the O–H and aliphatic C–H (2870 cm^{-1}) bonds which are the basic function groups in biomass were present in the raw material as well as in the products.

In order to analyse the structure of the hydrolysed products, SEM microscopy was applied to the samples. SEM images of the raw material are shown in Figure S.3-A and S.3-C in the Supporting Information for a magnification of 5000X and S.3-E for a magnification of 1000X. The images corresponding to the solids obtained after hydrolysis are shown in Figure S.3-B and S.3-D for a magnification of 5000X and S.3-F for a magnification of 1000X. In Figure S.3, it is observed that the raw material presented different shapes with a smooth surface. After

hydrolysis, the remaining solid showed a non-smooth surface. In addition, Figures S.3-D and S.3-F suggest that a porous solid was obtained after hydrolysis. Cellulose and hemicellulose that are located in the outer area of the particle would be rapidly hydrolysed in the process. However, the fractions of cellulose and hemicellulose situated in the inner part of the porous network of lignin would be the reason of the remaining 5% $w \cdot w^{-1}$ found (see Figure 1) at high reaction times (0.69 s).

The recovery of soluble sugars in the liquid products decreased when reaction time was increased from 0.19 s to 0.69 s. The yield of C-5 and C-6 obtained as soluble sugars after supercritical water hydrolysis is shown in Figure 2. The maximum recovery of soluble sugar reached was 73% $w \cdot w^{-1}$ at 0.19 s of reaction time. Although the highest yield of sugars was achieved at the lowest residence time, a decrease in the residence time would not produce a higher yield due to the uncompleted hydrolysis of cellulose and hemicellulose. In fact, the solid products after hydrolysis at 0.19 s of reaction time had a C-6 and C-5 composition of 22% $w \cdot w^{-1}$.

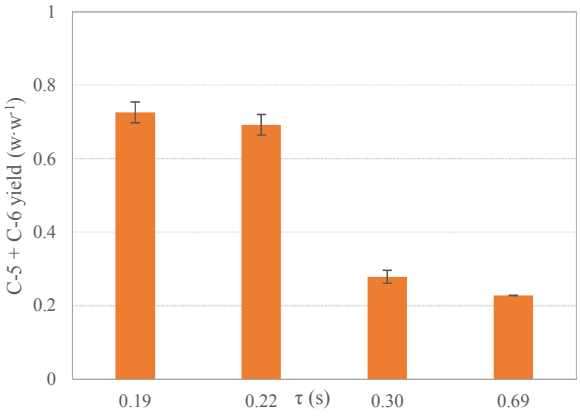
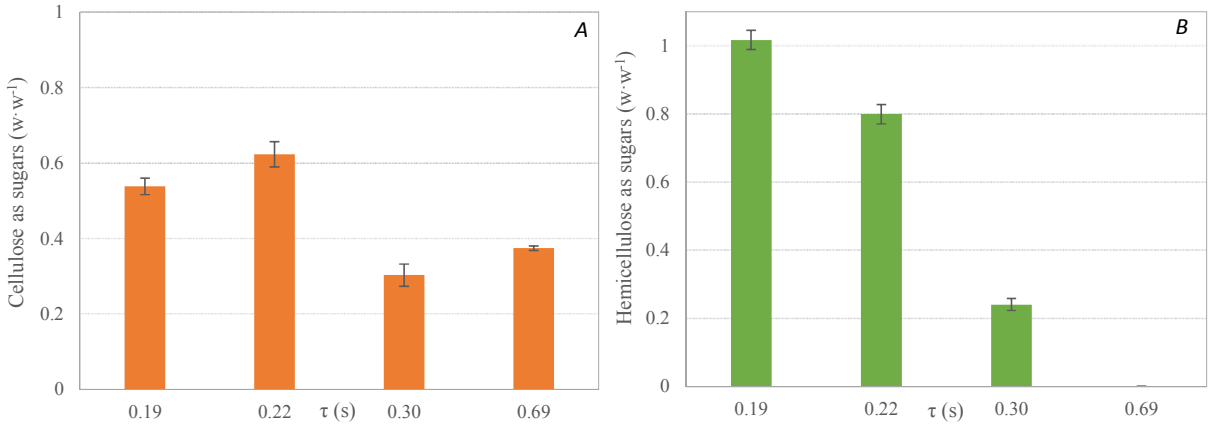


Figure 2. Yield of cellulose and hemicellulose recovered along residence time after supercritical water hydrolysis at 400ºC and 25 MPa.

The recovery of cellulose and hemicellulose fractions as soluble sugars along residence time are shown in Figure 3-A and 3-B respectively. The maximum yield of cellulose-derived sugars (63% $w \cdot w^{-1}$) was achieved at 0.22 s of reaction time. The hydrolysis of pure cellulose at the same conditions that the experimented in this work was able to produce a yield of 98% $w \cdot w^{-1}$ at

0.02 s of reaction time [13]. The difference in the yields of cellulose hydrolysis, suggest a strong effect of the cellulose interactions with the other components of the biomass over the kinetic. As explained above, it can be considered that cellulose in the natural biomass is embed into a 3-D matrix of lignin and hemicellulose. So, the dissolution of wheat bran cellulose will be more complex and slower than the dissolution of pure cellulose due to the mass transfer limitations. In fact, pure cellulose is dissolved in supercritical water [15, 38]. The kinetic of cellulose hydrolysis in pressurized water takes different behaviours depending on the reaction medium conditions. At subcritical temperatures, the hydrolysis reactions occur in the surface of the cellulose grains producing small oligosaccharides at low reaction rates. In addition, the cellulose particles obtained after partial hydrolysis at subcritical temperatures have the same crystallinity than cellulose before the treatment, which suggests that the cellulose hydrolysis takes place in the surface of cellulose grain at subcritical temperatures. However, if the reaction medium is near or supercritical water, the reaction rates are faster and the produced oligosaccharides are higher than at subcritical conditions, suggesting that the cellulose is dissolved (or partially dissolved) at those conditions. Cellulose is composed by several unit of glucose linked by β-1,4 bonds, which provides the molecules of many –OH groups. These groups form intramolecular hydrogen bonds that provide the cellulose molecules of chain stiffness and molecular stability. At supercritical conditions, water is a non-polar solvent with dielectric constant values lower than 10 and ionic products lower than $1 \cdot 10^{-8}$ $mol^2 \cdot kg^{-2}$. The fact that cellulose is dissolved in near critical water suggests that cellulose present a poor polar global structure. Although it is difficult to determine what is the governing parameter in cellulose dissolution, according to the results presented by Cantero et al[38] and Sasaki et al[15], it can be thought that cellulose is dissolved at density values lower than 600 $kg \cdot m^{-3}$ and dielectric constant values lower than 15. The cellulose dissolution might not occur with the same degree when cellulose is interacting with other components of biomass like lignin or when it is located inside a lignin network. Despite of the difference between pure cellulose and wheat bran, the results obtained in this work improves those found in literature (<25% $w \cdot w^{-1}$) for batch, semi-continuous or continuous fractionation [16-20, 23-31]. Hemicellulose was completely hydrolysed and recovered as sugars

238 (mainly xylose) at a reaction time of 0.19 s. An increase in the reaction time caused a lower
239 yield of C-5 recovered as sugars. In this case, a similar value of C-5 yield was found in
240 literature [16, 23].



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242 **Figure 3. (A)** Cellulose recovery along residence time. **(B)** Hemicellulose recovery along
243 residence time.

244 The sugars produced after cellulose and hemicellulose hydrolysis can follow different reaction
245 in supercritical water, such as retro-aldol condensation (RAC) or dehydration [45]. The main
246 products of cellulose hydrolysis are shown in the reaction pathway illustrated in Figure 4.
247 Cellulose is hydrolysed into oligosaccharides as first step. Then the oligosaccharides are
248 hydrolysed into glucose. Once glucose is produced, it can be isomerised into fructose. The rate
249 of fructose production is highly affected by the reaction mediums conditions [46], at supercritical
250 water conditions the production of fructose from glucose is lower than at subcritical water
251 conditions. These carbohydrates, glucose and fructose, can follow mainly two reaction
252 pathways: dehydration and RAC. The dehydration reactions (horizontal way) produce 1,6
253 anhydro-glucose from glucose or 5-HMF from fructose. In these reactions, the sugar loose
254 molecules of water. Glucose loose one molecule of water to produce 1,6 anhydro-glucose while
255 fructose loose three molecules of water to produce 5-HMF. On the other hand, glucose and
256 fructose can follow RAC reactions (vertical way) in which the molecules are split into two
257 compounds. The RAC reaction takes place in the alpha carbon of the sugar. Thus, an aldose like
258 glucose will produce a molecule of 2 carbons and a molecule of 4 carbons after RAC reaction.

On the other hand, a ketose like fructose will produce two molecules of three carbons after RAC reaction. The main product derived product obtained in the hydrolysis of wheat bran was glycolaldehyde. The yields of production along the reaction time are shown in Figure S.4 in the Supporting Information. The yield of this compound at 0.19 s (highest yield of C-5 and C-6 recovery) was 20% $w \cdot w^{-1}$. The maximum amount of glycolaldehyde was 14% $w \cdot w^{-1}$ at 0.22 s of reaction time. Small amounts of glycolaldehyde were also detected associated with the oligosaccharides. This behaviour was observed also in the hydrolysis of cellulose and cellobiose in supercritical water.

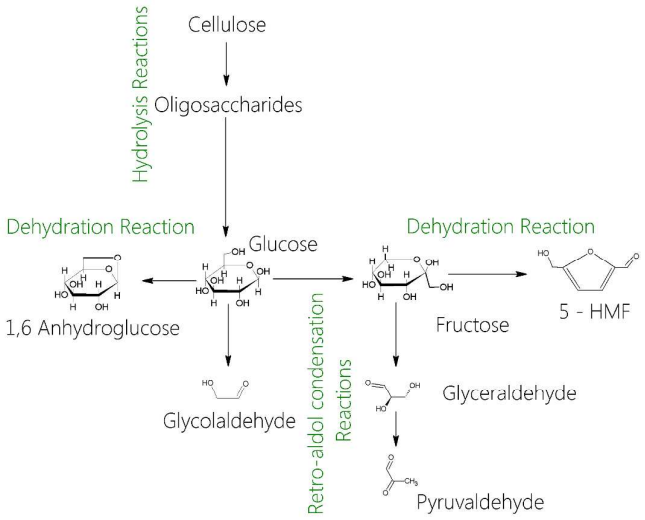

Figure 4. Main reactions of cellulose in supercritical water: hydrolysis, isomerization, dehydration and retro-aldol condensation.

The production of 5-HMF would be undesired if a microorganism post-processing of the obtained sugars is needed [24]. In Figure S.5 in the Supporting Information it is shown the obtained yields of 5-HMF in the experimented conditions. In the same way that it was developed in a previous work [13], the yield of 5-HMF over the whole range of residence time was lower than 0.05% $w \cdot w^{-1}$.

A fraction of the soluble lignin present in the starting material was hydrolysed and obtained together with the sugars in the liquid sample. The amount of soluble lignin was determined

following the method described in section 3.2.1. All the measurements of soluble lignin gave values below 1000 ppm which represents less than 10% $w \cdot w^{-1}$ of carbon in the liquid sample.

The proposed method in this paper for the fractionation and hydrolysis of wheat bran shows meaningful advantages over the traditional methods of acid or enzymatic hydrolysis. In 2009, Arai *et al* [1] developed the concept of decentralized production of fuels and chemical compounds using renewable resources like lignocellulosic biomass as starting material. The decentralized production proposes the use of the available biomass in the area to produce and supply this area of energy, fuels and chemical compounds. The main requirement to obtain this kind of production is the development of compact and versatile process that can be placed in the countryside, near the biomass, avoiding in this way the shipping costs. The technology developed in this work shows a promising alternative for biomass fractionation and hydrolysis due to the extremely low reaction time. The reaction time is directly bonded to the reactors volume, which will make the technology compact and versatile or not. The acid hydrolysis technology usually involves reaction times higher than 30 minutes and temperatures of around 170ºC [36, 37]. The enzymatic hydrolysis technology demands lower temperature but much higher reactions times, about 70 hours [35]. The reduction of the reaction time from 30 minutes or 70 hours (traditional methods) to 0.2 s (supercritical water hydrolysis) involves a substantial reduction in the reactor volumes from cubic meter to millilitres. In addition, the scale up of the process developed in this work makes easier the operation in some aspects in comparison with the lab scale: reactor volume, particle size and pumping. The reactor volume will be increased from microliters to millilitres, which will avoid problems of clogging in the reactor. In this work, the particle size was between 100 μm and 200 μm in order to avoid clogging in the reactor and the pump. A higher reactor diameter will allow higher particle size avoiding milling costs. In addition, the higher flows used in the industrial scale favour the pumping. The main issue for pumping solids at lab scale is the size of the pump check valves. At lab scale (1 – 3 $L \cdot h^{-1}$) the size of the characteristic ball inside the check valve is around 1 mm, which is only ten times higher than the particle size. On the other hand, the pump check valves at industrial scale

(1 – 3 $m^3 \cdot h^{-1}$) are much higher than at lab scale, which makes it 100 or 1000 higher than the particle size of the biomass. Thus, at industrial scale the clogging in the pump is highly reduced. Other important advantage of the developed method in this work is the possibility of increasing the product concentration. The reaction developed in the experimental setup described in this work is stopped through a flash decompression, lowering the temperature from 400ºC down to 100ºC. The flash operation produces two phases: a liquid high with high concentration of sugars (it can be modified by changing the decompression pressure) and a vapour phase with extremely low carbon concentration (the amount of vapour can be modified by changing the decompression pressure). This cooling method has at least 3 advantages: the reaction is effectively stopped, the product concentration can be increased by taking out water as vapour and the vapour obtained as product is almost free of carbon and contaminants, so it can be recycled to the system directly.

3. Experimental

3.1. Materials

A local supplier supplied the wheat bran used in the experiments. The particle size of the original biomass was 430 μm. In order to ensure an unstopped pumping, the particle size was reduced to 125 μm using a ball mill Retsch PM100. Distilled water was used as reaction medium to run the experiments. The standards used in High Performance Liquid Chromatography (HPLC) analysis were: cellobiose (≥ 98%), glucose (≥ 99%), xylose (≥ 99%), galactose (≥ 99%), mannose (≥ 99%), arabinose (≥ 99%), glyceraldehyde (≥ 95%), glycolaldehyde dimer (≥ 99%), lactic acid (≥ 85%), formic acid (≥ 98%), acetic acid (≥ 99%), acrylic acid (≥ 99%), furfural (99%) and 5-hydroxymethylfurfural (≥ 99%) purchased from Sigma. Milli-Q water and sulphuric acid (HPLC grade) were used as mobile phase in the HPLC analysis. For the determination of structural carbohydrates and lignin [47], sulfuric acid (≥ 96%) and calcium carbonate (≥ 99%) supplied by Sigma were used as reagents. Milli-Q water was used in this procedure.

3.2. Methods

3.2.1. Chemical characterization for raw material

Natural biomass (wheat bran) was used in the experiments, so first of all the composition of the sample was determined. For that purpose a Laboratory Analytical Procedure (LAP) from NREL was used to determine the structural carbohydrates and lignin in biomass [47]. Briefly, the sample was dried at 105 ºC in an oven for 24 hours in order to obtain composition in dry basis. After that, the sample was subjected to a Soxhlet extraction using hexane as solvent in order to remove the extractives from the sample. For carbohydrates and lignin determination, 300 mg of solid sample (after Soxhlet extraction) were weighed and 3 ml of 72 % sulphuric acid were added. The sample was incubated at 30 ºC for 30 minutes and after that, 84 ml of deionized water were added. Finally, the sample was heated at 120 ºC for 60 minutes. The final product was vacuum filtered and a 50 ml liquid aliquot was used to determine soluble lignin as well as carbohydrates. The remaining solid was collected to analyse the insoluble lignin and ash content. The liquid aliquot was analysed with UV-Visible spectrophotometer to determine soluble lignin. The wavelength was set at 280 nm and the used extinction coefficient had a value of 18.675 $L \cdot g^{-1} \cdot cm^{-1}$ [48]. A similar liquid aliquot was neutralized with calcium carbonate to a pH between 5 and 6 and then analysed with HPLC to identify and quantify structural carbohydrates. The solid was dried at 110ºC for 24 h and then cooled in a desiccator, weighting the solid. After that, the sample was placed in a muffle at 550 ºC for 24 h and the remaining residue was weighed to obtain the ash content.

3.2.2. Analysis

The solids in the product were separated by centrifugation and dried at 60 ºC for 24 h. Then, following the same procedure described in Section 2.2.1, the total lignin content was determined. The separated solids obtained after wheat bran hydrolysis were analysed by spectroscopy Fourier Transform Infrared (FTIR) and scanning electron microscopy (SEM). The FTIR experiments were carried out using a Bruker Tensor 27. Samples were analysed in the wavelength range of 4000 cm^{-1} – 600 cm^{-1} with a resolution of 4 cm^{-1}. The number of scans per sample was 32 being the scanner velocity 10 KHz. The interpherogram size was 14220 points.

The SEM experiments were conducted in a JSM-820 (JOEL, Japan) operated at 20 kV of accelerating voltage. A gold evaporator Balzers SCD003 with a gold thickness of 25 mm – 30 mm was used.

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

The soluble oligosaccharides concentration in the samples was determined by acid hydrolysis to glucose and HPLC determination. Briefly, to 10 ml of filtered liquid aliquots was added 4 mL of 96 % sulphuric acid. The sample was maintained at 30 ºC during 60 min in an oven. Then it was diluted with 86 ml of deionized water and incubated at 121 ºC for 60 min. Calcium carbonate was added to 20 ml of this sample to neutralize the medium and finally the supernatant was filtered and analysed with HPLC. It should be mentioned that the oligomers concentration and the RAC and dehydration products were determined using the acid hydrolysis method. The concentration of monomers and its derived products was also determined by direct analysis of the obtained sample from the pilot plant by HPLC. In this way, the quantity of glycolaldehyde or 5-HMF that can be produced from the hydrolysis of an oligomer with a degraded end-group can also be determined by difference.

3.2.3. Yield and reaction time

In this work, reaction time is one of the main parameters for controlling the hydrolysis process. The reaction time was calculated as shown in Equation 1, where *'V'* is the volume of the reactor (m^3), *'ρ'* (kg/m^3) is the density of the medium at the reactor conditions (considered as water due to the low concentration of biomass, ≈1% $w \cdot w^{-1}$) and *'F_m'* is the mass flow in the reactor (kg/s).

$$\tau = \frac{V\rho}{F_m} \quad (1)$$

The yield of main compounds (C-6 sugars, C-5 sugars, glycolaldehyde and 5-HMF) was determined by Equation 2, where *'Y_s'* is the yield of the compound *'s'*, *'C_s'* is the concentration of *'s'* in the liquid product in ppm and *'S_{in}'* is the concentration of sugars at the inlet of the reactor in ppm, calculated as shown in Equation 3. Soluble sugars derived from cellulose (cellobiose and glucose) were called C-6, the derived from hemicellulose (xylose, mannose, galactose and arabinose) were called C-5 and the rest of compounds were organic acids (acetic, lactic and acrylic acid), glycolaldehyde, glyceraldehyde and 5-HMF.

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

$$S_{in} = C_{in} \cdot S_T \quad (3)$$

In Equation 3, *'C_{in}'* is the concentration of wheat bran at the reactor's inlet in ppm and *'S_T'* is the cellulose and hemicellulose fraction in the raw material in mass fraction which represents the proportion of wheat bran susceptible of being hydrolysed into sugars (see Table 1). When the yield was referred to each fraction, cellulose or hemicellulose, *'S_T'* was the portion of each fraction in the raw material.

3.3. Experimental setup

The continuous pilot plant used for this work is shown in Figure 5. The hydrolysis pilot plan was designed to operate up to 400 ºC and 30 MPa using a sudden expansion micro-reactor (SEMR) developed in a previous work [13].

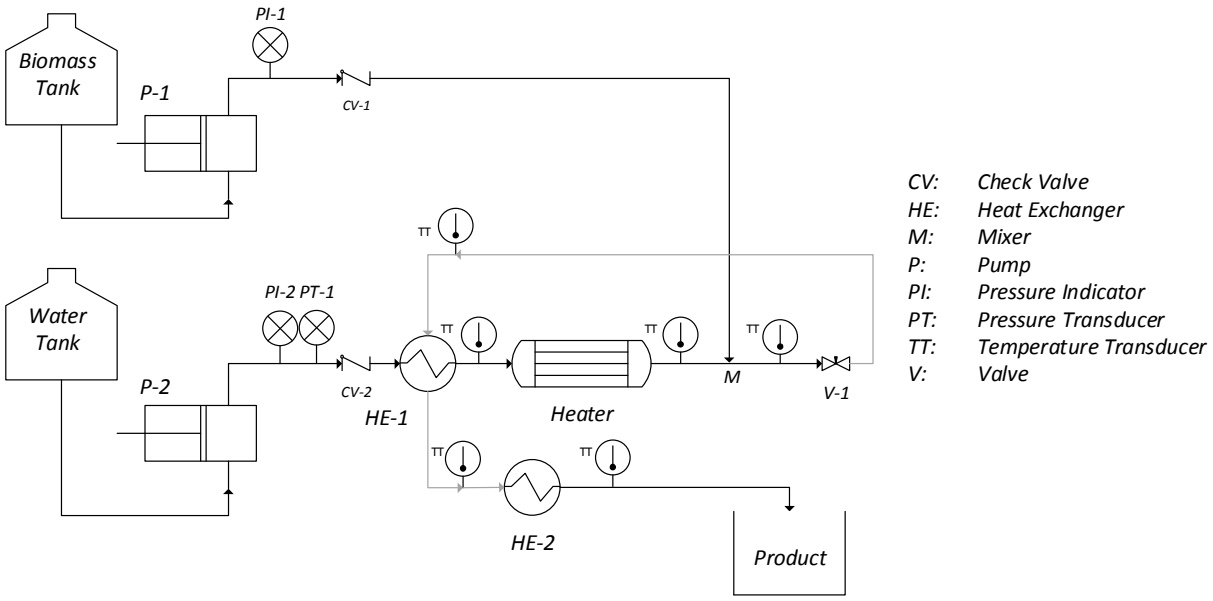

Figure 5. Plan of the Fast Sugars pilot plant in the University of Valladolid.

The main advantage of this reactor is the instantaneous cooling of the products stopping efficiently the reactions of hydrolysis in very low times. This allows the precise evaluation of the reaction time without diluting the products. In a similar way, the heating of the biomass stream is achieved instantaneously by a supercritical water injection at the reactor inlet. With this heating method, it is possible to change the temperature of a biomass stream from room temperature until 400ºC in a mixer which is placed at the reactor inlet. In addition, the reactor is thermally isolated with Rockwool insulation which makes possible to consider it as isothermal. A detailed description of the pilot plant as well as the operation procedure is presented in a previous work [38]. In this case, a wheat bran suspension (5% $w \cdot w^{-1}$) was continuously compressed and pumped up to the operation pressure (25 MPa), remaining at room temperature until the inlet of the reactor. In that point the suspension was instantaneously heated by mixing it with a supercritical water stream and the hydrolysis reactions start. Then the effluent was suddenly depressurized at the outlet of the reactor without previous cooling in order to instantaneously stop the hydrolysis. In this setup, a modification from the previous pilot plant [13] was tested. In this setup, the reactor outlet stream was driven to a heat exchanger (HE-1) to pre-heat the supercritical water stream. In order to ensure the cooling of the sample, a cooler (HE-2) was set after HE-1.

The aforementioned experimental setup was carefully designed following the security regulations for high pressure and temperature pilot plants. The hot and pressurised pipes of the pilot were confined inside a bunker for security reasons. This section of the setup is accessible by the back of the pilot plant as it is shown in Figure S.6 in the Supporting Information. In addition, the operation of the setup can be done completely by managing the control panel situated in the front of the pilot plant, the opposite to the bunker access. For further information about the security aspects in the design see a previous work of Cantero et al [38].

4. Conclusions

Wheat bran hydrolysis in supercritical water was analysed at 400ºC and 25 MPa at reaction times lower than 1 s. This method showed to be an effective procedure to hydrolyse both, cellulose and hemicellulose, at the same time with low concentration of degradation products. This result was achieved by working at high temperature (400ºC) and low residence time (0.19 s). The control of the reaction time was the key factor to stop the reaction before sugars degradation.

The recovery yield of cellulose and hemicellulose as C-6 and C-5 was 73 % $w \cdot w^{-1}$. The solid after the hydrolysis was composed of 85% $w \cdot w^{-1}$ of lignin. An increase in reaction time increased the lignin content of the solid. However, a cellulose fraction (5% $w \cdot w^{-1}$) seems to remain occluded inside a lignin network after a reaction time increment. The obtained solid product after hydrolysis consisted of an amorphous and porous material.

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