Study of Liquid Hot Water Fractioning Treatment using Wheat Straw Residues as Raw material in a Biorefinery Concept

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Abstract

The use of lignocellulosic material has been studied in the biorefineries context. However, the fractioning of agro-industrial residues, such as wheat straw, presents processing challenges due to its solubilization resistance. The pretreatment processes aim to overcome the inherent recalcitrant barrier by subjecting the biomass to physical-chemical processes and facilitating the extraction of sugars and lignin. The inherent production of inhibiting substances of fermentative processes needs to be minimized. In this thesis, the method of isothermal autohydrolysis (with a solid-liquid ratio of 1:11) was used at temperatures of 160, 180 and 200˚C and holding times of 30, 60 and 90 minutes. Mass balances of all extraction steps were also performed in order to evaluate the losses between them. The lignin, sugars and degradation products profile in the liquid fraction were evaluated for each of the experimental conditions. The condition that produced the highest concentration of monomeric sugars (3.4 ± 0.05 g/L) was 180˚C for 90 min. However, the one that produced the most oligomeric sugars (11.0 ± 0.5 g/L) was 160˚C for 90 min. This is also the highest temperature and holding time that can be imposed by without surpassing the degradation products concentration threshold. Hydrolyzing the oligosugars, this concentration of total sugars can reach 12.5 ± 0.2 g/L, 180˚C for 60 min. The condition satisfies the criterium for the production of hemicellulosic sugars, limits of toxic products and maintaining both cellulose and lignin in the solid fraction of the process for future valorization is 160˚C for 90 min.
Keywords: Biorefinery; Degradation products; Lignin; Liquid hot water; Pretreatment

1. Introduction

In the age of global warming and overconsumption - which are both a result of high worldwide resources demand and population growth - as well as the increase of the subsequent generated wastes (solid, liquid and gaseous) are damaging biodiversity all around the world by raising pollution levels in soil, oceans and atmosphere [1]. This environmental concern led to find sustainable alternatives for industrial processes, driven by the final consumer's awareness on this matter. This stakeholder is now open to the idea of paying more for a product that it is not so damaging for the environment. Therefore, it must be consider not only economical reasons but also ecological aspects in order to progressively replace non-renewable fossil stocks [2, 3] by changing the focus to the develop of alternative energy production platforms and producing chemicals using technologies capable of using biomass as a substrate [4]. Exploring a cheap, clean and renewable energy source has become a common goal since the 1970's fossil fuel crisis in which, as nowadays, the global economy was highly dependent on petroleum-based energy sources [5]. Initially, the use food crops as raw-material was presented as a solution. However, concerns about the feedstocks sustainability have risen, including the impact it may have on land use since the feedstock will compete directly with the food chain crops which originated the increase of the food prices in the past [5, 6]. There is also concerns about biodiversity impacts in communities such as destruction of natural life by this unfair competition between crops used for biofuels production and the natural habitats. More specifically, it is also discussed that bioethanol and biobutanol for gasoline and diesel mixtures, respectively, are not an efficient CO_2 emission abatement technology. This means that to achieve the goals of carbon neutrality in the future, the fixation of carbon and subsequent reduction on greenhouse gases emissions, it is necessary to have a more sustainable technology [6].

Lignocellulosic Biomass (LCB) from agricultural and forest residues are an example of a sustainable, self-renewable and low-cost resource that can be converted into a large spectrum of products including fuels and chemicals on a large scale due to its high content of polysaccharides [1]. Approximately 200×10^9 tons of

LCB are produced every year worldwide making this the most abundant renewable biological resource on earth [7, 8, 1]. Organic agricultural wastes (agricultural byproducts) are by definition: (1) renewable; (2) available in abundance; (3) source of fibers, chemicals and other industrial products; and (4) far less costly than other feedstocks (crude oil, natural gas, corn kernels, and soy oil) based on the Price/Energy ratio [5, 7]. Consequently, there as been an increase in LCB processing research, focusing particularly on forest and agricultural residues. The challenge that comes with using this type of raw materials consists on overcome the inherently complex and heterogeneous composition as long as its recalcitrance to conversion reactions [2, 4, 1].

Lignocellulose is composed by cellulose, hemicellulose, lignin, extractives and ash. Both the cellulose and hemicellulose fractions are polymers of sugars and thereby a potential source of fermentable sugars. Lignin and the organic and inorganic components usually referred as extractives and ash, respectively, can be used for the production of chemicals, combined heat and power or other purposes [8].

With the usage of LCB raw-materials in the bioproduction technologies solved the direct conflict between this type of substrate and the food chain and, by this, accomplishing the objective of global food security [9], but, the use of biomass as a resource for energy and fuel production is limited by maximum production rates and the supply of biomass, and so, nowadays, the technologies in place can not overcome the high energy and fuel demand [4]. With the actual available scientific know-how, even if all the worldwide LCB was only used to produce energy or fuels, it would only cover 20 % of the actual global demands [10]. The relatively low energy content, seasonality and non-uniform geographic availability of LCB have been identified as major obstacles to the large volume production of bioenergy and biosubstances [11], when compared to the traditional chemical production. It is well justified to pursuit this economic opportunity for the development of bio-sourced chemical products since this market niche value is comparable to the fuel industry, but only requires a fraction of the biomass [4].

The overall goal of a bio-based production complex is the generation of a variety of goods from different biomass feedstocks through the combination of different multi-step hybrid technologies from different fields of research including polymer chemistry, bioengineering and agriculture. The end goal, or the ideal biorefinery concept, is to integrate, in the same biomass platform, conversion into fuels, power, biomaterials and biochemicals, allowing also the development of waste valorization procedures in order to get as much value of as possible from all the outlet streams [12]. In this concept, the term waste as something to discard or deposit is completely obsolete and it should be rethinked and looked at as a resource for further valorization [4]. The purpose and aim of this master thesis work is to study the first step of biomass treatment right after raw material selection, which involves make it more amenable for sugar production, the pretreatment step, using a combination of a variety methods, being them physical, physicochemical, chemical or biological. Previous works have been done in order to characterize this platforms in terms of processing, advantages and disadvantages of several combinations of protocols, sugar profile, inhibitors concentration, with aim to a future fermentation and its specific requirements [13].

This work motivation is focused on acquiring if wheat straw, an agroindustrial residue, can provide the sugar extraction concentration and profile for specific fermentation processes with a threshold of cell growth inhibitors by changing the operation conditions of pretreatment that this LCB will be submitted. If this goal is achieved, further developments will consist in conducting a cost-effective sugar production study, having in consideration nutrient recycling, waste stream management, and carbon dioxide valorization in order to minimize the ecological footprint of the process.

2. Methodology

2.1. Raw-material

The LCB used in this work was wheat straw harvested in 2019 in the region of Margarethen am Moos, state of Lower Austria and stored at TU Wien lab, at room temperature in a closed polyethylene terephthalate (PET) box away from direct light. The particle size was reduced in a cutting mill, equipped with a 2 mm mesh, before pretreatment.

This wheat straw was previously described by Serna-Loaiza et. al (2020) [14], following the National Renewable Energy Laboratory (NREL) protocol [15] for the determination of structural carbohydrates, lignin, extractives and ash in biomass. This authors also determined this raw-material moisture content, useful not only, to determine the components weight percentage in a wet basis, but also for sample preparation. The moisture content was assessed using Sartorius® moisture analyser model MA 150.

2.2. Fractioning of wheat straw raw-material

LHW, or autohydrolysis, was carried out at laboratory scale in a stainless steel high pressure autoclave STR (Zirbus, HAD 9/16, Bad Grund, Germany) provided by TU Wien (Institute of Chemical, Environmental and Bioscience Engineering, Vienna, Austria). The reactor has a working volume of 1 L and maximum temperature and pressure of 250℃ and 60 bar, respectively. The autoclave is equipped with two external mantles for heat exchange, one electric for heating and other connected to the tap water grid for cooling. The stirrer used was a single turbine impeller with 4 vertical blades with a left sloped cut on the edge of each of them for better mixture. Each run was performed with a clockwise rotation of 150 rpm.

The LSR was 11 grams of type 1 water to 1 gram of dry wheat straw, for every run.

The experiments were carried out using isothermal conditions in which the reactor was set to be heated until it reaches the temperatures of 160, 180 and 200 ˚C and held at that set point for 30, 60 and 90 minutes for each run. This 9 combinations of experimental settings were made in triplicate, making the total number of 27 experiments. Once the holding time was achieved, the reactor was then cooled down until the inside product enters

thermal balance with the cooling stream. The heating profiles considering heating, holding and cooling phases were plotted. After the cooling step, the reactor was opened and the remaining liquid and solid phases were recovered. The pretreated mixture was weighted for further mass balances assessments. The separation between these two phases was conducted using a regular stockings nylon membrane (Clever ®) where the solids were putted inside the bag-like sock and separated using a hydraulic press model HAPA-Presse HPH 2.5 with work pressure up to 150 bar to avoid the filtering membrane bag disruption. The pretreated solid and liquid fractions were weighted for further mass balances assessments. The remaining liquid phase was centrifuged using the Sigma 4K15 ultracentrifuge (Linder Labortechnik ®) at 14,000 rpm for 20 min at the set up temperature of 20 ºC for the supernatant recovery. The remaining pellet was discharged.

The remaining liquid fraction was stored in a 500 mL Schott ® flask at 4 ºC for future use and assessment. The supernatant liquid was weighted for further mass balances assessments. On the other hand the remaining solid fraction, after the drying step (see subsection 2.3), was stored in a closed zip plastic bag at room temperature. All the weighting steps were performed using the Sartorius ® scale model GP 4102, with an maximum error of $\pm$ 0.01 g.

2.3. Remaining fractions moisture and solid content

A sample of both remaining solid and liquid phases were then assessed in terms of moisture and solid content, respectively. In order to do this, glass vials were used, previously dried and tared in a drying oven VENTI-Line VWR ® at the set up temperature of 105ºC. The vials were cooled down in a desiccator and both samples from the two fractions were put inside the vials and the total weight was noted. After this step, both samples were dried over night in the same oven under the conditions described before. It was used a single sample for the liquid fraction solid content assessment and a duplicate for the moisture content in the remaining solids. In the following day, the samples were cooled down in the desiccator, as before, and weighted again. All the weighting steps were performed using an analytical scale KERN ® ABT 320-4NM, with a maximum error of $\pm$ 0.001 g.

2.4. Liquid fraction density

The density of the remaining liquid fraction was measured in a Mettler Toledo ® DE45 Delta Range Density Meter by injecting the desired liquid through the inlet capillary tube in order to get a continuous liquid inside the equipment, specifically, without any air bubbles. Three measurements were made it was assumed the mean value was used for computation purposes.

2.5. Mass balance and losses report

With the objective of assessing the mass losses between in the downstream processing steps it was measured the various weight of both solid and liquid fractions, before and after each step. All the weighting procedures were performed using the Sartorius ® scale model GP 4102, with an maximum error of $\pm$ 0.01 g.

2.6. Ash Content

The determination of the ash content follows the NREL protocol [15]. For most of the experiments, the ash content weight that was obtained was always bellow the equipment determination error (data not shown). The only experiments that showed positive values indicated an ash content in the solid samples of approximately 2%wt and of 0.06 %wt in the obtained extracts. This value was considered constant for all the extractions and equal to 1.09 $\pm$ 0.07 %wt.

2.7. Lignin content in the liquid fraction

For the lignin quantification in the lignin fraction it was applied the NREL Protocol [16] based on a quantitative acid hydrolysis of the dry matter content of the extract. This protocol assess the acid soluble lignin (ASL) and the acid unsoluble lignin (AIL) concentration for each trial. After drying enough volume of liquid fraction sample in the drying oven as described before, it should be weighted 300 $\pm$ 1 mg of the remaining solids in an autoclave resistant Pyrex ® tube. After adding also 5 ml of 72 % sulfuric acid (H_2SO_4) the sample was kept at 30℃ for 1h in a water-bath and stirred with a glass rod every 10 minutes. The sample was then diluted in order to get an (H_2SO_4) concentration of 4 % in the total sample volume and then this mixture was autoclaved at 121℃ for 1h. After this, the samples were cooled down to room temperature and vacuum-filtered. The filtration was performed with a Büchner funnel and filter paper Sartorius ® grade 388, previously weighted, and a diameter of 110 mm. The first liquid fraction was collected and used for the determination of the ASL. Then, the tube was washed with water to collect all the solid in the filter paper. To obtain the value of ASL concentration, the permeate is then analysed in the spectrophotometer, at 205 nm, and diluted accordingly to be in the range of absorbance between the values of 0.6 and 1. Considering that the remaining solids in the retentate correspond to the AIL value, the paper filter and its content is dried over night as described before and, when it is back at room temperature, the final weight is noted and the concentration value assessed.

2.8. Degradation products in the liquid fraction

Furfural, 5-hydroxymethylfurfural (HMF), and acetic acid concentrations were determined accordingly to the NREL protocol [16] using high preformance liquid chromatography (HPLC) (LC-20A HPLC system, model SPD-M20A IVDD, Shimadzu, Japan) by UV and RI detection with a Shodex SH1011 analytic column at 40℃ with 0.005 M (H_2SO_4) as mobile phase and a flow rate of 0.6 mL/min. An ultra-centrifugation step was made, as described before, prior to sample insertion, so the does not get clogged. At least, 20 µL of the remaining supernatant volume was taken, transferred to the correspondent equipment glass vials and placed in the respective HPLC sample tray. A stock solution of the measured degradation products was prepared and diluted accordingly. These standards were used to calculate a calibration curve, from which the concentration of the analyzed samples was determined.

2.9. Sugar concentration in the liquid fraction

During the LCB pretreatment, carbohydrates, which make up the most of the biomass content, are released to the liquid fraction in the form of soluble sugars. These polysaccharides consist mainly of glucose, xylose, arabinose, galactose and mannose. In order to assess how the sugar concentration behaves with changes in the LHW fraccioning conditions, when pretreating LCB, the sugar concentrations within the LHW outlet liquid fraction were quantified by subjecting all arrays to a hydrolyzed and non-hydrolyzed sugars assessment, that provides information about the amount of total and monomeric sugars present in the solution, respectively. Monomeric sugars were analyzed using HPAEC-PAD (ICS-5000, Thermo Scientific, USA) with deionized water as eluent. Oligomeric sugars were hydrolyzed (diluted sulfuric acid) at 120 ºC and analyzed as monomers. A sugar recovery standard was used to account for losses. For monomeric sugars, a 1 mL sample was taken from each trial liquid fraction, diluted with a factor of 1:20, and then put to analyse in the HPAEC equipment. The hydrolization of the remaining oligomers must be made for the total sugar's concentration assessment. It was added 1 mL of a 4% (H_2SO_4) solution to 0.5 mL of each arrayand then make up the volume with water to 10 mL, obtaining a 1:20 dilution factor. An analytical set of Sugar Standard Solutions (SSS) was prepared, accordingly to the NREL protocol [16], for calibration and control of the area of integration *vs* sugar concentration behavior. Nevertheless, a proper assessment and correction of losses due to decomposition of sugars during dilute acid hydrolysis must be made. Therefore, a set of Sugar Recovery Standards (SRS) was also prepared, accordingly to the NREL protocol [16]. All of this mixtures must be done in autoclave resistant Pyrex ® vials with their respective lid in order to avoid liquid transfer losses or leaks. The samples, the SSS and the SRS, were then put to react in an autoclave at 121 ºC for 1h, similar to the procedure described in subsection 2.7. After cooling down, it followed another dilution step of 1:10, in order to make the final dilution factor 1:200, and an ultra-centrifugation step by an Eppendorf ® centrifuge model 5418 R for 20 min at 14 500 rpm with the goal of avoiding HPAEC equipment capillary lines from clogging with precipitated lignin that might be present in the liquid sample. The supernatant is then transferred to proper equipment glass vials and putted in the respective HPAEC equipment tray for sugar determination. For both assessments, if the obtained concentration picks were not within the range of the standards used for calibration, the samples were diluted to fulfil this criterium, accordingly to the NREL protocol [16].

2.10. Severity Factor

The Severity factor, proposed by Overend and Chornet (1987) [17], can be computed using the following mathematical expression:

$$R_0 = \int_0^{t_{op}} exp\left(\frac{T(t) - T_{ref}}{\omega}\right) dt \qquad (1)$$

In which T(t), in Celsius, gives the temperature profile with the reaction time, t, in minutes and T_{ref}, in Celsius, is the reference temperature. Previous authors have fixed the value of 100 ºC for this reference temperature. In order to be able to compare and discuss the obtained results with them, in this work, this value was also used in all the calculations. The time of operation, t_{op} (min) is also considered to be the time for which the reactional mixture is above T_{ref}.

The empirical parameter ω, is commonly assigned to the value of 14.75 (dimensionless), assuming an overall reaction following first-order kinetics and Arrhenius relation of temperature [18].

2.11. Empirical modelling

Several models based on the experimental data were tried making use of the Microsoft ® Excel program for Mac version 16.42, using the function Linest adapted to non-linear models. The degradation products, lignin and sugar concentrations were modeled, having in consideration the R_0 given by the operation conditions harshness towards the raw-material. However, due to the profile behavior of sugars and lignin, combined with a small number of data points to be modeled, polynomial models, represented by the general equation 2, were the only ones that could reproduce the experimental data for this components with some goodness of fit.

$$y(x) = a_n x^n + a_{n-1} x^{n-1} + ... + a_1 x + a_0 \qquad (2)$$

Regarding the models used to describe the three degradation products of this pretreatment step are one-phase association, represented by equation 3, and allosteric sigmoidal, decribed by equation 4, in the following equations:

$$y(R_0) = y_0 + (Plateau - y_0) \cdot (1 - exp(-K \cdot R_0)) \quad (3)$$

Where y_0 is the value when R_0 is null and it has the same units as $y(R_0)$; $Plateau$ is the y_0 value at infinite times, expressed in the same units as $y(R_0)$ and; K is the rate constant, dimensionless.

$$y(R_0) = \frac{a \cdot (R_0)^h}{(b + (R_0)^h)} \qquad (4)$$

Where a is the maximum product concentration, in the same units as $y(R_0)$. It is the highest given product concentration extrapolated to very high severity factor, and therefore, is almost always higher than any measured for a given experiment. The variable b equals $(K_{half})^h$, being K_{half} the severity factor that produces a half-maximal degradation product concentration and; h is the hill slope.

3. Results & discussion

3.1. Wheat straw fractioning

The evaluation criterium used to state if there the pretreated sugar solution was viable to be analysed was to observe if the respective temperature profile, with time, presented three well defined phases: heating; holding; and cooling. The resulted temperature profiles are present in figure 1.

It is clear that, considering the mean value for each set of conditions, the three phases are clearly defined and the resulted liquid fraction was used to quantify the components described before.

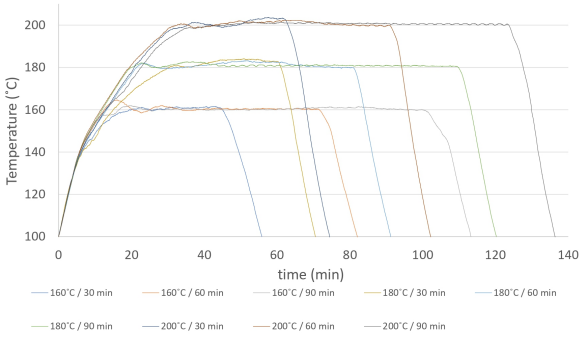

Figure 1: Inner product temperature profiles with the time of operation for a given set of holding conditions (temperature and time).

3.1.1 Monomeric sugars

The monomeric sugars concentration was evaluated in order to assess if this sugar enriched medium can be used in monomeric specific fermentation processes without the need of further depolymerization or detoxification steps, having in consideration the type microorganism that will use this sugar solution as substrate, for example, bacteria. The concentration profile with the severity factor for monomeric pentoses (C5), hexoses (C6), and the sum of both, are plotted in figure 2.

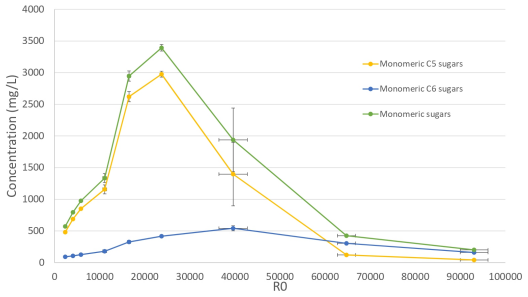

Figure 2: Profile of monomeric pentoses (C5), hexoses (C6) and the sum of both concentrations with the severity factor. All the experimental markers have their respective standard deviation bars.

When comparing the maximum C5 monomeric sugar concentrations in this work (3.0 $\pm$ 0.05 g/L) to the ones reported by Beisl et. al (2019) [19] (0.2 g/L), directly from the autohydrolysis liquid fraction and prior to the concentration step, it is interesting to observe that increasing the holding temperature from 120 to 180 ℃ and decreasing the holding time from 120 to 90 min results in a 15-fold increase in C5 monomeric sugar concentration values. When looking at maximum C6 monomeric sugar concentrations, Beisl et. al reports a 2.2 times higher value (1.2 g/L) when comparing to this work's concentration (0.5 $\pm$ 0.04 g/L), meaning that cellulose is being more deconstructed in Beisl et. al work than in this project. If the goal is to produce more monomeric sugars, either C5 or C6, then the protocol to follow should be the one used in this work, meaning, 180 ℃ and 90 min, with a severity factor of 23754.2 which gives a monomeric sugars concentration value of 3.4 $\pm$ 0.05 g/L. An important consideration to have, when applying this operational setup, is to combine the pretreatment with a detoxification step, if the microorganism used to ferment this sugars is susceptible to growth inhibition, namely, *P. stipitis* [20] and *S. acidocaldarius* [19] as exploited in Degradation products analysis. When analysing the concentration profile of monomeric sugars originated by the pretreatment step, it is clear that the mono C5 profile is similar to the total monomeric concentration for the conditions with a severity factor less than 64763.9 (200 ℃ and 60 min of holding conditions), which is expected since the main objective of this method is to produce hemicellulosic sugars, being them, mostly, C5-type sugars. When this severity factor value is surpassed, the C6 sugar concentration exceeds the one of C5, meaning that the pretreatment method is being so harsh on the raw-material that, by this point, not only the main sugar source not anymore hemicellulose, but instead, cellulose, but also that hemicellulose-derived sugars are already being degraded into HMF and furfural. Both this factors decrease the value-added product concentration in the solid stream, reducing the overall process revenue, and, make unusable the use of this sugar solutions to fermentation processes, respectively.

In order to modulate the monomeric sugars profile with the severity factor, using polymeric models, it was assessed how the experimental data fitted the polynomial models. The parameters for modeling monomeric sugars are described in table 1, as well as the respective correlation coefficient (R^2 and the severity factor domain of application.

Table 1: Monomeric (Mono) sugars modeling parameters, for pentoses (C5), hexoses (c6) and the sum of both, as well as the respective correlation coefficient (R^2) and the severity factor domain of application.

	a_5	a_4	a_3	a_2	a_1	a_0	R^2	Domain
Mono C5	-	-	-8.00E-10	3.00E-05	-0.1883	910.51	0.9708	[2385.2;23754.2[
	-	-	-	1.00E-06	-0.1578	6140.7	0.9992	[23754.2;93064.7]
Mono C6	-2.00E-21	6.00E-16	-5.00E-11	2.00E-06	-4.20E-03	9.41E-01	0.9938	[2385.2;93064.7]
Mono Sugars	-	-	-8.00E-10	3.00E-05	-0.2047	1033	0.9738	[2385.2;23754.2[
	-	-	-	9.00E-07	-0.1526	6522.6	0.9996	[23754.2;93064.7]

The correlation coefficient (R^2) is always higher than 0.97 which indicates a good fitting between the experimental values and the predicted model concentration, in the respective domain.

3.1.2 Total sugars

The total pentoses (C5), total hexoses (C6) and the sum of both concentrations profile with the severity factor are plotted in figure 3.

In this case, analysing the concentration profile of the total sugars, meaning, all the sugars in the less polymerised (more hydrolyzed) condition possible, it is clear that the total C5 profile is similar to the total sugar concentration for the conditions with a severity factor less than 39628.2 (200 ℃ and 30 min of holding conditions), which is again and indicator that LHW method is producing, mainly C5 sugars from hemicellulose. This concen-

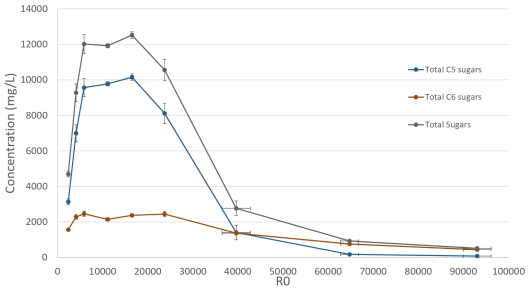

Figure 3: Profile of total pentoses (C5), total hexoses (C6) and the sum of both concentrations, with the severity factor. All the experimental markers have their respective standard deviation bars.

tration value is exceeded by the C6 sugars by the same reasons described in Monomeric sugars, nonetheless, it happened now at a lower aggressive condition (200˚C for 30 min) than before. This can be explained by changes in the degree of polymerization comparing both type of sugars. For the first case, there was a higher concentration of large polymer molecules (e.g.: oligosacharides) susceptible to be hydrolyzed and the energy supplied to the system, via heat exchange, was being used to depolymerize this molecules into monomers. Since in the total sugar assessment all the samples have been hydrolyzed *a priori* (see subsection 2.9) the only incidence of the thermal energy is into the hydrolyzed sugars (monomers) which will led to the conversion into pretreatment degradation products at a higher rate than before.

Comparing the maximum total C5 sugar concentrations in this work (10.1 ± 0.2 g/L) to the ones reported by Beisl et. al (2019) [19] (0.6 g/L), similarly to subsection 3.1.1, it is again observed that increasing the holding temperature from 120 to 180℃ and decreasing the holding time from 120 to 60 min results in a even higher 17-fold increase in total C5 sugar concentration values. For total C6 sugar concentrations, Beisl et. al now reports less concentration of this type of sugars (2.1 g/L) when comparing to this work's concentration (2.5 ± 0.15 g/L). An interesting assessment to make is that increasing the holding temperature from 120 to 160℃ and decreasing the holding time from 120 to 90 min results only in a 20% increase in total hexose sugar concentrations. If the goal is to produce more total sugars, either C5 or C6, then the protocol to follow should be the one used in this work, meaning, 180 ℃ and 60 min, with a severity factor of 16525.3 which gives a total sugars concentration value of 12.5 ± 0.2 g/L. Again, this condition is already above the thresholds reported previously by Nigam et. al (2001) for *P. stipitis* [20] and Beisl et al. (2019) for *S. acidocaldarius* [19].

The maximum C5 total sugar concentration (10.1 ± 0.2 g/L) was obtained at a severity factor of 16525.4 (180℃ for 60 min), however, it has a similar concentration value to other conditions with a lower severity factor, more specifically, using the set temperature at 180˚C and 60 min holding time (R_0 of 11131.5), and 160 ˚C for 90 min (R_0 of 5884.8). This indicates that, at 160 ℃ of temperature and 90 min holding time, the hemicellulose sugars possible to be solubilized into the liquid fraction reaches a plateau of maximum extraction. Since the rate of production is decreasing, despite of having the extraction maximum only at 180 ℃ and 60 min holding time, it indicates an increase in secondary reactions, forming degradation products from this hemicellulosic sugars. After the concentration maximum is reached, the pretreatment method is being so aggressive on the raw-material that, by this point, the rate of sugar production from hemicellulose hydrolysis is surpassed by the rate of degradation products formation. This degradation products surpass the total sugar concentration for the condition of 200℃ for 30 min (R_0 of 39628.2) which is a clear evidence of the shift in the reaction equilibrium towards degradation products formation.

When looking at the C6 sugar concentration profile, after it reaches is maximum at the severity factor of 5884.8, there is a decrease in the total sugar concentration by 13% when comparing to the next harsh operation condition (R_0 of 11131.5), however, the sugar concentration increases again, in 10 %, for the following condition, with the severity factor value of 16525.4. This could be an indicator of cellulose disruption and subsequent increase in the production of C6 sugars, mainly glucose, having this matrix has sugar source. The HMF formation, that has its origin from C6 sugars, needs a higher severity factor value to be produced than the case of furfural, which use C5 sugars as substrate. This can be justified since, in this conditions, the energy transferred into the reactional mixture is still being used for the disruption of cellulose and not for the production of degradation products.

After the condition that gives the maximum of total sugar concentration, the more harsh it was towards the wheat straw, the more this concentration decreases, meaning that the pretreatment method is being so aggressive on the raw-material that, by this point, the applied protocol is not producing sugars, but instead, degradation products.

In order to modulate the total sugars profile with the severity factor, it was assessed how the experimental data fitted the polynomial models. The parameters for modeling total sugars are described in table 2 as well as the respective correlation coefficient (R^2 and the severity factor domain of application.

Table 2: Total sugars modeling parameters, for pentoses (C5), hexoses (c6) and the sum of both, as well as the respective correlation coefficient (R^2) and the severity factor domain of application.

	a_5	a_4	a_3	a_2	a_1	a_0	R^2	Domain
Total C5	1.29E-19	-3.39E-14	3.28E-09	-1.39E-04	2.2041	-475.63	0.9709	[2385.2;93064.7]
Total C6	-	-5E-16	1E-10	-0.000007	0.1506	1528.7	0.9252	[2385.2;93064.7]
Total Sugars	1.26E-19	-3.38E-14	3.34E-09	-1.45E-04	2.3398	1092.8	0.965	[2385.2;93064.7]

The correlation coefficient (R^2) is always higher than 0.925 which indicates a good fitting between the experimental values and the predicted model concentration, in all of the domain of experimentation.

3.2. Degradation products analysis

Since degradation products may condition future fermentation steps, the next criterium to consider needs to be the already cited degradation products concentration thresholds. The degradation products experimental data and models with the corresponding severity factor (R_0) are plotted in figure 4.

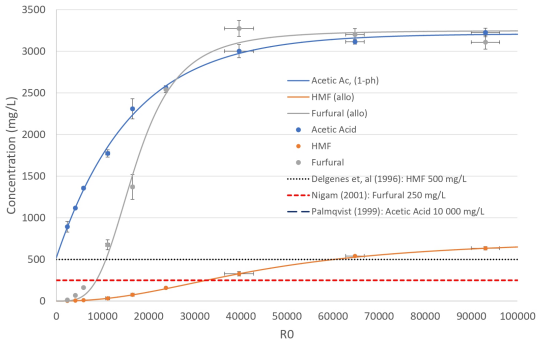

Figure 4: Degradation products experimental data and models with the corresponding severity factor (R_0). It is plotted the best fitting model for each by-product having in consideration the correlation coefficient (R^2), giving: one-phase association model (1-ph) for acetic acid and allosteric sigmoidal model (allo) for both HMF and furfural. Inhibitory concentration thresholds are also drawn using horizontal lines. Acetic acid limit is not represented since a concentration of 10 g/L of this degradation product is out of this project scope for all the experimental conditions. All the experimental markers have their respective standard deviation bars.

Considering the previous studies of Palmqvist et. al (1999) [21], that states that acetic acid presents inhibitory behavior on yeast growth only when it reaches the concentration of 10 g/L, all the conditions are within this limit. The raise on this acid concentration can even be helpful since it auto-catalyzes the hydrolysis of hemicellulose, resulting in a more effective hemicellulosic sugar solubilization and extraction [22]. With HMF, it seems that this degradation product also did not interfere, at least significantly, in the cellular growth. Sanchez & Bautista (1988) [23] stated that HMF only had the effect of increasing the yeast culture lag phase. Other approach was made by Delgenes et. al [24], cited by Mussato et. al (2004) [25], reported that when using the yeast *Pichia stipitis*, a 43 % reduction in cellular growth is achieved when the concentration of 0.5 g/L HMF is achieved. Nevertheless, even considering this more conservative approach for this study, this value is only achieved when using the condition of 200 ℃ and 60 min holding time (or higher). When analysing the outcome concentration for furfural it presents a higher concentration in all the operational conditions when compared to the previous component. This was expected since furfural is the decay product of C5 sugars which are the main type of sugars from the hemicellulosic matrix. On the other hand, HMF is the degradation product from C6 sugars, the main component of cellulose. For furfural, again, in Delgenes et. al (1996) work [24], still with the *Pichia stipitis*, reported a reduction of 25% on cellular growth when furfural concentration reached 0.5 g/L. For the same yeast type, Nigam et. al (2001) [20] claim that a concentration of 0.25 g/L reduces 10 % in the ethanol production yield. When analysing the concentration of furfural in this work, for both publications, this is only obtained when using the 160 ℃ as holding temperature. For the maximum holding time of this set of experiments (90 min) the furfural concentration is still within range. For another type of yeast, *Saccharomyces cerevisiae*, the work of Sanchez & Bautista (1988) [23] presents a higher threshold, stating that furfural only begins to affect cellular growth at 1.5 g/L. If this microorganism is used in further valorization processes, that could mean to use only a maximum of harshness conditions, for the pretreatment of wheat straw, of 180 ℃ and 60 min holding time.

In order to better predict the degradation products concentration with the severity factor it was evaluated how the data points fitted with the one-phase association and allosteric sigmoidal models. The model parameters for each of the models are presented in table 3.

Table 3: Degradation products models parameters and the respective correlation coefficient (R^2)

	One phase association				Allosteric sigmoidal		
	Acetic Acid	HMF	Furfural		Acetic Acid	HMF	Furfural
y_0 (mg/L)	519.7	-54.63	-778.5	a (mg/L)	3759	756.5	3250
Plateau (mg/L)	3210	1322	3385	h	0.8944	2,236	3.644
K	6.139E-05	7.855E-06	5.243E-05	b	3973	2.503E+10	2.701E+15
R^2	0.9926	0.9829	0.9548	R^2	0.9859	0.9970	0.9911

When analysing the models, it is clear that the parameters definitions for $Plateau$, in the case of one-phase association and a in the allosteric sigmoidal are similar, so the concentration values (mg/L), given by this parameters are expected to be similar. In fact, this parameters, only deviate from each other 16% and 4% for acetic acid and furfural, respectively. However, in the case of HMF there is a deviation of 65%. This can be explained since the HMF concentration did not yet reached a maximum (plateau) for the highest severity factor that was studied, which means that, applying the models out of the range of this operational conditions, regarding this degradation product, led to extrapolations, and consequently, discrepancies, in the values parameterization.

Specifically for one-phase association model, y_0 gives the degradation product concentration for a null severity factor, meaning, the condition of 100 ˚C and no holding time. In the case of acetic acid, this model predicts a concentration of 0.5 g/L for this condition, meaning, that the acetyl groups are already being detached from the hemicellulose matrix at this temperature. On the other hand, for HMF and furfural, this model value is negative, meaning that the production of this compound only starts at more severe operational conditions. Using the inverse function of this model, given by equation 5, and applying the parameter values previously described, it is possible to predict the severity factor value for which this type of degradation products start to be produced accordingly to this model. This equation is described by:

$$R_0(y) = \frac{ln(\frac{Plateau - y_0}{Plateau - y})}{K} \quad (5)$$

Applying equation 5, HMF and furfural start to be pro-

duced at the severity factor of 5155.0 and 3948.2, respectively. In this work, that would have meant that HMF and furfural would only start to be produced at the conditions of around 160˚C for 90 min and 160˚C for 60 min, respectively. Observing the figure 4, it is clear that, on those conditions, this degradation products are already being formed.

The one have given high correlation coefficients for every analysed by-product ($R^2 \geq 0.95$). The goodness of fit was also assessed for the allosteric sigmoidal model. This model produced also high correlation coefficient ($R^2 \geq 0.98$), although this parameter decreased for acetic acid when compared with the previous one, therefore, it was observed a general increase on the relative deviation values using this model, exception made for the severity factor of 16525.3 and 64763.9. For HMF, the allosteric sigmoidal model gave positive concentration values. Based on the previous analysis, it was plotted in figure 4 the models that presented a better goodness of fit to the experimental data, which means, one-phase association for the acetic acid modelling and allosteric sigmoidal model for the case of HMF and furfural.

$$R_0(y) = \left(-\frac{y-a}{b \cdot y}\right)^{-\frac{1}{h}} \quad (6)$$

To evaluate the capacity of the inverse models equations (5 and 6) to predict the maximum operational severity factor for a given degradation product threshold, it was taken the limit values using the previous works of Nigam (2001) [20] and Delgenes et. al (1996) [24], to compute the R_0 value with the for furfural (250 mg/L) and HMF (500 mg/L), respectively. Using the one-phase association model, the values of maximum severity obtained were 65646.5 for HMF and 5411.5 for furfural. In the first case, for HMF, the inverse model equation gives a higher severity factor threshold than the one considered by the experimental data (R_0 of 64763.9); for 200˚C and 60 min) and the most severe condition that can be applied, regarding only this degradation product, is the same. However, for furfural, the maximum condition that does not surpass the furfural concentration threshold is now 160˚C for 60 min (R_0 of 4109.3). For the allosteric sigmoidal model, the values of maximum severity obtained were 60272.3 for HMF and 8682.1 for furfural. For HMF, the inverse model equation gives a lower severity factor threshold than the one considered by the experimental data (R_0 of 64763.9; for 200˚C and 60 min) and the most severe condition that can be applied, regarding only this degradation product, is now 200℃ and 30 min (R_0 of 39628.2). For furfural, the maximum condition that does not surpass the furfural concentration threshold is the same as for the experimental data, which means, 160˚C for 90 min (R_0 of 5884.8).

3.3. Lignin concentration analysis

The main goal of using LHW is to extract hemicellulosic sugars while maintaining, as much as possible, the other value-added components structure, such as lignin. If substantial lignin solubilization takes place in this pretreatment step, a further lignin extraction method, such as organosolv, might not be, neither efficient, nor even economically viable. Therefore, the goal regarding lignin, should be to maintain, as maximum as possible, this component in the solid fraction; in other words, avoid lignin solubilization and solubilization into the liquid stream. Also, it is in this work' scope to assess whether there is a critical point in lignin solubilization when increasing the severity factor. The AIL, ASL and total lignin concentration profiles are present in figure 5.

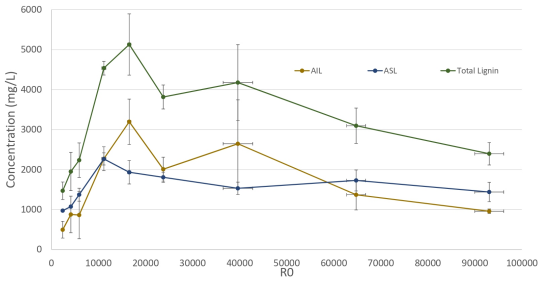

Figure 5: Profile of acid insoluble lignin (AIL), acid soluble lignin (ASL), total solubilized lignin (AIL + ASL) concentration, with the severity factor. All the points have their respective standard deviation bars.

This maximum point of lignin extraction occurs at the severity factor of 16525.3 (180℃ and 60 min holding conditions). After such conditions it is correct to state that the extracted lignin undergoes significant disintegration reaching, for the highest severity factor value, approximately 50% of the maximum total lignin extraction value. When analysis lignin it is clear that the total lignin concentration suffers a significant increase, about 2 times in the sum value of acid soluble and acid insoluble lignin, when increasing the severity factor from 5884.8 to 11131.5, which corresponds to an increase in the temperature of operation from 160 ℃ to 180˚C. For this project purposes, there is no interest on this increase in lignin solubilization into the liquid fraction since this sugar solution will be used for valorisation purposes, such as fermentations, which will not use lignin. Following this criterium, it is recomended to use a less harsh temperature than 180℃ to avoid an increase in lignin convertion.

In order to modulate the total lignin profile with the severity factor, using equation 2, it was assessed how the experimental data fitted the polynomial models. The parameters for modeling total lignin are described in table 4 as well as the respective correlation coefficient (R^2 and the severity factor domain of application.

Table 4: Lignin modeling parameters and the respective correlation coefficient (R^2) and the severity factor domain of application.

	a_4	a_3	a_2	a_1	a_0	R^2	Domain
Total Lignin	-1.21E-18	2.64E-13	-1.94E-08	0.000522	0.22	0.8559	[2385.2;93064.7]

The correlation coefficient (R^2) is 0.86 which indicates a reasonable fitting between the experimental values and the predicted model concentration, in all of the domain of experimentation.

4. Components simultaneous analysis

Since this work objective is to study sugar production in a biomass refinery context, an integrated analysis of all the assessed components must be made in order to chose the best fitting operational condition to use in larger scale applications.

This pretreatment method was able to solubilize a maximum monomeric sugars concentration of 3.4 g/L. In the work of Beisl et. al (2019) [19], a concentration of 1.4 g/L of monomeic sugars was not enough to fulfil the fermentation needs of *S. acidocaldarius*. After a concentration step, the same authors reported a 7.0 g/L concentration of total monomeric sugars. Applying a similar concentration procedure to the sugar solution obtained, in this work, at 180˚C for 90 min, would increase all of the components concentration, including degradation products, which, as described before, is inhibitory of fermentation procedures. However, when looking at the total sugar concentration values, it is observed that the maximum (12.5 g/L) is obtained at 180˚C and 60 min, without any concentration steps, which is close to the total sugar concentration stated by Beisl et. al (13.7 g/L), but in this case, after a concentration procedure. Nevertheless, for this condition, the degradation products threshold was already surpassed. The limiting concentration value considered in this work, as stated before, only allows the implementation of sugar solutions obtained from an extraction procedure with a severity factor of 5884.8, or less. The total sugar concentration at this severity factor (12.0 g/L) is around 96 % of the maximum total sugar concentrations that can be obtained and around 88 % of the concentration stated by Beisl et. al (2019), which means that this procedure it is close to fulfil this microorganism sugar requirments, since oligomeric sugars are used as substrate for some microorganisms (namely fungi), which includes *S. acidocaldarius*. The solubilized lignin, applying this pretreatment conditions (160˚C for 90 min), is around half of the maximum value for this work, which means, that lignin conversion is being kept to a minimum, as much as possible, and the solid fraction is still viable for further delignification processes.

This allows the statement that the best fitting conditions, of the ones that have been subjected to analysis, is to maintain a reaction temperature of 160 ˚C for 90 min.

5. Conclusions

The use of wheat straw as lignocellulosic raw-material for sugar production and further valorization in the biomass refinery context has been proven to be effective. This work assessment has successfully fractioned the LCB component of interest while maintaining the remaining valiable structure largely unscattered, by using an highly available agro-industrial residue that, due to its natural recalcitrance, presents significant disposable challenges in its source. It was proven that this secondary product of wheat crops can be reused into the biotechnology concept and it has open the spectrum of biobased substances production using a more sustainable process than the ones currently being used in classical chemical industry.

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