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Pretreatment and enzymatic hydrolysis for the efficient production of glucose and furfural from wheat straw, pine and poplar chips

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Abstract

A flexible approach to a two-step Biorefinery for the production of glucose and furfural from three different feedstocks is presented. Pretreatment conditions were selected to drive the production towards the generation of glucose or furfural. Harsh pretreatment conditions produced solids with highly accessible glycan contents for the enzymatic hydrolysis with 100 % glucose yields when wheat straw or poplar chips were used as feedstock. Mild conditions afforded xylan-rich hydrolysates that could be efficiently transformed to furfural, either under conventional or microwave heating in biphasic media. Yields for the transformation of xylan from feedstocks ranged between 45 % and 90 % depending on the feedstock, the thermal pretreatment and the cyclodehydration conditions. Up to 12.6 kg of glucose and materials and 2.5 kg of furfural can be produced starting from 50 kg of biomass. A new analytical methodology based on ^{13}C NMR that provided good quality analytical results is also presented.

Keywords: Biorefinery, thermochemical pretreatment, enzymatic hydrolysis, glucose, cyclodehydration, biphasic medium, microwave, scale up, NMR quantification

1. Introduction

In recent years, an increasing effort has been devoted to replace fossil fuels and fossil-fuel derived chemicals (Barta el al., 2014; Martin et al., 2017). Lignocellulosic biomass, such as forestry and agricultural residues, is widely available worldwide at competitive prices and, even more important, its use as feedstock does not compete with human nutrition requirements. High production rates and low feedstock cost of lignocellulosic

biomass make them very attractive raw materials for the production of 2G biofuels, chemicals and materials (De Bhowmick et al., 2018). Lignocellulosic biomass is roughly composed of cellulose, hemicelluloses, and lignin, as well as small amounts of extractives (Behera et al., 2014). The bioconversion process of lignocellulosic materials to produce bioethanol or other bio-fuels requires three main steps including (Kumar et al., 2010): i) pretreatment to increase the accessibility of cellulose, hemicelluloses and lignins while minimizing the formation of by-products; ii) enzymatic hydrolysis of the cellulosic components to monomeric sugars that can subsequently be iii) fermented to ethanol and others. Downstream fractions arising from the conversion of lignocellulosic materials to biofuels, hemicellulose and lignin, have been typically underused.

In the last few years, the Biorefinery concept has been defined as "the sustainable processing of biomass into a spectrum of marketable products and energy whilst encompassing a network of facilities that integrate different technologies for bio-based products generation" (Chandel et al., 2018). In this sense, biorefineries may enable the valorization of these downstream fractions into a wide variety of valuable chemicals, increasing the sustainability of the production of biofuels (Chatterjee et al., 2015; Werpy and Petersen, 2004; Liu et al., 2012). This definition points in the same direction than that defined in the circular economy objective from the European Commission (*Towards a circular economy: A zero waste Programme for Europe*, 2014).

The key step in the production of biofuels is the hydrolysis of the cellulosic materials. Enzymatic hydrolysis is an effective and green process to enhanced production of monosaccharides from polymeric sugars for further bioconversion processes. Low enzyme activity and high enzyme costs make this step a critical point in the overall process for the production of bioethanol (Chen and Fu, 2016; Guo et al., 2018). Recently, some studies have already shown the feasibility of recycling these enzymes (Rodrigues et al., 2014), which noticeably increases the sustainability of the process.

The development of efficient and economical pretreatments to break the lignocellulosic matrix is another challenge for the bioconversion of these raw materials. Hydrolysis in diluted sulfuric acid hydrolysis, combined with high temperature and pressure, is a very effective and well-known process to depolymerize and dissolve the hemicellulose by removing the acetyl groups, uranic esters or glycosidic bonds (Seidl and Goulart, 2016) to obtain a glycan-enriched fraction more prone to enzymatic hydrolysis.

Hemicellulose rich fraction is one of the most abundant downstream fractions after acidic hydrolysis of the lignocellulosic biomass, and is mainly composed of pentoses. Pentoses can be converted into furfural. Besides its use as a solvent, furfural can be used as a platform molecule for the synthesis of a number of derivatives: furanic compounds, pentanodiols or liquid alkanes that are potential biofuel components (Bond et al., 2014; Lange et al., 2012). During the last years, intensive research has provided excellent catalysts for the cyclodehydration of xylose to furfural as Lewis acids (Guenic et al., 2015), zeolites (Choudhary et al., 2011), supported sulfonic acids (Wang et al., 2017), or tungstophoric acids (Dias et al., 2006). Despite that, its industrial synthesis still relies on the Quaker Oat's process developed in the 1920's. This process is based on the treatment of biomass in reactors with a mineral acid (*i.e.* sulfuric acid) (Zeitsch, 2000) at 170–185 ºC, being maximum furfural yields comprised between 45 % and 50 % of the total xylan in the raw material. The low cost of feedstock still makes this process viable despite the low overall yield.

The production of furfural using downstream fractions in Biorefineries would be very attractive to improve its sustainability if cyclodehydration of xylose proceeded in much higher yields than in the current industrial process. This improvement in furfural yields must be based on the prevention of the potential formation of degradation products, such as humins, whose origin is attributed to the reaction of xylose with the as-formed furfural in the presence of the acidic medium (Garrett and Dvorchik, 1969; Montané et

al., 2002; Rose et al., 2000; Williams and Dunlop, 1948). Continuous extraction of furfural either by distillation (Mandalika and Runge, 2012), or using biphasic reaction media (Sádaba et al., 2014, Romo et al. 2018) is a promising methodology to prevent humins formation. Indeed, direct production of furfural from biomass has been already reported using aqueous mineral acids as catalyst in MIBK biphasic media with good yields (Delbecq et al., 2018; Zhang et al., 2013). Another challenge in the production of furfural is the intensive use of energy. In this sense, the use of microwave irradiation to activate the reaction may allow to reduce the energy expenses with good yields in the production of furfural either from xylose (Weingarten et al., 2010), xylan (Chheda et al., 2007) or corn stover (Li et al., 2017; Mittal et al., 2017).

Different approaches can be envisaged in the Biorefinery process. For instance, in the so-called one-stage process, furfural is produced from biomass by direct treatment of different feedstocks (Kim et al., 2012) like corncob (Mao et al., 2012), sugarcane bagasse (Mesa et al., 2014), olive stones (Montané et al., 2002), rice husk (Suxia et al., 2012), shorgum straw (Vázquez et al., 2007) or straw (Yemiş and Mazza, 2011). The residual solid is hopefully used as the raw material for the production of fermentable sugars. The two-stage process, however, separates both steps in different reactors allowing their separate optimization that results in a better overall control of the whole process with, *a priori*, higher furfural yields and better quality solid residues (Mandalika and Runge, 2012).

In this paper, we show the optimization of a two-stage process design, which could be used in a hypothetical Biorefinery focused on the production of glucose and on direct transformation of pentoses to furfural.

2. Material and Methods

2.1. Thermochemical Pretreatment

Biomass raw materials have been processed at CENER facilities of Biorefinery and Bioenergy Centre (BIO2C), located in Aoiz (Spain). The thermochemical pretreatment assays were made in a steam pressurized plug-flow horizontal reactor (Advancebio Systems LLC, USA) that allows a high solid load (50 %) and operation up to 192 ºC .The experimental conditions for thermochemical pretreatment were selected based on an experimental design using selected feedstocks (data not shown).

Three sources of biomass were used, poplar (*Populus sp.)*, pine (*Pinus radiata)* and wheat straw (*Triticum aestivum)*. For each feedstock, three parameters were combined at different levels: temperature, residence time and acid/feedstock ratio. Selected temperatures were 164 ºC, 178 ºC and 192 ºC for poplar and pine chips and 173 ºC, 180 ºC and 187 ºC for wheat straw. Residence times were 5 minutes, 10 minutes or 15 minutes for wheat straw and 5 minutes for pine chips and poplar chips. The preparation of the feedstock combinations was carried out previously based on dry matter (50 kg) content and adjusting the moisture up to 50 %. Sulfuric acid (98 %) was added at relations of 1 %, 2 % and 3 % for wheat straw and 0 %, 2 % or 4 % for pine chips and poplar chips to dry weight of the total solid content. Manual mixing was done to achieve homogenization and left overnight at room temperature before feeding the reactor.

Compositional analysis

Samples moisture content was calculated by drying at 105 ± 2 ºC up to constant weight according to CEN/TS 14774-3:2010 procedure. Ash content was determined after combustion for 2 h at 550 ± 10 ºC in a muffle, according to UNE-EN ISO18122:2015. Nitrogen determination was carried out following the UNE-EN ISO 16948:2015 and using a 5.25 conversion factor into protein. Quantification of cellulose, hemicellulose, and lignin content in the solid fraction resulting from thermochemical pretreatments was done according to NREL procedures nº 42618 and nº 42627 respectively.

Quantification of sugars in the liquid fractions resulting from thermochemical treatments was made either after filtration or after further acid hydrolysis of the oligomeric sugars to produce the monosaccharides. This second hydrolysis step was conducted at pH 1, upon addition of 72 % H_2SO_4 (w %), and autoclaving at 121 ºC for 1 h. Samples were stored at - 20 ºC until analysis.

Liquid samples were analyzed by liquid chromatography following three different methodologies $HPLC_1$, $HPLC_2$ and $HPLC_3$. In $HPLC_1$, NREL protocol (Sluiter et al., 2008), sugars were quantified using a HPLC system (Agilent 1200) equipped with a refractive index detector (RID). The stationary phase was an ICSep ION 300 column (7.8 x 300 mm, Transgenomics, Glasgow, United Kingdom) at 72 ºC and 8.5 mM H_2SO_4 aq. was used as mobile phase. $HPLC_2$ methodology was essentially similar, although RHM-Monosaccharide H+ (8 %) column (Phenomenex) at 75 ºC was used as stationary phase and H_2O at 0.4 mL/min flow rate as mobile phase (Sádaba et al., 2014),. Quantification using $HPLC_3$ was made on a Metrohm 940 Professional IC Vario equipped with amperometric detector using Metrosep carb2 150/4.0 (Metrohm) and Metrosep Carb2 guard at 30 ºC as stationary phase. 300 mM NaOH aq. and 1mM sodium acetate at 0.5 mL/min solutions were used as mobile phase.

Sugar degradation compounds after thermochemical pretreatment, such as furans (furfural, **FAL**, and 5-hydroxymethylfurfural, **HMF**) or acetic acid, were systematically quantified by HPLC with DAD index detection using a Zorbax column (250 x 4,6 mm, Agilent Technologies) at 50 ºC as stationary phase and a mobile phase consisting of water: acetonitrile at 80:20 ratio.

^{1}H and ^{13}C NMR were recorded at 300 K in a Bruker ASCEND 400 spectrometer equipped with a PABBO 5 probe, at 400 MHz and 101 MHz respectively. Chemical shifts are given in ppm and referenced using residual signal from C*H*Cl_3 at 7.26 ppm and 4.79 ppm for H_2O for ^{1}H NMR. ^{13}C NMR spectra were referenced using the signal

at - 2.74 ppm for trimethylsilylpropanesulfonic acid sodium salt (**TMPS**). The NMR signal was processed using the commercial Bruker Topspin 3.2 software. Determination of xylose, **XYL**, glucose, **GLC,** and mannose, **MAN**, in liquid samples by ^{13}C NMR was made in D_2O using **TMPS** as internal standard. Quantification of **FAL** and **HMF** was performed by ^{1}H NMR using 1,3,5-trimethoxybenzene (**TMOB**) as internal standard in $CDCl_3$.

2.2. Enzymatic Hydrolysis

The solid fraction obtained after the thermochemical pretreatment was submitted to hydrolysis, using a baseline enzyme cocktail (Cellic® CTec2) kindly provided by Novozymes Reaction conditions were set at 50 ºC, 10 % total solids content and pH 5 with stirring at 180 rpm for 72 hours; pH was readjusted after 24 hours of hydrolysis. Enzyme dosage was 60 g/kg ODM. Samples were analyzed by HPLC for soluble sugars quantification using $HPLC_1$.

Yields for the enzymatic hydrolysis were calculated as follow:

$$glucose\ yield = 100 * \frac{glucose\ obtained\ after\ enzymatic\ hydrolysis\ (g)}{glucose\ in\ the\ feedstock\left({}^{g}/_{100\ gODM}\right) * mass\ of\ feedstock\ (g)/100}$$

Statistical analysis were carried out using Statistica version 13.05.0.17 (TIBCO) software.

2.3. Cyclodehydration of xylose to furfural

Laboratory scale reactions activated by microwave irradiation were performed on a CEM Discover S Class reactor, equipped with IR temperature probe and APD pressure controller, using a constant temperature program. Microwave irradiation was conducted at constant temperature with dynamic temperature/pressure control and a maximum power of 300 W. After the reaction was completed, the reaction mixture was rapidly cooled to 60 ºC with air current. Reactions were made by triplicate. Scaled-up reactions under microwave irradiation were run in an ETHOS One ^{TM} Microwave Digestion

System (Milestone Inc)., kindly provided by GOMENSORO. The digestion system was equipped with 12 reaction vessels (100 mL each) and a microwave diffusor that homogenizes the irradiation in the microwaves chamber. Vessels were charged to a total volume of 50 mL, using 18.75 mL of **WST$_H$** and 31.25 mL of MIBK

Laboratory scale reactions with conventional heating were done in a 100 mL Autoclave Engineers apparatus controlled by PID using a total volume of 50 mL. Semi-pilot scale reactions were made using 4.0 L vessels at CENER facilities. Reactor was charged to a total volume of 2.0 L using 750 mL of **WST$_H$** and 1250 mL of MIBK

For both microwave and conventional reactors, reaction times are referred to the point target temperature is reached, this is the end of ramping.

XYL, **MAN** and **GLC** concentrations in the aqueous phases after cyclodehydration reaction were calculated using ^{13}C NMR. Quantification of **FAL** and **HMF** was done by ^{1}H NMR as described before.

$$FAL\ yield = 100 * \frac{MW_{FAL} * mol\,TMOB\left[\frac{\sum I_{FAL}}{4} \Big/ \frac{TMOB_{5.95}}{3}\right] - initial\ concentration\ of\ FAL\ \left(\frac{g}{L}\right) * V_{hydrolyzate}(L)}{concentration\ of\ xylose\ \left(\frac{g}{L}\right) * V_{hydrolyzate}(L)}$$

Where I_{FAL} refers to the integral values for the signals corresponding to **FAL** at 9.54 ppm, 7.96 ppm, 7.61 ppm and 6.59 ppm and TMOB to the integral value for the signal corresponding to the aromatic hydrogen atoms of 1,3,5- trimetoxybenzene at 5.95 ppm. **FAL** and **HMF** yields are referred to the **FAL** and **HMF** produced exclusively in the cyclodehydration reaction, considering the overall amount of **XYL** and xylan or hexoses in the hydrolysate samples.

3. Results and discussion

Three different feedstocks, wheat straw (*Triticum aestivum)*, poplar chips (*Populus sp.)* and pine chips (*Pinus radiata)*, were chosen for this study given their natural abundance in Navarre. Wheat straw is an abundant herbaceous residue in the agronomical sector.

Poplar and pine, which are abundant in Navarre forestry, were chosen as hard and soft wood models respectively. Compositional analysis of the biomass feedstocks is given in Table 1.

3.1. Thermochemical pretreatment of biomass feedstock

Thermochemical pretreatment of the biomass consisted in the acidic hydrolysis of the biomass using sulfuric acid with the aim of solubilizing the hemicellulosic fraction while keeping intact the lignocellulosic fraction. The slurry obtained after thermochemical pretreatment was filtered, affording a solid and a liquid fraction. Compositional analysis of the as-obtained solid fractions are gathered in (Figure 1). Thermochemical pretreatment of pine chips under mild conditions drastically reduced the presence of xylan-mannans in the insoluble fraction below 1.5 % and below 2.0 % in poplar chips. Nevertheless, in the case of wheat straw, xylan-mannan contents were above 3.2 % even under the harshest conditions (187 ºC, 15 min., 3 % of H_2SO_4), accounting for the different structures of herbaceous and woody biomass feedstocks. Because of this decrease in the xylan-mannan ratio in the composition of the insoluble fraction, a noticeable increase in the proportion of acid-insoluble lignin and glycan was observed in all cases. Indeed, highest lignin contents, 42.4 %, 34.1 % and 22.7 % for pine, poplar and wheat straw respectively, were obtained upon harshest pretreatment for each feedstock. More extensive xylan-mannan solubilization together with some glycan solubilization from the solid fractions was consequently observed. In the case of acid-insoluble lignin, highest contents were found in pine chips insoluble fractions (25.9-42.4 w %), although most of the values ranged from 30 % to 35 w %. On the other hand, lignin contents were similar in all the cases (*ca.* 26-28 %) in poplar insoluble fractions, regardless of the thermochemical pretreatment conditions. A similar effect could be observed in wheat straw insoluble fractions, whose lignin contents were very similar (*ca.* 16-18 %), excepting those where thermochemical pretreatment were

harshest. In that case, larger solubilization of the xylan-mannans fraction, and consequently an increase in lignin contents, was observed.

A slight decrease in glycan proportion in the insoluble fractions could be observed in all the biomass sources under all treatments with regard to that found in the feedstocks suggesting, at least, a partial hydrolysis of celluloses under the experimental conditions, as it is shown in liquid fractions analysis (Figure 2). The highest glycan concentrations in the insoluble fraction are reached by far using poplar chips, 41.4 w %, and the lowest using wheat straw, 25.2 w %. Anyway, it is important to note that, regardless of the pretreatment conditions, glycan contents presented a very narrow distribution, 33.0 ± 1.5 % for pine, 39.3 ± 1.8 % for poplar and 34.8 ± 1.2 % for wheat straw at 95 % confidence level.

Soluble fractions were analyzed using $HPLC_1$. As shown in Figure 2, the furans, organic acids and sugar concentration profiles had strong dependence on the feedstock used and the thermochemical treatment. More precisely, the highest soluble sugar concentration, 161 g/L, was obtained when pine chips were pretreated under relatively mild thermochemical conditions (165 ºC, 5 min, 2 % of H_2SO_4,). Relatively low furans concentrations were observed under these condition, although the concentration of acetic acid, from the removal of the acetyl groups in the raw material, reached 10 g/L. An increase either in the catalyst concentration, or in the reaction temperature was accompanied by a decrease in the sugar concentration, together with an increase of the furans concentration provoked by the sugar cyclodehydration to **FAL** or **HMF**. The increase in furans concentrations did not counterbalance the decrease in sugars that suggest that degradation of sugars to humins or levulinic acid may occur under harsh conditions. Other products from the degradation of lignocellulose as vanillin, fenol, syringol, ferulic acids or benzaldehyde (Wang et al., 2019), were detected in low concentrations. Noticeably, at relatively mild conditions in terms of the acid

concentration and the temperature, residence time is, by far, the most relevant factor in the production of soluble sugars and furans during pretreatment. Higher sugar productions are usually achieved at higher temperatures and residence times.

When temperature and/or acid concentration were increased to higher levels, the concentration of sugars experienced a steady decrease albeit with a concomitant increase of furans and acetic acid production. In the case of poplar feedstock, the composition of sugars in the hydrolysates followed an opposite trend. At 2 % catalyst, sugar concentration from poplar increased with temperature and residence time, reaching up to 90 g/L except at 192 ºC, when sugar concentration decreased due to degradation processes (no significant increase in furans was observed). An increase in catalyst to 4 w % concentration also produced a decrease of sugars in the hydrolysate without significant increase in the furans concentration. This indicates that degradation of sugars and furans might occur under these conditions. However, in the case of wheat straw, at 1 % and 2 % catalyst loading, a steady increase in soluble sugar and furan concentrations could be observed upon increasing reaction temperature and residence times, reaching up to 53 g/L for xylans. However, when acid concentration was 3 %, an increase of residence times to 15 minutes was detrimental to sugar production at all temperatures, and the maximum sugar concentration was 65 g/L (at 180 ºC for 5 min). In all cases, furans concentrations steadily increased with harsher reaction conditions.

3.2. Enzymatic hydrolysis for soluble sugar production

Insoluble fractions obtained after the thermochemical pretreatment were submitted to enzymatic hydrolysis with the aim of obtaining an enriched soluble sugar hydrolysate that can be used for fermentation into bioethanol, biobutanol (*From the Sugar Platform to biofuels and biochemicals - Final Report for the European Comission Directorate-General Energy*, 2015) or any other products. Enzymatic hydrolysis was done, in all cases, using a baseline enzyme cocktail, Cellic® CTec2 under previously optimized

conditions (not shown). Higher **GLC** contents related to ODM after enzymatic hydrolysis were obtained when poplar chips were pretreated at 178 ºC at 4 % acid catalyst during 15 min. with an overall 40 g/100 g ODM and 100 % yield of the ODM **GLC** in the solid fraction (Figure 3, Table 1). Similar yields were obtained in the enzymatic hydrolysis of wheat straw solid fractions, 36 g/100 g ODM and 100 % yield, whereas, in the case of pine chips only, 26 g/100 g ODM were obtained with 71 % overall yield under **GLC** priorization conditions (Table 2, Figure 3).

Milder conditions in the thermochemical pretreatment derived in lower conversion to **GLC** after enzymatic hydrolysis (Figure 3). Thus, in the case of poplar chips, a decrease in the residence time to 5 minutes at 178 ºC, at 2 % acid concentration caused a decrease in the yield of **GLC** to ca. 37 g/ 100g ODM. When the pretreatment temperature was 164 ºC the yield was significantly reduced to 32 g/ 100g ODM. Lower acid catalyst concentration during the thermochemical pretreatment was also a critical parameter, when similar operation temperatures and residence times were used. Indeed, at 178 ºC, 5 minutes and 2 % acid, the as measured **GLC** concentration was as low as *ca.* 27 g/ 100g ODM.

Pine solid fraction was much more sensitive to the thermochemical pretreatment conditions. Highest **GLC** concentration was 25 g/ 100g ODM when the pretreatment was run at 192 ºC and 4 % catalysts. A slight decrease in the temperature to 178 ºC induced a decrease in **GLC** concentration to 15 g / 100g ODM. Finally, in the case of the wheat straw, maximal **GLC** yield was 36 g/ 100g ODM when the thermochemical pretreatment had been performed with residence times of 15 min. at 187 ºC at 2 % or 3 % acid concentration. **GLC** yields were higher than 80 % under these or harshest pretreatment conditions, obtaining quantitative hydrolysis for the solid fractions obtained at 180 ºC and 2 % acid catalyst.

As a general trend, best results in terms of solubilized **GLC** were observed when harsh reaction conditions were applied in the thermochemical pretreatments. Poplar biomass produced the highest yields of **GLC** after enzymatic hydrolysis (40 g/ 100g ODM) followed by wheat straw (36 g/ 100g ODM) and pine (25 g/ 100g ODM). This difference shall be explained by a more comprehensive breakage of the biomass structure in poplar and wheat straw during the pretreatment, which eased the access of the enzymes to the cellulose.

3.3. Cyclodehydration of xylose to furfural

3.3.1. Analysis of the xylose contents

Reliable quantification of **XYL** is one key issue concerning the optimization of the cyclodehydration reaction conditions, as well as the evaluation of the whole process. As it is shown in Table 2 and Figure 2, $HPLC_1$ methodology provided the quantification of the combined amounts of **XYL** and **MAN**. Good separation of these analytes could not be achieved and hence, individual data could not be obtained. Quantification of **XYL**, **MAN** and **GLC** was tried using a different stationary phase, $HPLC_2$ (Sádaba et al., 2014), and, although **GLC** could be satisfactorily resolved, **XYL** and **MAN** could not be separated neither. Indeed, it is well known that most of the stationary phases commonly used in HPLC analysis do not perform good resolution of **MAN**, galactose and **XYL** that usually present very similar retention times. Besides that, some other monosaccharides such as fructose, altrose or galactose may be present as interferences leading, either to long analysis times (Kiemle et al., 2004), or to non-selective methodologies. Ionic chromatography, $HPLC_3$, was also tried and **MAN** could be well resolved but, in the case of **XYL** and **GLC**, although retention times were different (6.42 min for **GLC**, 6.67 for **XYL**) good resolution could not be achieved neither. Additionally, a third compound, galactose, eluted at 6.96 min., that made quantification of **XYL** even more complicated. This did quantification of **XYL** a non-trivial issue,

particularly when dealing with hydrolysates from pine and poplar chips whose contents in **MAN** and **GLC** were significant.

Quantification of monosaccharides in aqueous solution using ^{1}H NMR is an alternative to chromatographic determination. This methodology has been described in biomass hydrolysate samples treated with deuterated H_2SO_4 and D_2O as solvent. Despite chemical shifts of anomeric hydrogen atoms are very close to that of residual H_2O, using NMR suppression of residual water signal is usually enough to obtain excellent results after careful integration of the signals (Altaner and Saake, 2016; de Souza et al., 2013; Mittal et al., 2009). However, the need of using deuterated reactants and solvents prevents the analysis of representative amounts of sample.

Direct ^{1}H NMR quantification of the **XYL**, **MAN** and **GLC** contents in the obtained hydrolysates was directly tried adding the minimal amount of D_2O for locking the NMR field. This methodology was unsuccessful in our samples, as the H_2O signal made integration of anomeric hydrogen atoms not reliable even using water signal suppression pulse programs. However, when $^{13}C\{H\}$ NMR was used, very well defined signals for the anomeric carbon atoms of D-**XYL**, D-**MAN** and D-**GLC** in their α and β configurations could be observed. Peak assignment for the anomeric carbon of D-**XYL**, D-**MAN** and D-**GLC** in their pyranose form was made by comparison with pure standards prepared in similar conditions than those of the hydrolysates. Signals at 92.2 ppm and 96.6 ppm where assigned to α and β-D-**XYL**; signals at 94.0 ppm and 93.7 ppm to α and β-D-**MAN**; signals at 92.1 ppm and 95.9 ppm to α and β-D-GLC respectively. The presence of the corresponding furanose and open forms were not considered, as it is known that their amount is about 1 % in aqueous solution (Kiemle et al., 2004). Galactose and fructose were also detected in small amounts by NMR, but their quantification is out of the scope of this study. 3-(Trimethyl)silylpropane-1-

sulfonic sodium salt (**TMPS**) was chosen as internal standard because, under our analysis conditions, showed good solubility in the medium. The intensities of the signals for the anomeric carbon atoms were calibrated with those corresponding to **TMPS** at -2.7 ppm, 14.9 ppm, 19.0 ppm and 54.3 ppm with excellent correlation for the three studied monosaccharides. Calibration standards were prepared to emulate the solutions arising from the acidic hydrolysis process: 25 μL of H_2SO_4 (6 % w/v) and 50 μL of a solution of **TMPS** in D_2O were added to 400 μL aqueous solutions of the corresponding hydrolysate samples.

Wheat straw hydrolysates, with very low amounts of **MAN** and **GLC**, were chosen to evaluate the accuracy of this method. In this way, **WST** and **WST$_H$** were first analyzed by HPLC using $HPLC_2$ and $HPLC_3$ methodologies and $^{13}C\{H\}$ NMR (Table 3). The as-determined **XYL**, **MAN** and **GLC** contents were similar between NMR and HPLC when the HPLC peaks are well-resolved (**GLC** for $HPLC_2$, **MAN** for $HPLC_3$). In the case of **XYL**, $HPLC_3$ values were higher than those determined either using $HPLC_2$ or NMR because **XYL** and **GLC** are not well resolved and the **GLC** content is relatively high whereas values are very similar for NMR and $HPLC_2$. In the case of poplar and pine samples, similar considerations can be made for the accuracy in the concentration of **GLC** and **MAN**, **XYL** determined by NMR. These concentrations were, in pine and poplar samples, lower than those determined by HPLC, which can be attributed to the mentioned resolution problems in HPLC. Furthermore, although in low concentrations, the presence of other carbohydrates as galactose or fructose previously detected by NMR may cause interference in HPLC quantifications. Therefore, it was considered that quantification of **XYL**, **MAN** and **GLC** by ^{13}C NMR is reliable in the rest of this study.

XYL content in **WST** and **WST$_H$** was 30.0 g/L and 53.0 g/L respectively. The difference in **XYL** content between these fractions is due to xylan oligomers (^{13}C NMR signals at 101.8 and 101.6 ppm for the anomeric carbon) that were fully depolymerized

upon autoclaving of **WST** to produce **WST$_H$**. A similar reasoning can be applied to the content of **GLC** that raised from 3.9 g/L in **WST** to 6.2 g/L in **WST$_H$**. Concentration of **MAN** in pine sample **PIN$_H$** was 43.8 g/L, followed by **XYL**, 24.1 g/L and GLC 14.1 g/L. **XYL** content in **POP** samples was 73.8 g/L, which is slightly higher than that found in **POP$_H$**, 60.0 g/L, while **FAL** content increased from 0.8 g/L to 5.3 g/L upon autoclaving. If dilution effects are not considered, almost 63 % of the difference in **XYL** had been converted to **FAL** during autoclaving at 121 ºC. **GLC** contents were about 15.0 g/L in both **POP** and **POP$_H$** showing that almost all accessible glycan and xylan had been already hydrolyzed upon the thermochemical pretreatment. **MAN** contents were slightly increased showing that some mannan remained intact upon thermochemical pretreatment.

3.3.2. Optimization of the cyclodehydration reaction.

Two major challenges must be faced in order to improve the efficiency of the cyclodehydration reaction of **XYL**: i) the formation of degradation products from **FAL**, namely humins and ii) the intensive use of energy. Biphasic reactions conditions using methyl isobutyl ketone (**MIBK**) or toluene have proven to be effective to prevent humins formation given that **FAL** can be efficiently transferred to the organic phase upon its formation (Romo et al., 2018). Concerning to the use of energy, microwave reactors allow ramping in short times (*ca*. 2 min) that reduces the energy costs increasing the efficiency of the process. In addition, rapid cooling of the reaction mixture is achieved assuring excellent control of the reaction time that prevents the formation of byproducts. Another drawback associated to conventional heating and long ramping times is that **XYL** cyclodehydration occurs at temperatures as low as 150 ºC, which favors the formation of humins upon heating to higher temperatures, that can be also circumvented using microwave irradiation.

According to the work of Mittal (Mittal et al., 2017) and to previous studies on the kinetics of biphasic dehydration of **XYL** to **FAL** (Weingarten et al., 2010) the sulfuric acid, that is already present in the downstream fractions arising from thermochemical pretreatment, could be a good catalyst to perform cyclodehydration. In the case of pine and poplar feedstocks, hemicellulosic fractions with highest **XYL** concentrations (**XYL** priorization in Table 2) where chosen, whereas in the case of wheat straw, a high concentration sample, after treatment at 173 ºC at 2 % catalyst during 15 min. was used. Fully depolymerized pine samples, **PIN$_H$**, were used for reaction optimization under microwave irradiation. This election was not casual, because, in our opinion, the relatively high content in **MAN** (43.8 g/L) and GLC (14.1 g/L) would made optimization more difficult. Four different parameters were tuned in the cyclodehydration reaction of **XYL** to **FAL**: target temperature, pH, reaction time and **MIBK**: aqueous phase ratio. These are the most relevant parameters according to previous studies on the kinetics of **XYL** dehydration in biphasic media (Weingarten et al., 2010). NMR analysis of the resulting organic phases showed that **FAL** and **HMF** were almost the sole reaction products transferred to the organic phase. Although in some cases the measured **FAL** yields are slightly higher than **XYL** conversions, experimental error made these discrepancies not significant (Table 4). The effect of MIBK:H_2O ratio was first studied at 170 ºC and 300 s reaction time (Table 4, entries 1-5) at pH 0.73. Final concentrations of hexoses in the aqueous phase were similar in all the experiments, although **HMF** yield steadily increases with MIBK:H_2O ratios reaching up to 21 % (entry 5) that proved efficient transfer to the organic phase. **HMF** yield did not justify the hexoses conversion. ^{13}C NMR analysis of the aqueous phase evidenced the presence of levulinic acid (signals at 213.5 ppm and 177.4 ppm) as a degradation product and HPLC analysis confirmed the formation of small amounts of succinic acid, lactic acid, levulinic acid and acetic acid. **FAL** yield steadily increased

from 53 % at MIBK:H_2O 1:1 to 84 % at 5:1 whereas **XYL** conversion raised from 73 % to 85 %. As dehydration reaction takes place in the aqueous phase, **XYL** conversion is not so affected by MIBK:H_2O ratio as **FAL** yield, which is much better extracted to the organic layer. Given the slight difference in terms of **XYL** conversion between 3:1 and 5:1 ratios, the first was chosen in the rest of the optimization.

An increase in reaction temperature from 150 ºC to 210 ºC (entries 3, 6, 7, 8) caused a steady decrease in the sugar concentration in the aqueous phase. **FAL** and **HMF** yields followed a similar trend, reaching their maximum at 190 ºC (100 % and 13 % respectively) and decreasing at 210 ºC, while conversion of **XYL** kept constant, 95 %. Faster degradation in the aqueous phase occurs at 210 ºC, whereas the rate of **FAL** and **HMF** transfer rate to the organic solvent kept almost constant, that explains the decrease in yields. The effect of reaction time was also studied, and upon ramping of the reactor to 190 ºC or 210 ºC, excellent **FAL** yields, 90 % and 97 %, can be observed (entries 9 and 10). At 190 ºC **FAL** yield reached its maximum 300 s after ramping (100 %, entry 7) with a slight decrease after 600 s (92 %, entry 12). A similar trend was observed in the production of **HMF**, 14 %, 300 s after ramping, that dramatically decreased after 600 s. Finally, concerning the effect of the pH, no significant differences in the reaction performance could be found when the reaction was run at either pH 1.2 or pH 0.73 in terms of **FAL** or **HMF** yields. A slight decrease in **FAL** yield was observed when the reaction was run at pH 0.63 athough **XYL** conversion kept constant (entries 7, 13 and 14).

Optimized reaction conditions (190 ºC, 300 s, MIBK:H_2O 3:1) were tested in fully hydrolyzed $\mathbf{WST_H}$ and $\mathbf{POP_H}$ (entries 15 and 16) as received. Quantitative conversion of **XYL** and 84 % **FAL** yield were obtained in both cases. Similarly, **WST** and **POP** were directly reacted without any addition of sulfuric acid, pH 3 (entries 17 and 19) with low **XYL** conversions, (50 % and 16 %), and 28 % and 33 % **FAL** yields

respectively that can be attributed to relatively high pH values that caused slower kinetics in the cyclodehydration. Acidification of these samples to pH 1 and 1.8 respectively provided similar yields (entries 18 and 20) than those obtained with **WST_H** and **POP_H** under the same reaction conditions. In the case of **POP** samples, **FAL** yields were very similar to those from **WST**. It is worth noting that xylan had been already fully depolymerized in **POP** but not in **WST**. Therefore, it can be assumed that cyclodehydration is strongly dependent on the pH and that is much slower than xylan depolymerization. The lower selectivity towards **FAL** in poplar and wheat straw samples can be explained by the higher **XYL** concentration that may accelerate the formation of degradation products in the aqueous phase.

For the sake of comparison, reaction temperature was optimized for **WST_H** as received using conventional heating and a 1.7:1 MIBK/H_2O (optimized conditions with pure **XYL**, data not shown). Maximum reaction yield was obtained after ramping at 170 ºC, 73 % yield (entries 21-24), while humins formation provoked a decrease in the yield at higher temperatures. Noteworthy, this maximum is observed at lower temperature than under microwave irradiation, which can be attributed to the longer time for ramping (32 min. against 2 min.). These results were similar or even superior to those recently reported by Mittal (Mittal et al., 2017) who reported 85 % **FAL** yield from corn-stover hydrolysates under microwave irradiation using acidic catalysts.

However, scaling the optimized laboratory parameters is another challenge in developing chemical processes, particularly under microwave heating because of the difficulties to ensure homogeneous irradiation of microwaves in large reaction volumes. Scaling of the cyclodehydration reaction under microwave irradiation was done using **WST_H** hydrolysate without any addition of sulfuric acid. In order to improve the sustainability of the process, reactions were run at 1.7:1 MIBK:aqueous ratio, as it had been done using conventional heating. Reaction temperature optimization was made

using only one vessel (Table 4, entries 25-28). Ramping was much slower although irradiation was made at 500 W, hence we decided to stop irradiation after reaching the target temperatures to prevent humins formation. Under these conditions, best **FAL** yields, 73 % (entries 4 and 5), were obtained when ramping to 200 ºC and 210 ºC. Then, the reliability of the system was tested using up to 5 reaction vessels simultaneously ramping to 200 ºC (entry 29). Temperature was reached after 5 minutes of irradiation at a maximum of 900 W, providing an average yield of 70.2 ± 2.2 %. This showed the low variability between the different reaction vessels, evidencing that microwave irradiation was homogeneous along the microwave chamber. Overall production of **FAL** in this batch was 2.23 g, and thus, 5.35 g could be expected using the whole 12 vessels system. Cyclodehydration reaction was also scaled-up under conventional heating using $\mathbf{WST_H}$ as received as starting material, under the same reaction conditions (Table 4, entries 30-32) in a 4.0 L vessel. Ramping time was much longer than under microwave irradiation, hence, reaction was also stopped after reaching the target temperature. Maximum yields, 90 % (entry 31), were obtained after ramping to 180 ºC and decreased, 68 % (entry 32), when ramping to 190 ºC. Overall **FAL** productions were relatively high, reaching 22.7 g/batch when the reaction was run at 180 ºC. It is worth noting that large-scale reaction yields were higher or noticeably higher than those found in small-scale experiments with conventional heating, that was due to the shorter time needed to reach the target temperature in scaled-up systems that prevented the formation of undesired side products. Optimum ramping temperature was also different between microwave and conventional irradiation that is due again to the different ramping time. Thus, because of much slower rate in ramping, lower temperatures were needed under conventional heating to reach maximal productivity, whereas in the case of microwave irradiation, higher temperatures were required to reach the maximum.

Productivity of **FAL** with the different methodologies was estimated in terms of grams of **FAL** produced by time of operation of the used reactors. It was assumed 70 % **FAL** yield in scaled-up microwave heating and 90 % yield using conventional heating. Highest productivity was estimated using microwave digester with all the feedstocks, reaching up to 88 g **FAL**/h when using poplar chips as feedstock, 63.1 g **FAL**/h using wheat straw and 28.8 g **FAL**/h using pine chips. Given that the optimized yields for wheat straw were higher when using conventional heating, the estimated overall yields were also higher in the scale up of the reaction, 90 % for pine chips, 76 % for poplar chips and 58 % for wheat straw against 77 %, 59 % and 45 % respectively for the scaling in microwave digester. Therefore, using pine chips may afford almost quantitative conversion of xylans to **FAL** under conventional heating, although the highest productivity in terms of grams of **FAL** per hour is the lowest within the three studied feedstocks given their low composition in xylan. Highest productivities were estimated using poplar chips, 88 g/h but with lower overall efficiency 59 %.

Our results, are better than those typically obtained in industry with 45 % overall **FAL** yield. The results from the **XYL** cyclodehydration are slightly superior to those found in the direct production of **FAL** from maple wood under conventional heating (Zhang et al., 2013) that reached up to 85 % **FAL** yield, but using lower xylan loadings. Few studies, however, have reported using directly Biorefinery derived downstream or syrup fractions for the production of **FAL** from **XYL**. 45 % **FAL** yield was reported using sulfonic ionic liquid as catalyst on a Biorefinery derived mixture from wheat and/or barley straw after 4 h of microwave irradiation (Serrano-Ruiz et al., 2012). Our results in the cyclodehydration are in the range of the 86 % overall **FAL** yield reported for the conversion of xylans in switchgrass using batch reactive distillation (Mandalika and Runge, 2012). In our study, however, more concentrated hydrolysates were used (20-50 g/L vs 5.0 g/L xylan) that shows the efficiency of the biphasic system in transferring

FAL and hence, presents higher productivities. A very similar approach using sugarcane bagasse has been reported (Mesa et al., 2014) but, in this case, acidic thermal treatment is focused either to the production of **FAL** or **XYL**, obtaining 53 % and 75 % overall yields for **FAL** and **XYL** depending on the conditions, clearly under those reported in our study.

Biorefinery concept implies a multiple use of one substrate for upgrading each fraction obtained after thermochemical pretreatment. According to this, the middle point in the thermochemical pretreatment for obtaining balanced yields for **GLC** or **XYL** production (Table 2) is different for each feedstock. In terms of the thermal pretreatment efficiency for glycans in the solid fraction, the best yields were obtained under harsh pretreatment conditions. In this sense, high temperatures together with long residence times in the reactor provided solid fractions with high glycan contents. Additionally, in view of the enzymatic hydrolysis yields, these glycans are far more accessible to the enzymes, promoting higher overall **GLC** yields. Indeed **GLC** yields were almost quantitative under these conditions for wheat straw and poplar chips.

In the case of **XYL** extraction, harsh reaction conditions promoted the degradation of the as-extracted xylan (Figure 2). Hence, milder conditions were preferred in the thermochemical pretreatment, reaching excellent extraction yields for poplar (88 %) and pine (100 %) although in this case concomitant extraction of mannose was observed (Table 4). Higher **GLC** production can be achieved using wheat straw and poplar chips under optimized conditions. Similarly, in the case of the production of **FAL**, cyclodehydration of wheat straw and poplar chips hydrolysate would provide excellent yields in terms of g **FAL**/ 100 g ODM. On the other hand, overall yields in terms of available xylans are higher for pine chips, but their relatively high content in **MAN** reduce the yield in terms of g **FAL**/ 100 g ODM. Finally yet importantly, it has been demonstrated that this excellent yields in terms of **FAL** production can be achieved with

only a slight acidification of the hydrolysate sample after the thermochemical pretreatment of the feedstock.

An overview picture for our two-steps Biorefinery (Figure 4) and the extraction conditions (Table 2) show the versatility of the proposed scheme. Overall yields for **FAL** production were estimated either using the actual concentrations of **XYL** in the liquid fraction, or using the overall extraction yields that are gathered in Table 2. This latter approximation shows the potential of the scheme. This way, starting from a 50 kg ODM batch, and considering 15 kg losses during thermochemical pretreatment operation, up to 12.6 kg of GLC could be obtained using poplar as feedstock, 12 kg using wheat straw and 8.3 kg using pine chips. Concerning to **FAL**, best feedstock would be pine chips, which would provide up to 2.5 kg using conventional heating and 2.2 kg under microwave irradiation. **FAL** production from wheat straw and poplar chips would be similar when using conventional heating but noticeably lower under microwave irradiation. Obviously, estimated yields using the actual concentrations of **XYL** in the filtrate are lower, but in the case of using pine chips under conventional conditions, are not very far than those estimated under ideal extraction. Extensive washing of the solid fraction will, undoubtedly, allow to reach ideal extraction values. Last, but not least, in the case of poplar chips, up to 14.1 kg of material is recovered under the non-ideal conditions from a 50 kg ODM batch (35 kg considering mass loss), that shows the potential of the proposed scheme.

4. Conclusions

Thermochemical pretreatment conditions of the feedstock are critical in the production of fermentable **GLC** and **FAL** in a two-step Biorefinery approach using wheat straw, pine and poplar chips. This approach allowed easy optimization of the different steps. Quantitative fermentable **GLC** yields were obtained after enzymatic hydrolysis. High **FAL** yields were obtained from the downstream fractions in biphasic medium, either

using conventional or microwave heating. Finally, a new analytical methodology based on ^{13}C NMR has been proposed to quantify the concentration of sugars in aqueous media. This methodology provided excellent results in terms of precision and allowed the analysis of representative amounts of hydrolysate.

E-supplementary data of this work can be found in e-version of this paper online

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7. Figures captions

Figure 1. Compositional analysis of insoluble fractions after thermochemical pre-treatments using H_2SO_4 as catalyst for pine (up), poplar (middle) and wheat straw (down). Sugar contents (g/L) and acetic acid (g/L) (left axis); Furans (5-hydroxymethylfurfural and furfural) (mg/L) (right axis)

Figure 2. Soluble furans (5 hydroxymethylfurfural and furfural), acetic acid and xylose concentration detected in pine chips (up), poplar chips (middle) and wheat straw (down) slurry samples.

Figure 3. Soluble glucose (g/100 g ODM) (Left axis) and Glucose yields (%) (right axis) after 72 h of enzymatic hydrolysis for pine (up), poplar (middle) and wheat straw (down)

Figure 4. Scheme for the two-step Biorefinery.

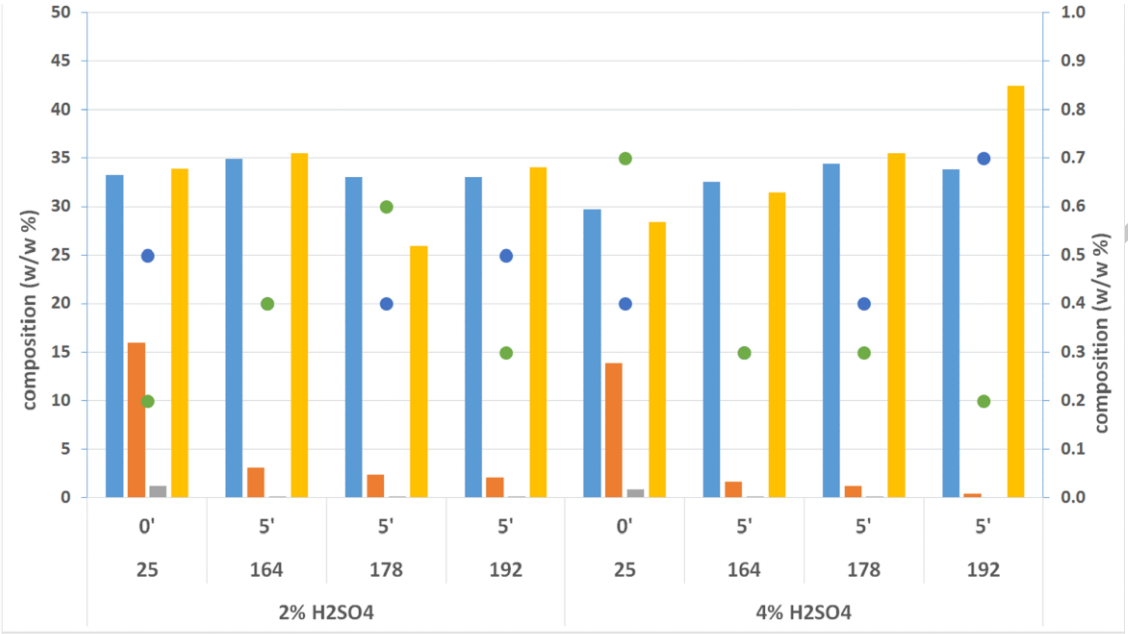
8. Tables

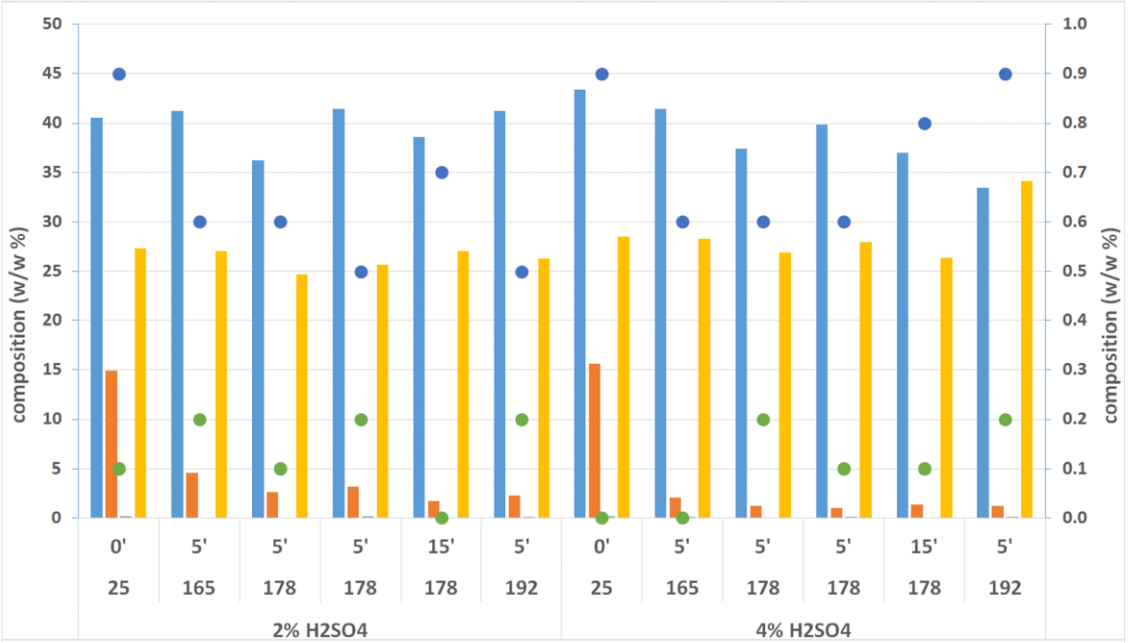
Table 1. Compositional analysis of the biomass raw materials

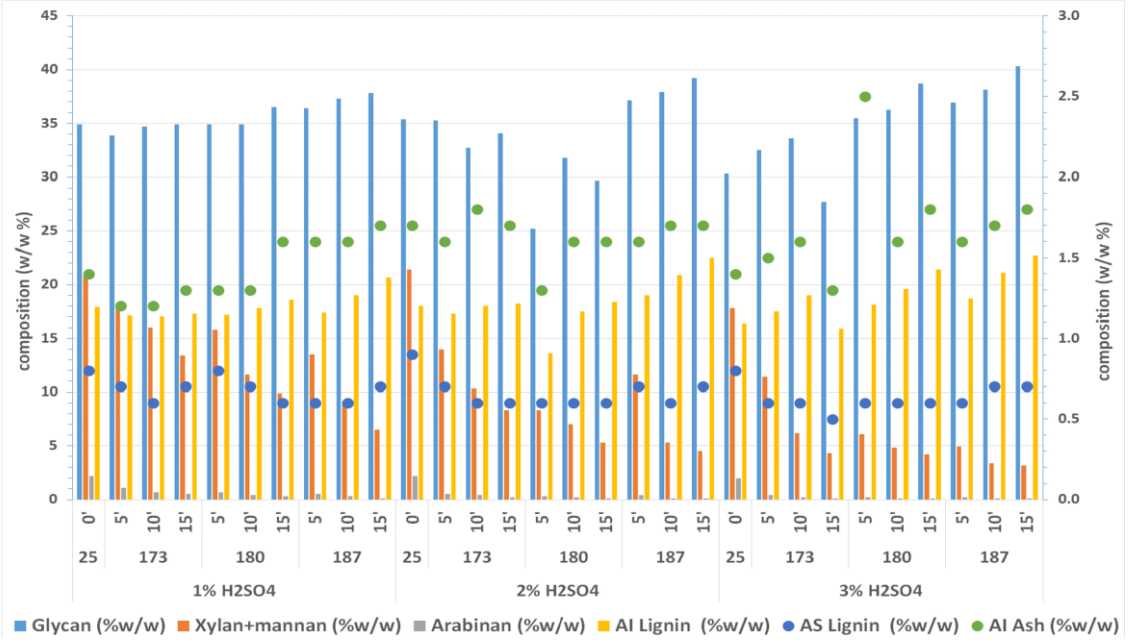
Table 2. Thermochemical pretreatment conditions for glucose and xylose production optimization

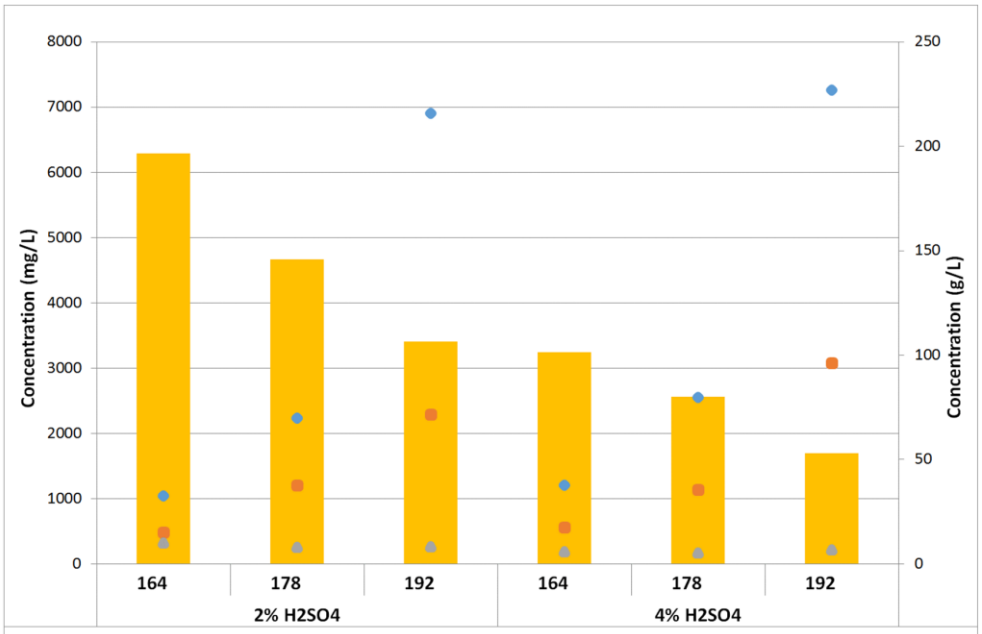
Table 3. NMR and HPLC measured concentrations for glucose, xylose and mannose in hydrolyzed biomass samples. NMR data are expressed at 95 % confidence level.

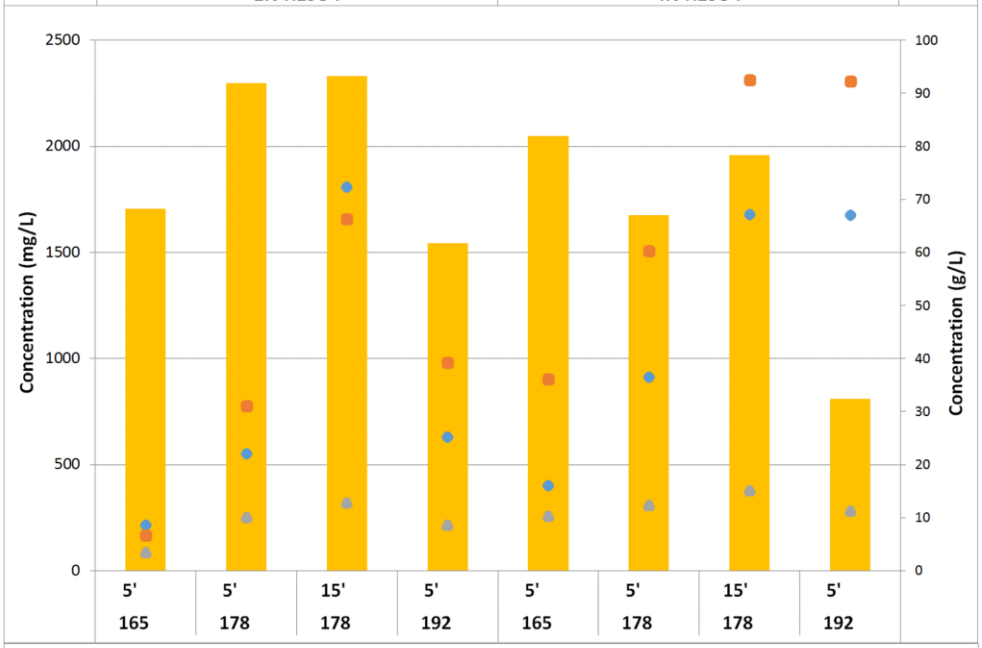
Table 4. Optimization of cyclodehydration reaction under microwave irradiation.

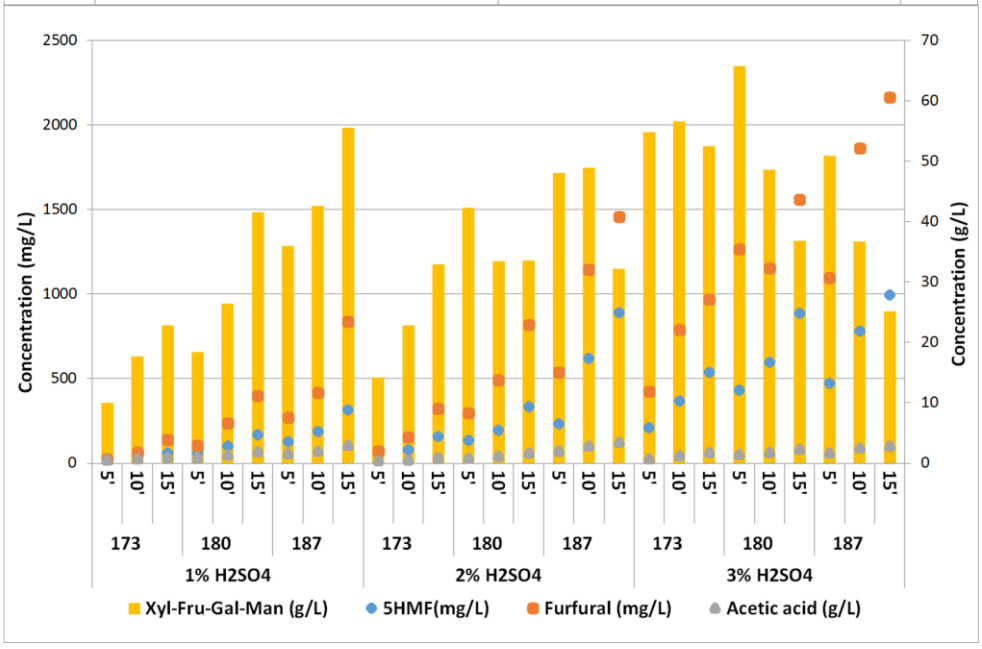


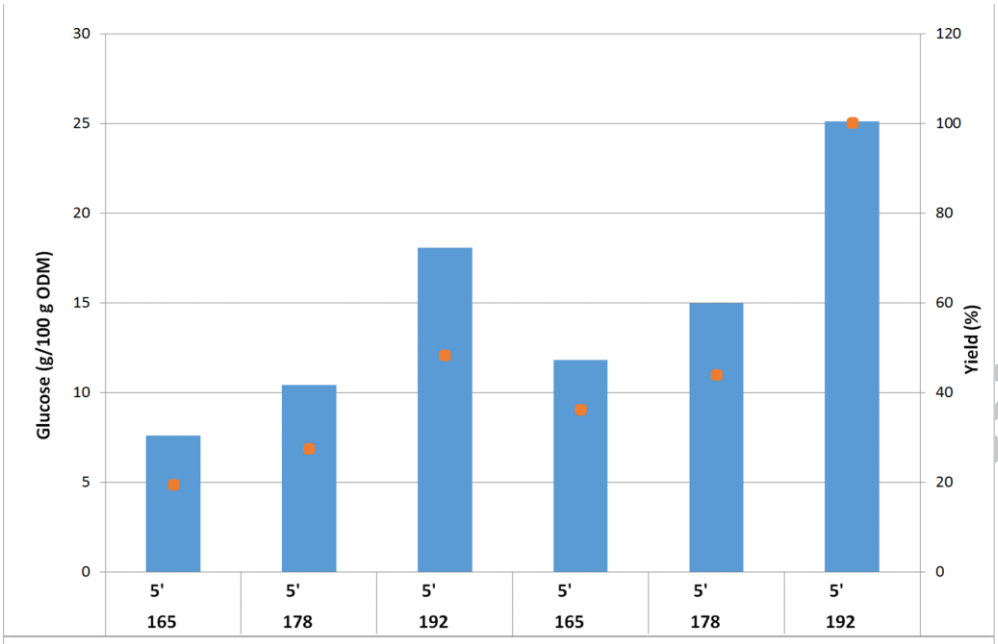


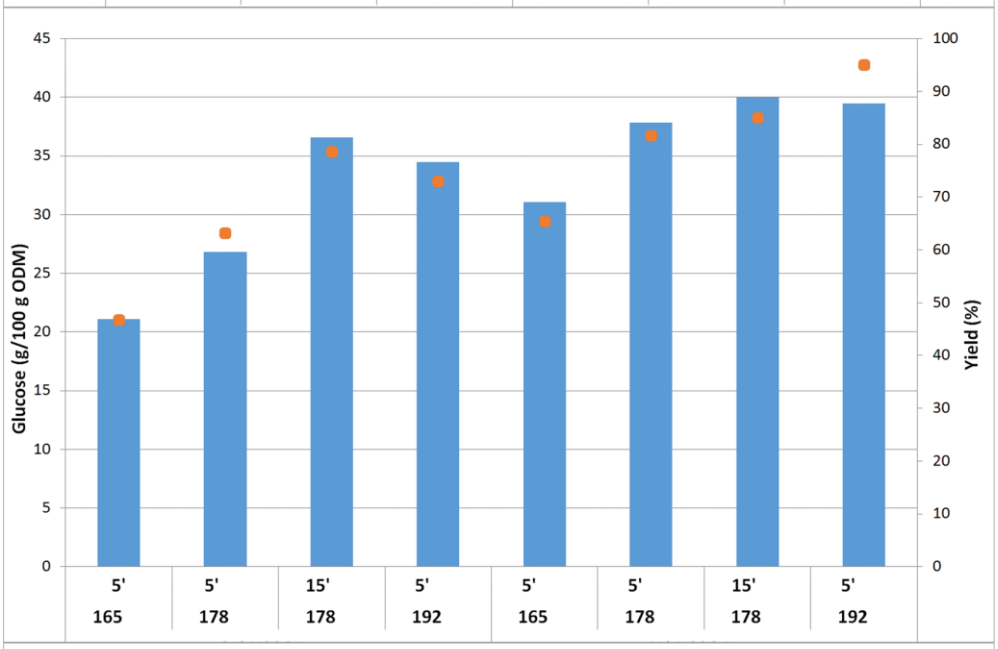


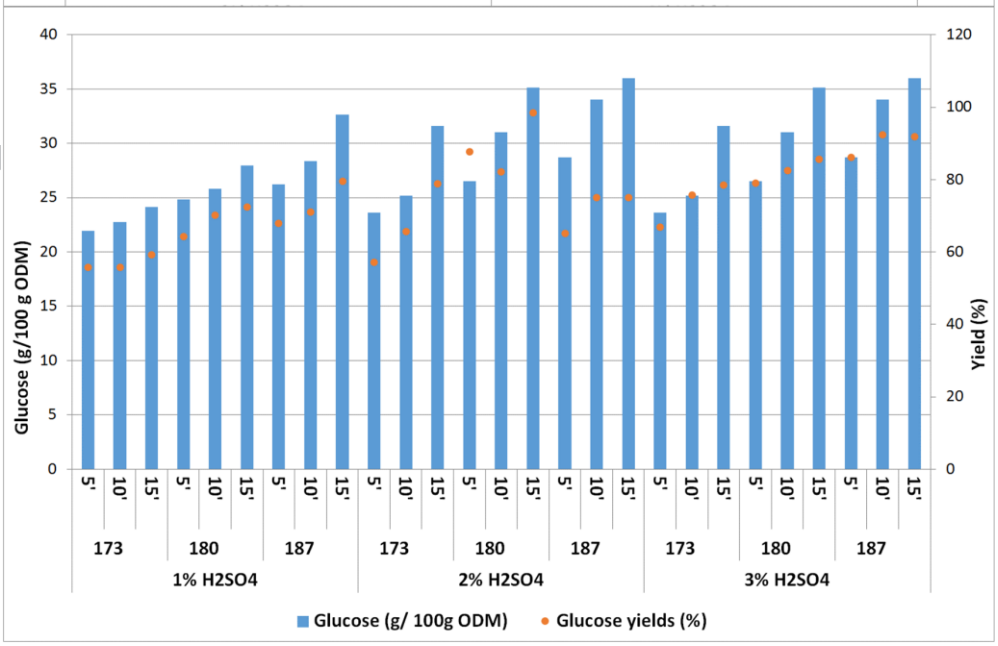


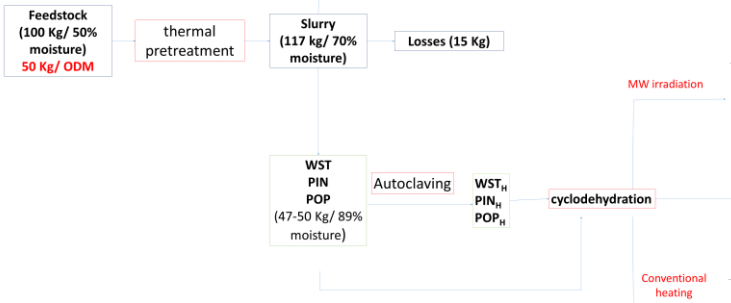












Feedstock	hydrolysis yield (%)	kg glucose
Wheat Straw	100	12.0
Pine chips	71	8.3
Poplar chips	99	12.6

Feedstock	Kg FAL/ batch[1]	Overall yield[2] (%)	kg FAL/ batch[2]
Wheat Straw	0.6	45	1.9
Pine chips	1.5	77	2.2
Poplar chips	1.1	59	1.9

Feedstock	kg FAL/ batch[1]	Overall yield[2] (%)	kg FAL/ batch[2]
Wheat Straw	0.8	58	2.5
Pine chips	1.9	90	2.5
Poplar chips	1.5	76	2.4

1) estimated using xylose contents in the filtrate; 2) estimated using extraction yield in the hydrolysates

Table 1. Compositional analysis of the biomass raw materials

Determination (%weight/ DM[a])	Wheat Straw		Pine chips		Poplar chips	
	Avg.	SD	Avg.	SD	Avg.	SD
Total Extractives	14.8	2.6	10.6	0.6	8.3	0.2
Ethanol-Soluble Extractives	3.2	0.5	1.9	0.3	2.2	0.3
Total ash	4.3	0.8	1.9	0.0	1.0	0.0
Glycan	34.1	1.4	37.1	0.9	40.6	0.4
Xylan-Mannan	20.8	1.1	14.1	0.5	16.0	0.1
Arabinan	2.1	0.3	0.3	0.0	0.5	0.04
AI[b] Lignin	14.3	0.8	20.8	0.8	21. 8	0.4
AS[c] Lignin	0.7	0.0	0.8	0.0	0.6	0.1
AI[b] Ash	0.7	0.3	0.1	0.0	0.6	0.3

a) DM: dry matter; b) AI: Acid insoluble; c) AS: acid soluble

Table 2. Thermochemical pretreatment conditions for glucose and xylose production optimization.

Feedstock	Priorization	Pretreatment conditions			Enzymatic hydrolysis		Hydrolysate	
		T (ºC)	Acid catalyst (%w/w)	Residence time (min)	Glucose (g/100g ODM)	Overall Glucose yield (%)	Xylose + mannose (g/100 ODM)	Xylose + mannose (% extraction yield)
Wheat straw	Middle	180	2	10	29.0	85	7.5	36
	Glucose	187	2	15	36.0	100	7.3	35
	Xylose	180	2	5	26.5	78	13.4	64
	Xylose[a]	173	2	15	-		10.2	49
Pine chips	Middle	178	4	5	18.2	49	10.5	74
	Glucose	192	4	5	26.3	71	7.4	52
	Xylose[a]	164	2	5	7.6	20	14.1	100
Poplar chips	Middle	178	3	15	40.0	99	13.1	82
	Glucose	178	3	5-15	40.0	99	13.1	82
	Xylose[a]	178	2	15	36.6	90	14.1	88

a) Sample used in cyclodehydration optimization.

Table 3. NMR and HPLC measured concentrations for glucose, xylose and mannose in hydrolyzed biomass samples. NMR data are expressed at 95% confidence level.

Sample	Analysis method	XYL (g/L)	MAN (g/L)	GLC (g/L)	XYL+ MAN +GLC (g/L)	FAL (g/L) [b]
WST	**NMR**	27.0 ± 0.5	0.5 ± 0.4	3.9 ± 1.0	31.4	0.9
	HPLC$_2$ [a]	30.0 ± 1.9	0.4 ± 0.0	3.4 ± 1.2		
	HPLC$_3$	31.8 ± 0.4	0.2 ± 0.1	1.2 ± 0.2	33.2	
WST$_H$	**NMR**	52.7 ± 1.7	1.0 ± 0.6	6.2 ± 0.9	59.9	1.0
	HPLC$_2$ [a]	53.0 ± 2.0	1.2 ± 0.1	7.0 ± 0.4		
	HPLC$_3$	61.2 ± 0.6	0.9 ± 0.1	6.3 ± 0.3	68.4	
PIN$_H$	**NMR**	24.1 ± 1.9	43.8 ± 3.0	14.1 ± 0.6	82.0	1.5
	HPLC$_2$ [a]	28.6 ± 0.6	49.4 ± 3.2	12.0 ± 1.3		
	HPLC$_3$	28.4 ± 0.6	37.6 ± 0.4	12.6 ± 0.7	78.6	
POP	**NMR**	73.8 ± 3.5	9.1 ± 0.4	14.8 ± 0.1	97.7	0.8
	HPLC$_2$ [a]	89.4 ± 4.2	11.6 ± 0.6	14.5 ± 1.8		
	HPLC$_3$	81.1 ± 0.2	9.0 ± 0.0	11.1 ± 0.3	101.2	
POP$_H$	**NMR**	60.0 ± 2.8	12.8 ± 0.6	14.7 ± 1.4	87.5	5.3
	HPLC$_2$ [a]	74.4 ± 1.9	15.3 ± 0.4	15.4 ± 3.3		
	HPLC$_3$	80.0 ± 0.4	11.6 ± 0.2	15.0 ± 0.3	106.6	

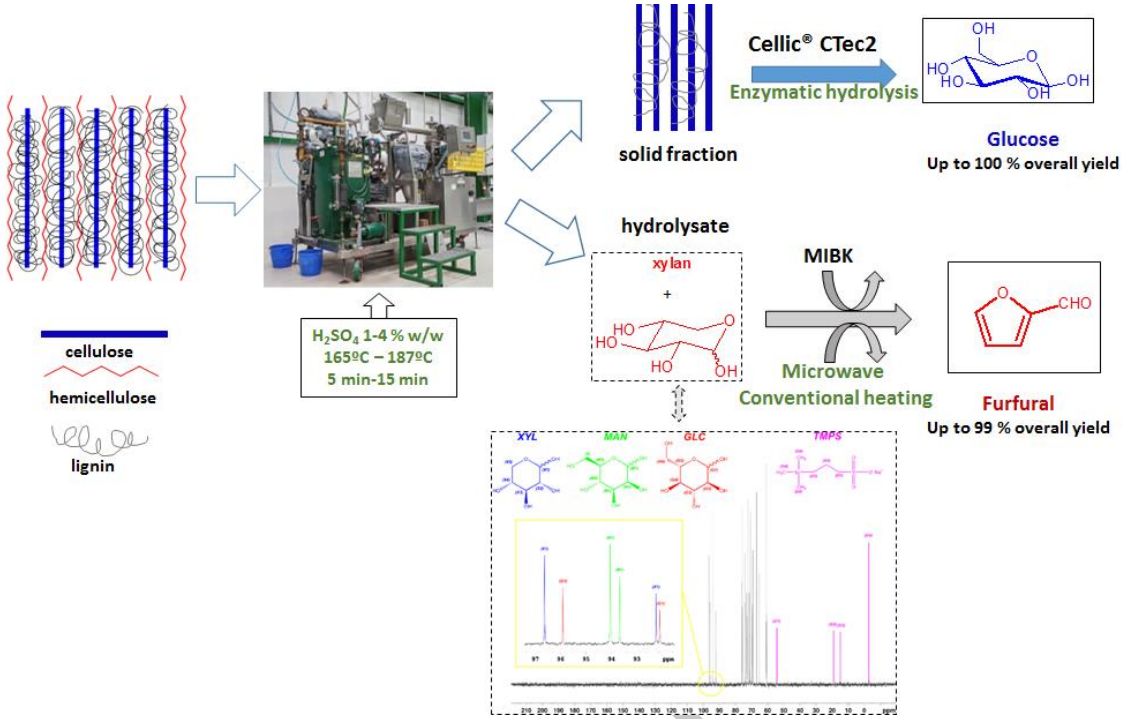
a) the xylose and mannose contents in the sample were determined by a tentative partition of the signal assigned to xylose and mannose by HPLC$_2$ using the ratios previously determined by NMR. b) FAL was quantified by NMR upon extraction with MIBK and addition of 1,3,5-trimethoxybenzene.

Table 4. Optimization of cyclodehydration reaction under microwave irradiation. Microwave optimization reactions (entries 1-20, 22 and 29) were run by triplicate and are expressed at 95% confidence level.

entry	**sample**	**Heating**	**T (ºC)**	**t (s)**	**MIBK/aq**	**pH**	**Conv Xil. (%)**	**FAL yield** (%)	**HMF yield** (%)	**[XIL]** (g/L)	**[MAN]** (g/L)	**[GLC]** (g/L)	**[XIL]** (g/L)
1	**PINH**	MW	170	300	1	0.73	73 ± 5.1	53 ± 4.9	5± 0.3	6.5 ± 1.2	24.9 ± 1.1	8.0 ± 2.6	6.5 ± 1.2
2	**PINH**	MW	170	300	2	0.73	75 ± 6.6	71± 18.9	9± 2.7	5.9 ± 1.4	25.1 ± 3.2	8.0 ± 1.2	5.9 ± 1.4
3	**PINH**	MW	170	300	3	0.73	80 ± 1.5	76 ± 8.9	10±0.8	4.9 ± 0.3	22.5 ± 1.2	7.2 ± 0.3	4.9 ± 0.3
4	**PINH**	MW	170	300	4	0.73	81 ± 5.0	75± 12.7	17± 3.3	4.6 ± 1.0	21.9 ± 1.3	6.9 ± 1.4	4.6 ± 1.0
5	**PINH**	MW	170	300	5	0.73	83 ± 6.3	84± 18.8	21±5.1	4.2 ± 1.3	20.2 ± 5.0	6.3 ± 1.4	4.2 ± 1.3
6	**PINH**	MW	150	300	3	0.73	36 ± 4.8	20 ± 1.5	5±0.8	15.3 ± 1.2	40.5 ± 1.0	10.8 ± 0.5	15.3 ± 1.2
7	**PINH**	MW	190	300	3	0.73	95 ± 1.6	100 ± 9.6	13±2.0	1.3 ± 0.4	2.6 ± 0.6	3.6 ± 0.4	1.3 ± 0.4
8	**PINH**	MW	210	300	3	0.73	94 ± 4.4	87± 2.26	3±0.8	1.3 ± 1.1	2.0 ± 1.	0.6 ± 0.9	1.3 ± 1.1
9	**PINH**	MW	190	1	3	0.73	84 ± 3.5	90 ± 6.2	14±0.7	3.8 ± 0.8	13.5 ± 1.3	5.4 ± 1.0	3.8 ± 0.8
10	**PINH**	MW	210	1	3	0.73	93 ± 3.8	97 ± 5.4	15±0.7	1.6 ± 0.9	3.6 ± 1.8	3.3 ± 1.9	1.6 ± 0.9
11	**PINH**	MW	190	120	3	0.73	91 ± 2.2	97 ± 6.2	11±2.4	2.3 ± 0.5	5.9 ± 7.0	3.4 ± 1.5	2.3 ± 0.5
12	**PINH**	MW	190	600	3	0.73	93 ± 2.2	92 ± 2.4	2±0.9	1.7 ± 0.5	1.7± 0.6	1.4 ±0.7	1.7 ± 0.5
13	**PINH**	MW	190	300	3	1.20	94 ± 2.5	98 ± 3.1	14±0.9	2.2 ± 0.6	2.1 ± 1.0	2.5 ±1.2	2.2 ± 0.6
14	**PINH**	MW	190	300	3	0.63	91 ± 3.1	89 ±2.3	5±1.5	2.1 ± 0.7	2.2 ± 1.2	3.2 ± 1.6	2.1 ± 0.7

15	**WS T_H[a]**	MW	190	300	3	1.00	99 ± 0.5	84 ± 1.5	17± 10.0	0.6 ± 0.2	0.9 ± 1.1	1.4 ± 1.4	0.6 ± 0.2
16	**PO P_H[a]**	MW	190	600	3	0.60	99 ± 0.1	84 ± 1.4	10± 1.5	0.8 ± 0.1	0.1 ± 0.3	0.5 ± 0.0	0.8 ± 0.1
17	**WS T[a]**	MW	190	300	3	3.01	50 ±6.0	28 ±2.7	17± 5.3	27 ± 3.2	1.1 ± 0.4	2.8 ± 0.5	27 ± 3.2
18	**WS T**	MW	190	300	3	0.97	99 ± 0.7	86 ± 2.7	22 ± 4.2	0.7 ± 0.3	0.8 ± 0.2	1.1 ± 0.7	0.7 ± 0.3
19	**PO P[a]**	MW	190	600	3	3.01	16 ± 0.8	33 ± 0.7	16± 0.8	59.2 ± 2.3	8.3± 0.2	14.2 ± nd	59.2 ± 2.3
20	**PO P**	MW	190	600	3	1.8	99 ± n.d.	87 ± 0.2	16 ± n.d	0.8	1.2	1.3	0.8
21	**WS T_H[b]**	conv	160	1	1.7	1.0		67					
22	**WS T_H[b]**	conv	170	1	1.7	1.0		73.2 ± 4.0					
23	**WS T_H[b]**	conv	180	1	1.7	1.0		66					
24	**WS T_H[b]**	conv	190	1	1.7	1.0		60					
25	**WS T_H[b]**	MW[c]	180	396[d]	1.7	1.0		41					
26	**WS T_H[b]**	MW[c]	190	402[d]	1.7	1.0		65					
27	**WS T_H[b]**	MW[c]	200	472[d]	1.7	1.0		73					
28	**WS T_H[b]**	MW[c]	210	555[d]	1.7	1.0		73					
29	**WS T_H[b]**	MW[c]	200	300[d]	1.7	1.0		70 ± 2.2					
30	**WS T_H[b]**	Conv[c]	170	2580[d]	1.7	1.0		74					
31	**WS T_H[b]**	Conv[c]	180	2160[d]	1.7	1.0		90					
32	**WS T_H[b]**	Conv[c]	190	3480[d]	1.7	1.0		68					

a) Sample reacted as obtained from thermochemical pretreatment b) Sample reacted as obtained from thermochemical pretreatment and autoclaving at 121ºC c) scaled up reactions d) refers to ramping time



Highlights

A process for the production of fermentable glucose and furfural was optimized.

Biomass pretreatment conditions determined the yield of enzymatic hydrolysis.

The use of biphasic media was critical for obtaining high furfural yields.

Separate process optimization provided quantitative yields for glucose and furfural.

^{13}C NMR spectroscopy has been satisfactorily used in sugar quantification.