ORIGINAL ARTICLE

Optimization of hydrolysis conditions for xylans and straw hydrolysates by HPLC analysis

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Abstract

Oligosaccharide analysis is commonly done by acid hydrolysis and following HPLC analysis. A major problem is the incomplete hydrolysis of oligosaccharides and disaccharides and the increasing formation of volatile furfural from pentose monomers and hydroxymethylfurfural (HMF) from hexose monomers. This paper optimizes the conditions of hydrolysis approaches and proposes a method for oligosaccharide quantification. The optimal condition for hydrolysis of model xylan from corn cob was found to be for 100 °C hydrolysis temperature, 120 min hydrolysis time, and 2 wt% sulfuric acid concentration. Under these conditions, the total free and bound xylose yield was 77.4% and hemicellulose conversion 87.4% respectively; no degradation products were found. The optimal conditions for hydrolysis of model xylan from beech wood were found to be for 120 °C hydrolysis temperature, 120 min hydrolysis time, and 2 wt% sulfuric acid concentration. Under these conditions, the total free and bound xylose yield was 65.1% and hemicellulose conversion 70.5% respectively; no degradation products were found. For pentosan hydrolysate, conditions were further optimized (110 °C, 60 min, 2 wt% H_2SO_4). Standard addition of xylan from the corn cob for hydrolysation showed similar conversion rates (< 2% deviation); no matrix effects were detected.

Keywords Pentosans · Oligosaccharides · Quantification · HPLC · Xylose

1 Introduction

Carbohydrates represent a major portion of biomass samples and consist mainly of polysaccharides constructed primarily of glucose, xylose, arabinose, galactose, and mannose monomeric subunits. In general, carbohydrates with a degree of polymerization (DP) lower than 10 are called oligosaccharides, while polysaccharides show a DP above 10 [1].

During hydrothermal pretreatment of lignocellulosic biomass, a certain share of these polysaccharides is partly hydrolyzed to water-soluble oligomers and monomers. They are hereafter referred to as sugar monomers being mainly xylose and glucose [2]. HPLC with refractive index detection enables the quantification of these soluble sugars as well as the subsequent carbohydrate degradation products (e.g., HMF and furfural). If the sugars are present in an oligomeric structure, an additional hydrolysis to separate the monomeric units is required.

Important parameters affecting the hydrolysis of pentose oligomers are temperature, time, and acid concentration. The challenge is that a complete hydrolysis of oligomeric structures is aimed for, while the further degradation of the released saccharides should be avoided as much as possible. This is true because typically, the combination of extreme temperatures (140° C) and long treatment time periods (> 3 h) during hemicellulose and cellulose hydrolysis leads to the degradation of the released monomeric sugars into insoluble humin. This final degradation product is typically challenging to quantify [3].

Most studies investigating lignocellulose pretreatment follow the NREL method for the quantification of overall C5 sugar content [4]. The problem with this measurement method is that the optimum conditions for the recovery of C5 and C6 sugars are not the same, since cellulose requires temperatures

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Fig. 1 Partial chemical structure of an arabinoxylan pentosan a linear β-1.4-D-xylopyranosyl backbone structure linked with randomly attached side chains of α-L-arabinofuranosyl at 2 and/or 3 position of the xylose chain [8]
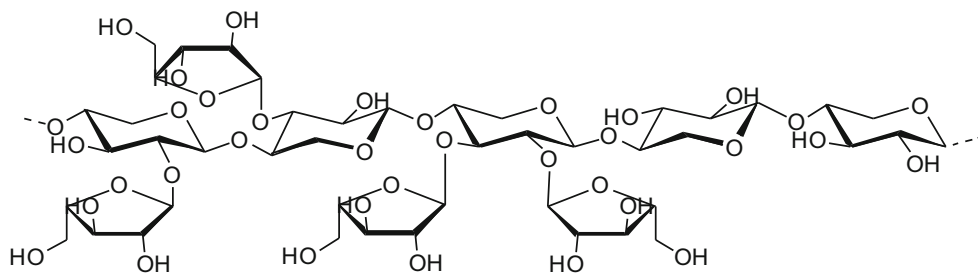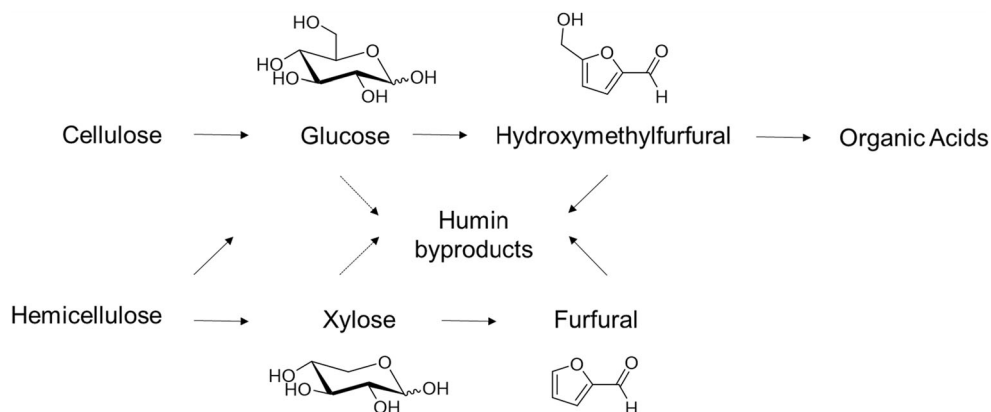

higher than hemicellulose for a complete hydrolysis. Thus, these higher temperature level results in more frequent degradation of C5 sugars. Furthermore, the interaction effects of temperature and time during the hydrolysis of C5 and C6 oligomers are hardly fully understood.

In this work, two model xylans as well as an unknown C5 sample from liquid hot water hydrolysis are investigated and optimized in terms of temperature, time, and acid concentration using HPLC-RI detection and response surface methodology with Box-Behnken design. The aim is to find the optimal hydrolysis conditions for a maximum recovery of total (free and bound) xylose and glucose from the different oligomers, respectively. The additional inclusion of the degradation products HMF and furfural enables full monomeric recovery. Furthermore, this paper aims to adjust the calculation of the total xylose recovery by adding a correction factor considering monomeric losses and degrading product formation, following the method of Lamp et al. [5]. Solid-phase analysis of products was not considered.

1.1 Pentosan structure

Pentosans are polymeric anhydrides of pentoses, mainly consisting of xylans, with an occurrence in wooden biomass [6]. Xylan itself is synthesized by enzymes in the Golgi apparatus. Xylan synthesis thus requires the coordinated action and regulation of these enzymes as well as others that synthesize and transport substrates [7].

Pentosans from plant tissues and cereals are carbohydrates consisting of a linear β-1.4-D-xylopyranosyl backbone structure linked with side chains of α-L-arabinofuranosyl at 2 and/or 3 position of the xylose chain (Fig. 1) [8].

Lignocellulosic biomass mainly consists of lignin, cellulose, and hemicellulose. During acid-catalyzed thermal hydrolysis, polymeric saccharides are depolymerized into their monomeric forms. Figure 2 illustrates the conversion paths of lignocellulosic biomass from polymeric structure via monomeric sugars—glucose from cellulose and hemicellulose, xylose from hemicellulose—towards 5-hydroxymethylfurfural from glucose and furfural from xylose. A prominent example of the conversion process is the acid-catalyzed thermal hydrolysis of the polymeric saccharides, releasing C5 and C6 saccharides. For example, harsh reaction conditions like temperatures above 140 °C lead to the formation of undesired humin byproducts [9].

The formation of aqueous xylose monomers from solid xylan is described in a simple model of reversible reactions. However, the decomposition of xylan to xylose is in fact a multistep process. The xylan chain is first decomposed to xylo-oligosaccharides, then decomposition proceeds to xylose monomer, followed by the further decomposition into furfural [10].

1.2 Pentosan quantification methods

Quantitative pentosan analysis is mainly done by the quantification of the sugar monomers within the pentosan molecules

Fig. 2 Conversion paths of cellulose and hemicellulose during acid hydrolysis [9]

Table 1 Calculation of hydrolysis outcomes

Definition (%)	Calculation	Equation
Xylose yield	$\frac{\text{Xylose (mol) } 0.88}{\text{Hemicellulose (mol)}} 100$	(2)
Arabinose yield	$\frac{\text{Arabinose (mol) } 0.88}{\text{Hemicellulose (mol)}} 100$	(3)
Glucose yield	$\frac{\text{Glucose (mol) } 0.9}{\text{Cellulose (mol)}} 100$	(4)
Furfural yield	$\frac{\text{Furfural (mol) } 1.375}{\text{Hemicellulose (mol)}} 100$	(5)
HMF yield	$\frac{\text{HMF (mol) } 1.286}{\text{Cellulose (mol)}} 100$	(6)
Hemicellulose conversion	$\frac{\text{Xylose (mol) } 0.88 + \text{Arabinose (mol) } 0.88 + \text{Furfural (mol) } 1.375}{\text{Hemicellulose (mol)}} 100$	(7)
Cellulose conversion	$\frac{\text{Glucose (mol) } 0.9 + \text{HMF (mol) } 1.286}{\text{Cellulose (mol)}} 100$	(8)
Furfural selectivity	$\frac{\text{Furfural (mol) } 1.375}{\text{Xylose (mol) } 0.88 + \text{Arabinose (mol) } 0.88 + \text{Furfural (mol) } 1.375} 100$	(9)
HMF selectivity	$\frac{\text{HMF (mol) } 1.286}{\text{Glucose (mol) } 0.9 + \text{HMF (mol) } 1.286} 100$	(10)
Correction factor		
0.88	$M_{\text{xylan}}/M_{\text{xylose}}$	(11)
0.9	$M_{\text{glucan}}/M_{\text{glucose}}$	(12)
1.375	$M_{\text{xylan}}/M_{\text{furfural}}$	(13)
1.286	$M_{\text{glucan}}/M_{\text{HMF}}$	(14)
Combined severity factor	$\log\text{CSF} = \log t + \log\left(e^{\left(\frac{T-100}{14.75}\right)}\right)$	(15)

Table 2 Experimental plan

	Temp (C°)	Acid (wt%)	Time (min)	Temp (C°)	Acid (wt%)	Time (min)	Temp (C°)	Acid (wt%)	Time (min)
Run	Corn cob xylan			Beech wood xylan			C5 straw hydrolysate		
1	100	4	180	100	4	180	100	4	180
2	100	2	120	100	2	120	100	2	120
3	100	6	120	100	6	120	100	6	120
4	100	4	60	100	4	60	100	4	60
5	120	4	120	120	4	120	110	4	120
6	120	4	120	120	4	120	110	4	120
7	120	4	120	120	4	120	110	4	120
8	120	6	180	120	6	180	110	6	180
9	120	2	180	120	2	180	110	2	180
10	120	4	120	120	4	120	110	4	120
11	120	4	120	120	4	120	110	4	120
12	120	6	60	120	6	60	110	6	60
13	120	2	60	120	2	60	110	2	60
14	140	4	60	140	4	60	120	4	60
15	140	2	120	140	2	120	120	2	120
16	140	6	120	140	6	120	120	6	120
17	140	4	180	140	4	180	120	4	180
18	100	6	60						
19	100	6	60						
20	100	6	120						
21	100	6	120						
22	100	6	180						
23	100	6	180						
24	100	4	180						

Fig. 3 Design of experiments 2D-response surface plot of released xylose from corn cob xylan hydrolysis versus temperature (**a**) and time (**b**) at 2 wt% (top left), 4 wt% (top right), 6% (bottom left) acid concentration
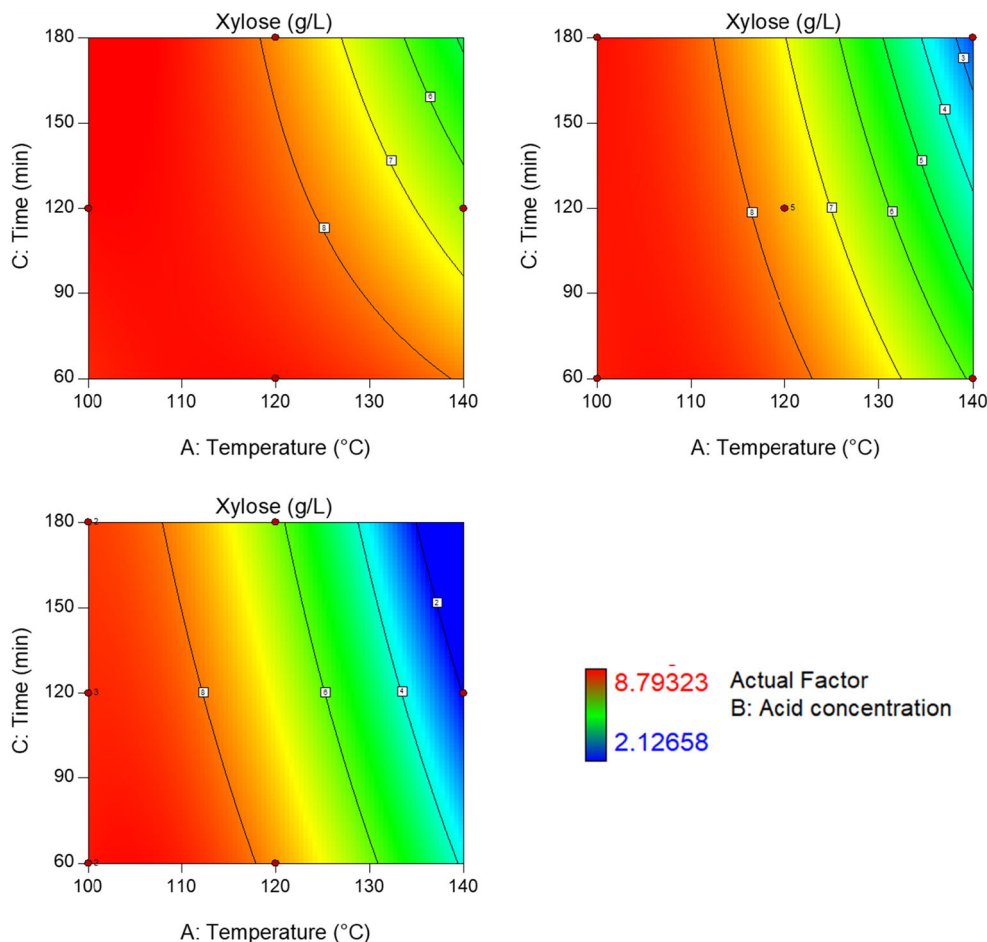

and a subsequent recalculation step [11]. Generally, pentosan analysis includes the following fundamental steps:

- Extraction of pentosans from sample matrix,
- Purification of pentosan extract,
- Hydrolysis of pentosan chains,
- Determination and quantification of the released monomeric sugars xylose and arabinose for pentosans, and
- Calculation of the original pentosan amount.

A widely spread procedure for sugar determination was established by Sluiter et al. [12], covering sugar, byproducts, and degradation products in liquid fraction process. The subsequent calculation of oligosaccharide concentration after acid hydrolysis is based on the method described by Huynh et al. [13] and was extended by Verspreet et al. [14] (Eq. (1)).

$$S\,(\%) = \frac{M_s\,V_e\,(S_b - S_a - S_x)}{m_s} \tag{1}$$

Fig. 4 Combined severity factors of temperature and time of released xylose yield form corn cob xylan (CCX) hydrolysis, displaying 2 to 6 wt% acid concentration
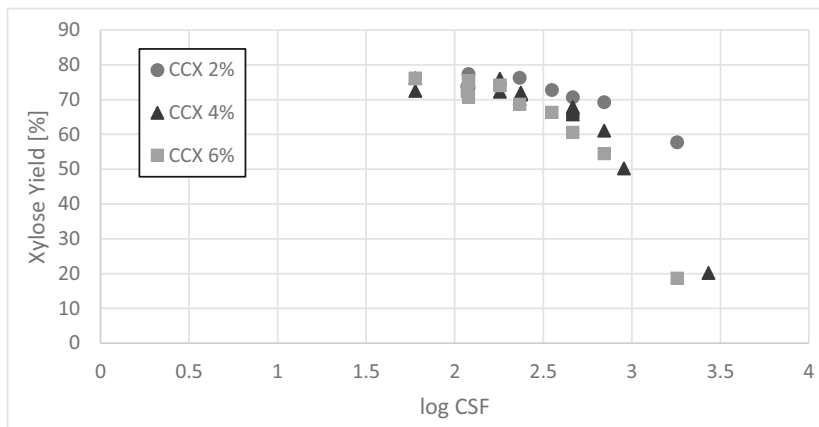
Fig. 5 Design of experiments 2D-response surface plot of released xylose from beech wood xylan hydrolysis versus temperature (**a**) and time (**b**) at 2 wt% (top left), 4 wt% (top right), and 6 wt% (bottom left) acid concentration
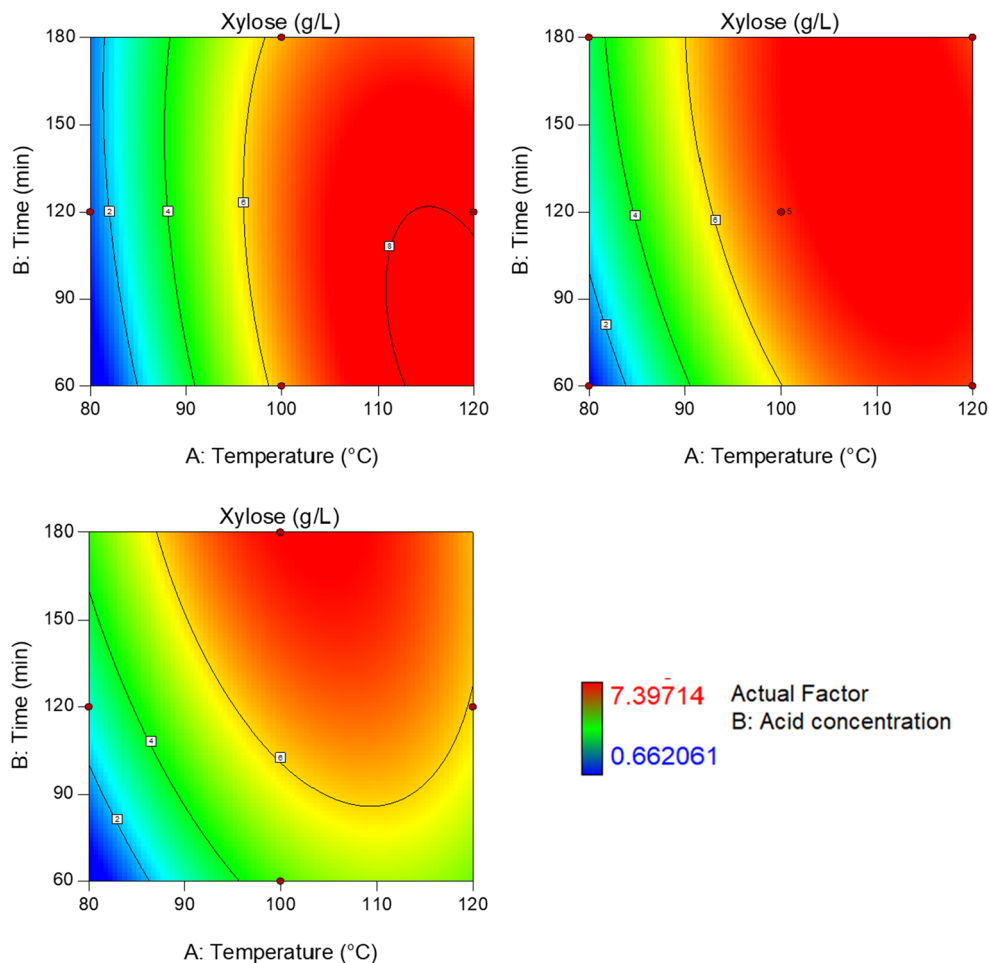

Equation (1) describes the concentration of the monomeric saccharides released from the oligomeric structure, where

M_s (g/mol)   is the molecular weight of the saccharide monomer,

V_e (mL)   is the volume of the extract,

m_s (g)   is the sample mass,

S_a and S_b (g/L)   are the saccharide monomer concentrations in the non-hydrolyzed and the hydrolyzed sample, respectively, and

S_x is the concentration of disaccharides containing the searched monomer.

This calculation does not account for a monomer yield loss due to the formation of HMF and furfural during oligomer hydrolysis. An alternative method for pentosan quantification is the colorimetric UV/Vis detection after acidic hydrolysis [15].

Fig. 6 Combined severity factors of temperature and time of released xylose yield form beech wood xylan (BWX) hydrolysis, displaying 2 to 6 wt% acid concentration
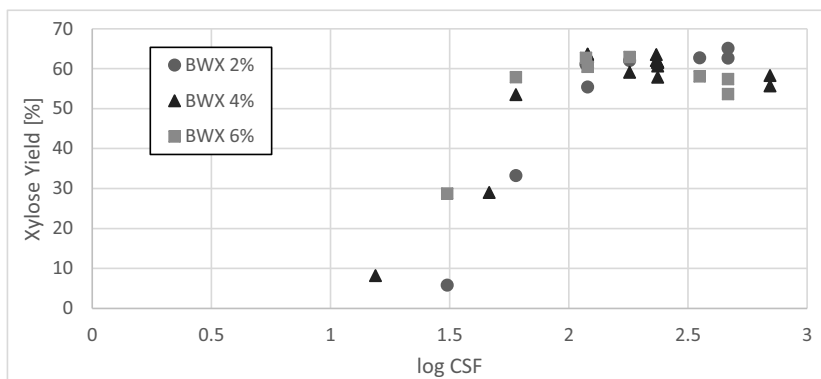
Fig. 7 Design of experiments 2D-response surface plot of released xylose from C5 hydrolysate hydrolysis versus temperature (**a**) and time (**b**) at 2 wt% (top left), 4 wt% (top right), and 6 wt% (bottom left) acid concentration
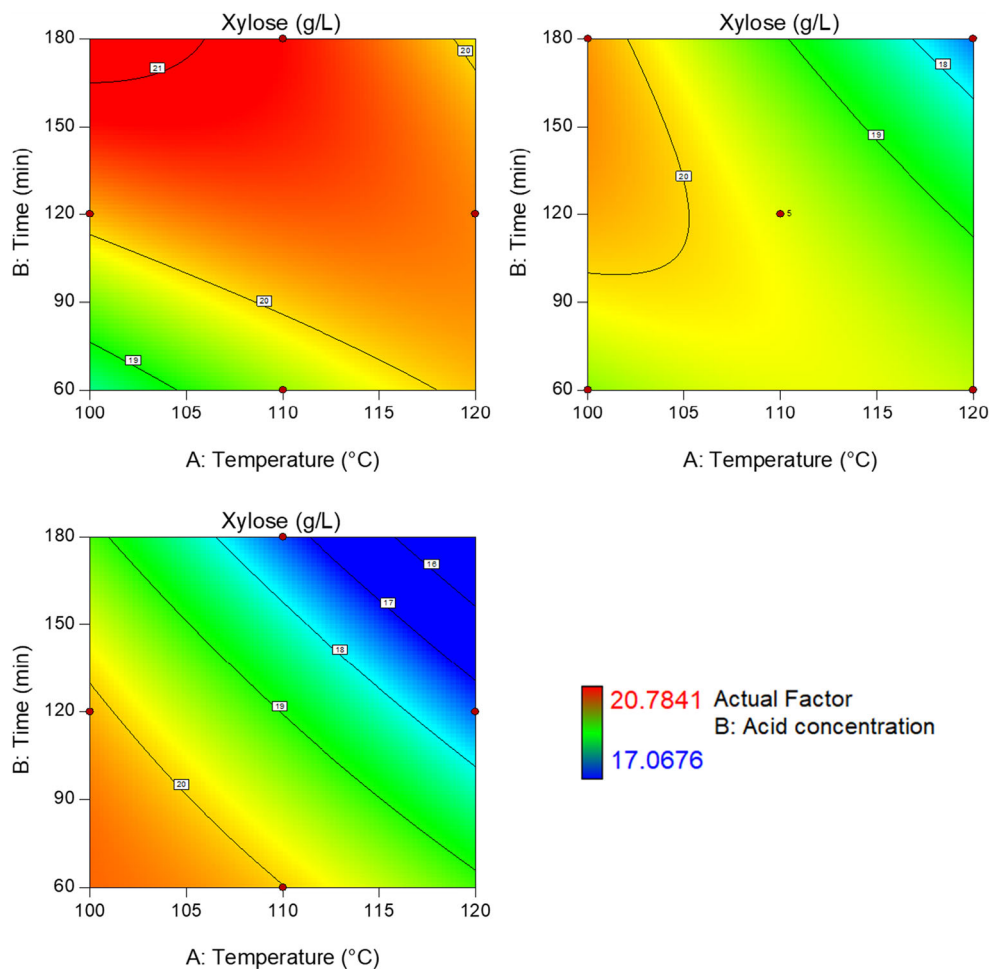
2 Experimental methods

2.1 Materials

Monomeric and oligosaccharide standards were purchased from Carl Roth GmbH. Beech wood and corn cob xylans (132 g/mol) were provided with a 95% purity and used without further purification. Wheat straw extract from liquid hot water treatment (LHW) was used as an unknown sample material [16].

2.2 Hydrolysis

The optimization of the hydrolysis conditions has been executed in two steps. First, standard samples of beech wood xylan and corn cob xylan were investigated to find the optimal

Fig. 8 Combined severity factors of temperature and time of released xylose concentration form C5 hydrolysate hydrolysis, displaying 2 to 6 wt% acid concentration
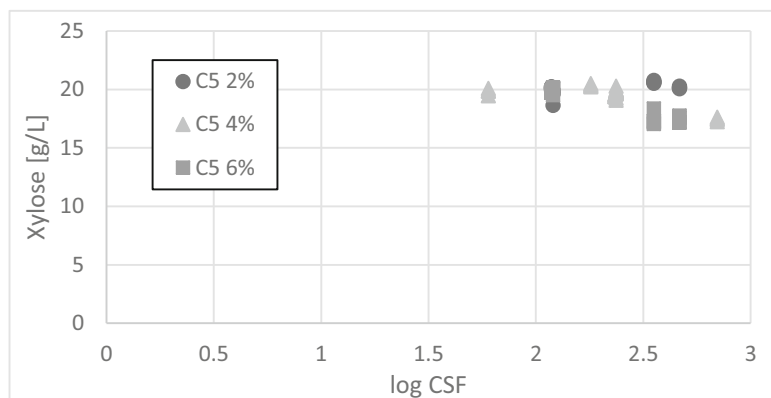
**Fig. 9** Summed up hydrolysis results from combined corn cob xylan dilution row and C5 hydrolysate displaying the determined concentrations of released monomers; the hydrolysis was executed at 110 °C, 2 wt% sulfuric acid, and 60-min run time. With corn cob xylan solution (bottom bar, dark gray), C5 hydrolysate (top bar, light gray), and combined solution of both substrates (line)
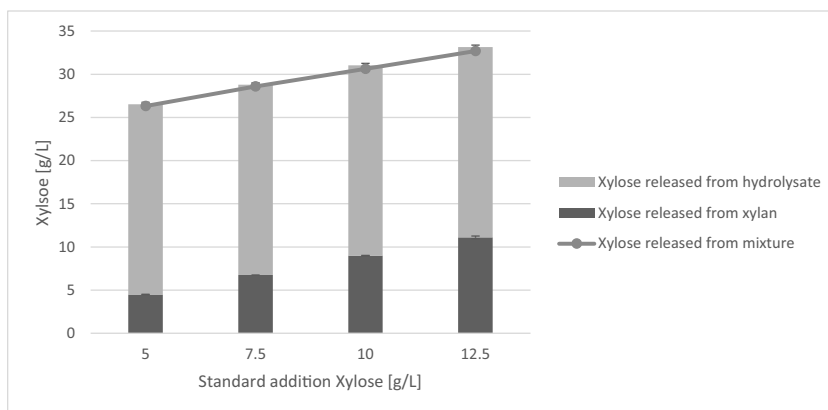
hydrolysis conditions for maximum total sugar recovery. Second, these conditions have been applied to an unknown sample of a liquid hot water pretreated wheat straw extract (see the "2.1. Materials" section).

– In case of the standard xylans, 2 g (66.6 mmol) was diluted in 200 mL demineralized water. For standard hydrolysis procedure, 175/350/525 μL (2, 4, 6 wt%) sulfuric acid (H₂SO₄ 72%) were added to the sample (10 mL) in a 10-mL cuvette with screw cap. The cuvette was incubated at 80, 100, 110, 120, and 140 °C in a thermal reactor (TR420 thermal reactor from Merck) for 60, 120, and 180 min. The temperature ranges from 80 °C as to determine the minimum temperature that enables hydrolysis of xylan structures, while at 140 °C humin byproducts are formed rapidly, hence inhibiting the aim of complete monomer release and recovery. Immediately after incubation, the cuvette was cooled in an ice water bath at 0 °C until room temperature was reached. The solution was transferred to a 10-mL centrifuge tube and neutralized to pH 7 by adding CaCO₃. The samples were stored at 4 °C for 24 h, then centrifuged (15 min, 4500 min⁻¹), and the supernatants were stored for HPLC measurement. For the unknown sample (LHW-C5 hydrolysate), the same procedure has been executed in the range of 100 to 120 °C found to be the optimal range of hydrolysis parameters for xylans.

– For standard addition of xylan to hydrolysate, stem solutions were prepared. In case of corn cob xylan, 5.0, 7.5, 10.0, and 12.5 g were diluted in 1000 mL demineralized water. An identical dilution row was prepared with the unknown hydrolysate from the wheat straw (LHW-C5 hydrolysate). Treatment of the samples followed the standard procedure in triplicates, at 110 °C, 2 wt% sulfuric acid and 60 and 90 min reaction time.

2.3 HPLC analysis

The amount of xylose and glucose as well as the degradation products—furfural and acetic acid from xylose, 5-hydroxymethylfurfural and formic acid from glucose—were measured by HPLC (Agilent Infinity II HPLC Series [5.3]). Sugar separation was achieved using a Biorad Aminex HPX-87P column with ionic form H⁺/CO₃⁻ deashing guard column and an Agilent Hi-Plex H⁺ column. A total of 5 mmol sulfuric acid was used as the mobile phase. Detection has been realized by a refraction index detector. The calibration process followed the adjusted method of NREL [12]. Standard solutions of glucose, xylose, arabinose, formic acid, acetic acid, 5-hydroxymethylfurfural, and furfural were prepared in the range between 0.024 and 24 mg/mL (Table 3).

2.4 Yields and conversions of saccharides and degradation products

The yields of released xylose and arabinose from hemicellulosic xylans as well as the conversion of the aforementioned saccharides towards furfural were determined by the method of Chen et al. [17] and expanded by the addition of arabinose yield. Chen et al.'s pretreatment combines sulfuric acid and moderate temperatures by conventional heating, followed by microwave irradiation heating. The equations for monomer yields and polymer conversion calculations as well as degradation product selectivity, correction factors for saccharides, reaction products, and the combined severity factor are displayed in Table 1. The hemicellulose stands for the total percentage of xylose, arabinose, and furfural converted from hemicellulose; the cellulose conversion designates the total percentage of glucose and HMF converted from cellulose.

2.5 Box-Behnken experimental design

The Box-Behnken design with response surface method was used to optimize the hydrolysis conditions to obtain the maximum amount of converted pentosan and xylose yield, achieving full monomeric recovery. Furthermore, interaction effects of the independent factors on the amount of total converted hemicellulose and xylose yield were studied. The independent variables were hydrolysis temperature (80 to 140 °C), hydrolysis time (60 to 180 min), and acid amount (2 to 6 wt%), which resulted in a three-factor three-level design. Statistical experiment planning was executed by using a commercially available software (Design Expert 9.0 [18]) with a central composite approach. The experimental plan is shown in Table 2 and consists of the abovementioned parameters. A total of at least 17 experimental runs were performed, and the actual predicted values of the response along with the experimental conditions are summarized in Tables 4, 5, 6, 7, 8, and 9.

3 Results and discussion

3.1 Hydrolysis optimization of corn cob xylan

Results Application of the abovementioned conditions on corn cob xylan illustrated in Fig. 3 shows the released xylose concentrations. Bright (red) colored areas show the highest released xylose concentrations, while decreasing concentrations and eventually the lowest concentration are show in darkened colors, dark (blue) respectively.

The highest amount of converted hemicellulose (97.0%) was detected at 140 °C, 2 wt% sulfuric acid, and 120-min reaction time. The highest amount of xylose (77.4%) was obtained at 100° C, 2 wt% sulfuric acid, and 120-min reaction time. Lowest amounts of xylose were found at 140 °C, 6 wt% sulfuric acid, and 120-min reaction time (18.7%) and 140 °C, 4 wt% sulfuric acid, and 180-min reaction time (20.3%) (Table 4). Correspondingly, the highest furfural concentrations were found (33.3 and 33.4%). Disregarding the <5% impurities of the starting material, the full conversion is achieved and the highest amount of detected xylose increases to 81.4%.

The calculated combined severity factors (CSFs) for corn cob hydrolysis are displayed in Fig. 4. The maximum xylose yields were achieved in the severity range of 1.5 to 2.5 CSF. Increasing severity factors higher than 2.5 CSF show a decline in xylose yields. The decrease of xylose yields at high CSFs indicates the degradation of xylose over time at high temperatures.

Discussion The results are indicating that higher temperatures and longer reaction times favor the conversion of hemicellulose to water-soluble products up to a maximum temperature and time; hereafter, humin formation is heavily favored [9]. The hemicellulose conversion is directly linked to temperature and time. Increased temperature provides more thermal effects (e.g., molecular collisions) resulting in more frequent cleavage of gylcosidic bonds between xylans. Catalyzing sulfuric acid provided highest conversion yields at 2 wt% addition. Temperatures above 100 °C favor the formation of furfural from xylose and arabinose over time. Hence, the maximum released xylose concentration results in incomplete hemicellulose conversion, while complete hemicellulose conversion lowers the xylose yield due to degradation. The amount of converted furfural can be corrected by the factor of Eq. (13). Compared to the method of Sluiter et al. [12] the optimized parameters (100 °C, 2 wt% acid, 120 min) provide higher released xylose concentrations from corn cob xylan (+14%) by reducing the degradation towards furfural (−22%) at similar hemicellulose conversion rates.

3.2 Hydrolysis optimization of beech wood xylan

Results Released xylose monomers from beech wood xylan are illustrated in Fig. 5. Following this graphic, the highest amount of converted hemicellulose (74.2%) was detected at 120 °C, 4 wt% sulfuric acid, and 180-min reaction time. The highest amount of xylose (65.1%) was obtained at 100° C, 2 wt% sulfuric acid, and 120-min reaction time. The lowest amounts of released xylose have been measured at 80 °C, 2 wt% sulfuric acid, and 120-min time (6.3%), as well as 80 °C, 4 wt%, and 60 min (8.7%) (Table 5). A yield of ~30% released xylose was detected at 80 °C, 6 wt%, and 120 min and 80 °C, 4 wt%, and 180 min. Thus, the temperature of 80 °C does not provide a complete hydrolysis of xylan structures at the chosen period and acid concentration. The highest amount of furfural (~10%) was found at 120 °C, 6 wt%, 120 min and 120 °C, 4 wt%, 180 min. Disregarding the <5% impurities of the starting material, the hemicellulose conversion the highest amount of detected xylose increases to 74% and 68.5% respectively.

The calculated combined severity factors for beech wood hydrolysis are displayed in Fig. 6. The maximum xylose yields were achieved in the severity range of 2 to 3 CSF. Decreasing severity factors smaller than 2 CSFs show a decline in xylose yields. The lowest CSFs indicate that temperatures below 80°C are insufficient for xylose hydrolysis.

Discussion In total, beech wood xylan is possibly harder to hydrolyze than corn cob xylan at the chosen conditions. At these conditions, it shows significantly lower xylose concentrations. In addition, beech wood xylan showed poor solubility in water. Potentially unsolved xylan in the stem solution might have contributed to the decreased xylose yields. A solution for this problem could be the inclusion of optimized catalytic

ionic acids which show increased conversion rates for oligosaccharides from hard wood [19]. Compared to the method of Sluiter et al. [12], the optimized parameters (120 °C, 2 wt% acid, 120 min) provide a minorly higher released xylose concentrations from beech wood xylan (+2.3%) by reducing the degradation towards furfural (−1.7%) at similar hemicellulose conversion rates.

3.3 Hydrolysis optimization of C5 hydrolysate

Results The detected monomer and corresponding concentrations of hydrolyzed C5 hydrolysate are displayed in Fig. 7. Hydrolysis of standard xylans showed highest xylose yields at low temperatures and acid concentrations within the chosen parameters. However, the screened conditions for C5 hydrolysis display no particular maximum of the released monomers. Treated C5 hydrolysate consists mainly of xylose (20 g/L) and partly glucose (4 g/L), as well as degradation products HMF (0.3 g/L), furfural (1.3 g/L), and released organic acids (3.8 g/L). The untreated C5 hydrolysate contains an average concentration of 3 g/L monomeric xylose. The highest amounts of released xylose (20.7 g/L) were detected at 110 °C, 2 wt% sulfuric acid, and 180-min reaction time with a furfural concentration of 1.25 g/L (Table 8). NREL conditions (120 °C, 4 wt%, 60 min) resulted in 6.3% fewer released xylose (19.4 g/L) and 21.3% higher concentrations of furfural (1.6 g/L).

The calculated combined severity factors for C5 hydrolysate hydrolysis are displayed in Fig. 8. Xylose concentrations were found in the severity range of 1.5 to 3 CSF. No particular maximum was detected; the reaction parameters have been narrowed for a corridor of optimal hydrolysis conditions.

Standard addition of xylans to C5 hydrolysate was executed to determine the degree of conversion and effects of matrix material. Corn cob xylan was chosen as an additive agent in standard addition, because of its better comparability as stemlike biomass with C5 hydrolysate. The dilution row was prepared for 5.0 to 12.5 g in C5 hydrolysate from wheat straw. Conditions were settled at 110 °C, 2 wt% sulfuric acid, and 60-min and 90-min reaction time.

Discussion Hydrolysis at 2 wt% acid concentration showed the most promising results with a maximum of released xylose ranging along time and temperature axis in a broad area. This is confirmed by the severity factor which was calculated for C5 hydrolysate and plotted versus the xylose yields for all added sulfuric acid concentrations. Acid concentrations at 2 and 4 wt% result in a similar severity factor inside the optimum corridor, while at 6 wt%, degradation of xylose rapidly increases.

The released xylose concentrations from corn cob xylan standards with a concentration range from 5 to 12.5 g/mol (bottom bar), C5 hydrolysate averaged from 5 samples (top bar), and the combined solutions of both substrates (line) are displayed in Fig. 9 in triplicates including standard deviation. The recovery of monomers indicates a yield of at least 90% for each added amount of xylan to the hydrolysate. The deviation of the sum of added xylan and hydrolysate compared to the mixture is in any case lower than 2% for 60 min hydrolysis and lower than 9% for 90 min hydrolysis. This indicates that no matrix effects from either substrate inhibited the complete hydrolysis. As a result of standard addition, the transfer of the optimal hydrolysis conditions from standard xylans was achieved.

4 Conclusion

The aim was to find the optimal hydrolysis conditions for a maximum recovery of total xylose and the additional inclusion of the degradation products HMF and furfural enabling full monomeric recovery. The hydrolysis of corn cob and beech wood xylan as well as an unknown sample of a C5 hydrolysate has been analyzed by using response surface methodology based on a Box-Behnken design. Quadratic regression was used to define the effects of the variables, temperature, time, and acid concentration as well as their interaction effects on the responses of hemicellulose conversion and xylose yield. Analysis of variance developed a significant model using quadratic regression and predicted both responses with a high level of confidence. The optimized hydrolysis parameters for model xylans and unknown samples enabled the increased recovery of xylose and reduction of furfural formation. For corn cob xylan, the maximum xylose yield was found to be 77.3% (100 °C, 2 wt% acid, 120 min) at 87.5% hemicellulose conversion, and for beech wood xylan, the maximum xylose yield was found to be 65.1% (100 °C, 2 wt% acid, 120 min) at 70.5% hemicellulose conversion. The hydrolysis parameters (110 °C, 60 min, 2 wt% H_2SO_4) were found optimal for the application on liquid phase hemicellulose xylan structures. Standard addition of xylan from corn cob to C5 hydrolysate showed similar conversion rates (< 2% deviation); no matrix effects were detected. All substrates show increased yields of released xylose and reduced furfural compared to established methods. Recovery of monomers from oligosaccharide hydrolysis was achieved at conversion rates higher than 90% including the application of correction factors for HMF and furfural.

In future works, the hydrolysis condition for hydrolysates from wheat straw and applicability of the presented method will be further investigated focusing on the conversion of wheat straw hydrolysate from steam as promising follow-up substrate.

Appendix

Acid hydrolysis thermal (oven)

For standard hydrolysis procedure, 355 µL hydrolysis mixture (H_2SO_4 96%) was added to the sample (10 mL) in a 100-mL glass bottle with screw cap. The bottle was incubated at 121 °C in an oven for 1 h. Immediately after incubations, the bottle was cooled in an ice-water bath at 0 °C to room temperature. The solutions were transferred to 50-mL centrifuge tubes and neutralized by adding $CaCO_3$ until pH 7 was adjusted. The samples were centrifuged (15 min, 4500 rpm), stored at 4 °C for 24 h, again centrifuged and stored for HPLC measurement.

Optimization of thermal acid hydrolysis

Hydrolysis of oligosaccharides in the thermal oven produced undesired results. Reaction vessels suffered permanent leakage. One hundred-millimeter glass flasks cannot be sealed to fullly extend at a temperature of 140 °C; all contained fluid evaporates within 180 min, thermally decomposing the desired product saccharides. Hence, 130 °C oven temperature is the maximum temperature applicable in the presented method, though still compromised by loss of volume. Hence, thermal oven hydrolysis results are disregarded.

HPLC Agilent Infinity II HPLC series details

- Pump: product no. G7162A, Serial no. DEAC90341, made in Germany
- Auto sampler: product no. G7116A, Serial no. DEAEM05467, made in Germany
- Thermostat: product no. G7167-60101, Serial no. DEBBP07859, made in Germany
- RI detector: product no. G7111A, Serial no. DEAEZ01310, made in Germany

Table 3 Concentrations of the serial dilution standard saccharides, byproducts, and degradation products

Component	C_1 (mg/mL)	C_2 (mg/mL)	C_3 (mg/mL)	C_4 (mg/mL)	C_5 (mg/mL)	C_6 (mg/mL)	C_7 (mg/mL)	C_8 (mg/mL)	C_9 (mg/mL)
Cellobiose	24	12	6	4.8	2.4	1.2	0.48	0.24	0.024
Glucose	24	12	6	4.8	2.4	1.2	0.48	0.24	0.024
Xylose	24	12	6	4.8	2.4	1.2	0.48	0.24	0.024
Arabinose	24	12	6	4.8	2.4	1.2	0.48	0.24	0.024
Acetic acid	24	12	6	4.8	2.4	1.2	0.48	0.24	0.024
HMF	5	2.5	1.25	1	0.5	0.25	0.1	0.05	0.005
Furfural	5	2.5	1.25	1	0.5	0.25	0.1	0.05	0.005

Table 4 Hydrolysis yields from corn cob xylan

Temp (C°)	Acid (wt%)	Time (min)	Hemicellulose conversion (%)	Glucose yield (%)	Xylose yield (%)	Arabinose yield (%)	Furfural yield (%)
100	4	180	89.35	9.66	76.04	1.94	2.05
100	2	120	87.48	5.90	77.42	1.81	0
100	6	120	88.63	9.85	75.21	1.86	2.20
100	4	60	86.27	6.07	76.27	1.79	0
120	4	120	90.97	10.36	67.87	1.66	8.14
120	4	120	94.38	10.28	66.50	1.65	9.03
120	4	120	93.86	10.28	67.10	1.63	8.12
120	6	180	90.07	9.72	54.50	1.46	15.89
120	2	180	95.03	10.52	69.34	1.65	7.45
120	4	120	90.51	10.27	65.80	1.63	9.23
120	4	120	90.25	10.30	67.72	1.64	7.84
120	6	60	92.05	10.22	68.70	1.66	6.33
120	2	60	90.52	10.28	76.31	1.97	2.52
140	4	60	95.45	9.48	50.31	1.48	19.67
140	2	120	97.00	10.01	57.77	1.56	15.99
140	6	120	81.91	7.36	18.72	0.86	33.39
140	4	180	83.04	7.61	20.25	0.88	33.29
100	6	60	87.49	7.05	76.06	1.86	0.97
100	6	60	87.74	7.37	76.22	1.87	1.04
100	6	120	88.25	9.34	75.47	1.87	1.85
100	6	120	88.05	9.23	75.26	1.85	1.85
100	6	180	88.83	10.21	74.27	1.91	2.79
100	6	180	88.63	10.21	74.06	1.91	2.87
100	4	180	89.35	9.66	76.04	1.94	2.05

Table 5 Hydrolysis yields from beech wood xylan

Temp (C°)	Acid (wt%)	Time (min)	Hemicellulose conversion (%)	Glucose yield (%)	Xylose yield (%)	Arabinose yield (%)	Furfural yield (%)
80	4	180	29.56	0.37	29.04	0.52	0.00
80	2	120	6.31	0.00	5.83	0.48	0.00
80	6	120	29.30	0.44	28.80	0.50	0.00
80	4	60	8.69	0.00	8.22	0.47	0.00
100	4	120	63.70	2.33	63.12	0.58	0.00
100	4	120	63.69	2.35	63.12	0.57	0.00
100	4	120	64.14	2.36	63.56	0.57	0.00
100	6	180	63.47	2.52	62.93	0.54	0.00
100	2	180	62.60	1.81	62.11	0.49	0.00
100	4	120	64.29	2.32	63.72	0.57	0.00
100	4	120	62.22	1.83	61.74	0.48	0.00
100	6	60	58.41	1.57	57.92	0.49	0.00
100	2	60	33.82	0.53	33.30	0.52	0.00
120	4	60	68.91	2.55	63.58	0.57	3.04
120	2	120	70.48	2.62	65.13	0.56	3.06
120	6	120	73.08	2.79	57.45	0.69	9.56
120	4	180	74.16	2.90	58.30	0.71	9.70

Table 6 Hydrolysis yields from corn cob xylan duplicate

Temp (C°)	Acid (wt%)	Time (min)	Hemicellulose conversion (%)	Glucose yield (%)	Xylose yield (%)	Arabinose yield (%)	Furfural yield (%)
100	4	180	84.91	8.51	72.34	1.47	1.78
100	2	120	83.25	4.88	73.51	1.52	0.00
100	6	120	89.04	8.34	70.76	1.47	2.34
100	4	60	82.03	5.12	72.55	1.51	0.00
110	4	120	90.31	10.14	71.77	1.63	3.27
110	4	120	88.52	10.15	71.51	1.62	3.63
110	4	120	86.65	10.15	71.57	1.63	3.30
110	6	180	87.84	10.07	66.39	1.57	7.24
110	2	180	87.50	10.07	72.85	1.64	3.03
110	4	120	87.64	10.26	72.04	1.68	3.41
110	4	120	87.74	10.16	71.67	1.64	3.30
110	6	60	86.95	10.00	72.37	1.64	2.97
110	2	60	83.46	6.10	73.83	1.53	0.00
120	4	60	87.78	10.19	70.24	1.66	4.59
120	2	120	87.70	10.18	70.75	1.65	4.53
120	6	120	91.01	9.82	60.61	1.54	11.25
120	4	180	90.05	9.90	61.09	1.54	11.62

Table 7 Hydrolysis yields from beech wood xylan duplicate

Temp (C°)	Acid (wt%)	Time (min)	Hemicellulose conversion (%)	Glucose yield (%)	Xylose yield (%)	Arabinose yield (%)	Furfural yield (%)
100	4	180	65.59	2.19	59.16	0.29	0.00
100	2	120	55.73	1.13	55.42	0.31	0.00
100	6	120	60.81	2.03	60.52	0.30	0.00
100	4	60	53.82	1.10	53.51	0.31	0.00
110	4	120	66.29	2.29	61.70	0.30	0.00
110	4	120	65.94	2.32	61.70	0.30	0.00
110	4	120	66.17	2.31	61.50	0.31	0.00
110	6	180	67.51	2.62	58.12	0.31	0.00
110	2	180	66.81	2.44	62.77	0.30	0.00
110	4	120	65.55	2.29	60.69	0.30	0.00
110	4	120	66.72	2.62	57.91	0.31	0.00
110	6	60	66.97	2.87	62.78	0.31	0.00
110	2	60	61.42	1.70	61.12	0.31	0.00
120	4	60	67.91	2.94	62.05	0.31	0.00
120	2	120	68.32	2.87	62.69	0.32	0.00
120	6	120	70.74	2.30	53.70	0.35	0.00
120	4	180	71.29	2.39	55.71	0.34	0.00

Table 8 Hydrolysis C5 hydrolysate

Temp (C°)	Acid (wt%)	Time (min)	Glucose conc [g/L]	Xylose conc [g/L]	Arabinose conc [g/L]	Furfural conc [g/L]	HMF conc [g/L]
100	4	180	3.68	20.41	2.11	1.22	0.27
100	2	120	2.06	19.94	2.00	1.00	0.17
100	6	120	3.66	20.13	2.07	1.22	0.26
100	4	60	2.19	19.46	1.97	1.00	0.17
110	4	120	4.04	19.77	2.06	1.40	0.32
110	4	120	4.02	19.63	2.06	1.43	0.32
110	4	120	4.02	19.81	2.06	1.40	0.32
110	6	180	3.96	17.07	1.77	1.82	0.19
110	2	180	3.66	20.78	2.03	1.25	0.29
110	4	120	3.99	19.82	2.05	1.37	0.31
110	4	120	4.03	19.71	2.06	1.41	0.32
110	6	60	3.57	19.74	2.00	1.27	0.27
110	2	60	2.20	20.00	1.97	1.03	0.18
120	4	60	4.06	19.42	2.00	1.59	0.35
120	2	120	4.69	20.28	2.02	1.58	0.38
120	6	120	4.53	17.60	1.73	2.32	0.14
120	4	180	3.96	17.45	1.75	2.33	0.15
120	0	180	0.62	4.03	0.91	0.82	0.08
Untreated sample				0.50	3.47	0.82	2.02	0.75

Table 9 ANOVA (analysis of variance)

Substrate	Source	Sum of squares	df	Mean square	F-value	p-value prob > F	Significance
Xylose CCX	Model	75.14	9	8.35	650.89	< 0.0001	Significant
Furfural CCX	Model	10.53	9	1.17	226.47	< 0.0001	Significant
Xylose BWX	Model	82.51	9	9.17	21.02	0.0003	Significant
Furfural BWX	Model	0.82	9	0.091	11.18	0.0022	Significant
Xylose C5	Model	17.71	9	1.97	44.15	< 0.0001	Significant
Furfural C5	Model	2.42	9	0.27	33.36	< 0.0001	Significant

Xylose CCX = concentration of released xylose from corn cob xylan; furfural CCX = concentration of converted furfural from corn cob xylan; xylose BWX = concentration of released xylose from beech wood xylan; furfural BWX = concentration of converted furfural from beech wood xylan; xylose C5 = concentration of released xylose from liquid hot water wheat straw hydrolysate; furfural C5 = concentration of released and converted furfural from liquid hot water wheat straw hydrolysate

Declarations

Conflict of interest The authors declare no competing interests.

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