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Dilute Acid Hydrolysis of Wheat Straw Oligosaccharides

Luís C. Duarte · Talita Silva-Fernandes · Florbela Carvalheiro · Francisco M. Gírio

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Abstract The dilute acid posthydrolysis of wheat straw hemicellulosic oligosaccharides obtained by autohydrolysis was evaluated. An empirical model was used to describe the effect of catalyst concentration (sulfuric acid, 0.1–4% *w*/*w*) and reaction time (0–60 min) based on data from a Doehlert experimental design. Catalyst concentration is the main variable influencing posthydrolysis performance, as both its linear and quadratic coefficients are statistically significant for the majority of the studied variables, namely, the ones related to sugar and byproducts production. Reaction time influences xylose and furan derivatives concentrations but not phenolics or acetic acid content. Catalyst concentration and reaction time interact synergistically, minimizing sugar recovery and promoting furan derivatives production. Based on the proposed models, it was possible to delimit an operational range that enables to obtain high monosaccharides recovery together with a slight decrease in inhibitors content as compared to the standard acid hydrolysis treatment. Furthermore, this is achieved with up to 70% less acid spending or considerable savings on reaction time.

Keywords Autohydrolysis · Dilute acid posthydrolysis · Experimental design · Hemicellulosic hydrolyzate · Wheat straw · Xylooligosaccharides

Introduction

The use of lignocellulosic biomass within the biorefinery implies the use of pretreatment processes that should effectively and selectively separate hemicellulose and/or lignin to facilitate the subsequent cellulose hydrolysis. Furthermore, these processes should also enable the highest recovery of those fractionated components in order to enable their upgrade for increasing the biorefinery economical viability. Among the promising biomass pretreatment options available, namely, autohydrolysis, steam explosion, alkaline, organosolv, and oxidation treatments, or their combinations, many produce a sugar-rich liquid

L. C. Duarte (✉) · T. Silva-Fernandes · F. Carvalheiro · F. M. Gírio
Departamento de Biotecnologia, INETI, Estrada do Paço do Lumiar 22, 1649-038 Lisboa, Portugal
e-mail: luis.duarte@ineti.pt

stream derived from the selective hemicellulose solubilization. A significant portion of these sugars is typically in oligomeric form [1, 2], as many studies have shown that preservation of sugars as oligomers can be beneficial (e.g. [3]). Actually, the partial hydrolysis of hemicellulose can enable a reduction both on energy requirements and especially on the formation of many relevant sugar degradation compounds, particularly, 5-hydroxymethylfurfural (HMF) and furfural, that can inhibit the upgrade of both the liquid and solid fractions. Furthermore, oligosaccharides, and particularly xylooligosaccharides produced from herbaceous and hardwood feeedstocks, may have a high added value as marketable products presenting many interesting properties for application in the food, pharma, and cosmetic industries as specialty chemicals [4–6]. As these applications are rather restricted in volume, further solutions are still needed to upgrade the high amounts of oligosaccharides that are envisage to be produced in a biorefinery. Within this framework, the oligomeric form imposes some upgrade problems, as it renders these sugars almost unusable, as few biotechnologically relevant microbial catalysts can directly metabolize them. Therefore, a posthydrolysis step of the hemicellulosic oligosaccharides is practically a compulsory requirement for its upgrade.

The posthydrolysis options can be reduced to acid [7–14] or enzymatic [14–16] catalyzed hydrolysis. Acid hydrolysis typically presents both higher yield and productivity when compared to the enzymatic hydrolysis processes. Furthermore, as much of the hemicellulose complex structure is still present in the oligosaccharides [1, 17], the action of several enzyme activities are usually required for the complete hydrolysis (e.g., for hardwood type materials: endoxylanase, exoxylanase, β-xylosidase and accessory activities like acetyl xylanesterase, α-glucuronidase, α-arabinofuranosidase, and feruloyl esterase); therefore potentially turning the process uneconomical. In contrast to enzymatic hydrolysis, significant monosaccharide degradation reactions may occur during acid posthydrolysis. Examples of such reactions are the degradation of pentoses to furfural, hexoses to HMF, and of both these furans to aliphatic acids such as formic and levulinic acids. Therefore, to obtain a high monosaccharide recovery, a careful optimization of the operational conditions is required. Nevertheless, this is seldom done. The most studied factors are catalyst concentration, ranging from 0% to 4% [11–14], reaction time, ranging from 0 to 602 min [12, 14], and temperature, ranging from 100.5 to 135 °C [12, 13]. The optimal conditions identified are dependent on pretreatment type and raw material. Specifically, catalyst concentration seems to be markedly influenced by pretreatment.

In this work, the dilute acid posthydrolysis of wheat straw hemicellulosic oligosaccharides obtained by autohydrolysis was evaluated, given that autohydrolysis is a promising technology for the integrated upgrade of this abundant material [18–20]. We have used a Doehlert experimental design to study the effect of catalyst (sulfuric acid) concentration and reaction time in order to maximize monosaccharide recovery and minimize byproduct formation and reagents cost.

Methods

Feedstock Material

Wheat straw was supplied *in natura* (10.6% moisture) by Estação Nacional de Melhoramento de Plantas (Elvas, Portugal). Upon arrival, it was ground with a knife mill to particles smaller than 1.5 mm, homogenized in a defined lot, and stored in plastic containers at room temperature. The processed lot contained (*w*/*w*, dry basis) 38.9% glucan

(cellulose), 18.1% xylan, 3.0% arabinan, 2.5% acetyl groups, 18.0% Klason lignin, 9.7% ash, 4.5% protein, and 5.5% of extractives and others.

Autohydrolysis

The feedstock material was thermally treated with water (autohydrolysis) in 600-mL stainless steel reactor (Parr, Moline, IL, USA), as optimized before [20]: liquid-to-solid ratio, 10:1 (*w*/*w*); agitation speed, 150 rpm under non-isothermal conditions (maximum temperature 215 °C, approximately 30 min heating time) that corresponds to a log R_0=3.96 [21, 22]. When the desired temperature was attained, the reactor was rapidly cooled down to room temperature by water circulating through a serpentine coil together with in an ice bath. The liquid and solid phases were recovered by filtration (Whatman filter paper no. 1).

Posthydrolysis

Posthydrolysis assays were performed in autoclave at 121 °C, in universal Schott flasks capped with blue stoppers. The effect of H_2SO_4 concentration and reaction time (isothermal period) were studied according to an experimental design (see below). Autohydrolysis liquor and H_2SO_4 72% (*w*/*w*) were mixed at different ratios to obtain the prescribed acid concentration. The total initial mass was kept constant at 20 g for every condition tested. When the reaction time was attained, the autoclave was rapidly cooled down to 100 °C (approximately 3 min).

The standard posthydrolysis conditions are defined by 4% H_2SO_4 and 60 min at 121 °C (condition Z, see below), as proposed in [2, 8, 14, 23] for the quantitative acid hydrolysis of oligosaccharides.

Experimental Design

A Doehlert uniform design [24] was used to establish the effects of H_2SO_4 concentration (X_1) between 0.1 and 4.0% (*w*/*w*) and reaction time (X_2) between 4.0 and 60.0 min. Five levels were selected to study H_2SO_4 concentration and three levels for reaction time, which enables the estimation of curvature effects for each independent variable. The design results in seven combinations (Table 1). All assays were carried out at least in duplicate to provide a measure of the inherent experimental error.

Table 1 Codified matrix for the Doehlert experimental design for two variables and the corresponding experimental matrix.

Trial	Variables			
	Coded		Real	
	X_1	X_2	H_2SO_4 (%)	Time (min)
A	0.00	0.00	2.05	30.0
B	1.00	0.00	4.00	30.0
C	−1.00	0.00	0.10	30.0
D	0.50	0.866	3.03	56.0
E	−0.50	−0.866	1.08	4.0
F	0.50	−0.866	3.03	4.0
G	−0.50	0.866	1.08	56.0

Each row represents an experimental trial

The model used to express the responses was a second order polynomial equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \varepsilon \quad (1)$$

where, Y is the response, X the independent variables, and the subscripts 1 and 2 are referred to H_2SO_4 concentration and time, respectively. β_0 is the regression coefficient at centre point; β_1 and β_2 are the linear coefficients of the variables 1 and 2, respectively; β_{12} is the second-order interaction coefficient between variables 1 and 2; and β_{11} and β_{22} are the quadratic coefficients for variables 1 and 2; and ε are independent random errors, assumed to be normally and independently distributed.

The linear multiple regression to Eq. 1 and its analysis of variance (ANOVA) were carried out using Microsoft® Excel 2003 regression tool pack, using all replicates. The best hydrolysis conditions were determined by using the Microsoft Excel® 2003 Solver tool based on the best-fit equations using a constrained model. Coded representation of the variables was used for all calculation purposes.

Analytical Methods

The monosaccharides, aliphatic acids, and furan derivates were quantified by high-performance liquid chromatography (HPLC). Monosaccharides (glucose, xylose, arabinose, mannose, and galactose) were quantified using a Bio-Rad Aminex HPX-87P column (Hercules, CA). A Merck Hitachi HPLC system (Tokyo, Japan) equipped with a refractive index detector (L-7490) controlled at 35 °C was used. The mobile phase was H_2O, the column temperature 85 °C, and the flow rate 0.6 mL/min. Injection volume was 20 μL. Formic and acetic acids, furfural and HMF were quantified using a Bio-Rad Aminex HPX-87H column. A Waters LC1 module 1 Plus (Millford, MA) equipped with both a refractive index (controlled at 45°C) and an ultraviolet detector (set at 280 nm) was used. The mobile phase was H_2SO_4 5 mM, the column temperature 50°C, and the flow rate 0.4 mL/min. The system was equipped with a Micro-Guard Cation-H Refill Cartridge from Bio-Rad before the HPX-87H column. Injection volume was 20 μL. It is believed that formic acid quantification is somewhat inaccurate, as it is suspected that it co-elutes with an unidentified compound under the reported conditions. Arabinose can be quantified by both systems. All samples were filtered with 0.45-μm Gelman membrane filters prior to analysis.

Total phenolic compounds content was assayed spectrophotometrically by the modified Prussian blue method as described in [25] using a Thermo Electron Corporation spectrophotometer model Genesys 6 (USA). Tannic acid was used as calibration standard.

Results and Discussion

Composition of Autohydrolysis Liquor and Posthydrolyzates

Table 2 presents the composition of the liquors obtained from wheat straw autohydrolysis under optimized conditions for hemicellulose recovery. Sugars are mainly in oligomeric form (82.5%), which is typical for autohydrolysis processes, as the conditions leading to the highest recovery of soluble hemicelluloses do not lead to its complete hydrolysis [20]. Xylooligosaccharides account for almost 80% of the total oligosaccharides, with arabinooligosaccharides and glucooligosaccharides appearing in equivalent amounts. All

Table 2 Composition of the wheat straw autohydrolysis liquors obtained under optimized conditions for hemicellulose recovery.

Component	Concentration (g/L)
XOS[a]	8.99
AOS[a]	1.17
GlcOS[a]	1.18
Xylose	0.61
Arabinose	0.86
Glucose	0.18
Mannose	0.76
Galactose	0.59
Acetic acid	2.23
Formic acid	1.74
Total phenolic compounds	2.16
Furfural	0.21
HMF	0.01

XOS xylooligosaccharides, *AOS* arabinooligosaccharides, *GlcOS* glucooligosaccharides

[a] Calculated as the increase in monosaccharide content after standard posthydrolysis ($X_1=1$, $X_2=1$)

monosaccharides have similar concentrations, never exceeding 1 g/L, except for glucose that presented a lower concentration (close to 0.2 g/L). Acetic acid and phenolic compounds, which are structural constituents of lignocellulosic biomass, are the main byproducts present. Furfural content is low, but it is still the main furan derivative, as HMF appears in almost negligible amounts.

The concentration of sugars and by-products obtained after the different posthydrolysis conditions applied to the wheat straw autohydrolysis liquor, as defined by the experimental design, are presented in Table 3 (trials A–G). The additional reported data are the trials V–Y, which were carried out to validate the models (see below) and the data for the standard hydrolysis condition (trial Z). In the posthydrolyzates, sugars are mainly present as monosaccharides, except for trial C, which corresponds to the lower severity tested (defined as a function of reaction time, temperature, and catalyst concentration [14]). For this trial, sugars were mainly maintained in the oligomeric form, although apparently with a lower degree of polymerization (data not shown). For all other conditions, xylose was the most relevant monosaccharide in the posthydrolyzates, being the one whose concentration is more markedly increased. Arabinose is the second major monosaccharide, but it never exceeds 2.1 g/L. Mannose, galactose, and glucose were also produced during posthydrolysis, but total hexoses concentration never exceeded 4.0 g/L (trial X). Actually, pentose sugars are dominant, typically accounting for close to 75% of all monosaccharides.

Acetic acid concentration only slightly increased for higher severity conditions, not exceeding 2.43 g/L (trial A), which is explained by the low acetylation level of wheat straw XOS obtained under these optimized operational conditions for the autohydrolysis process (1.5 acetyl substitutents per 100 xylose units) [20]. Formic acid concentration decreased as compared to the autohydrolysis liquor, which can probably be related to analytical problems, and it will not be discussed further. Furan derivatives are clearly produced during autohydrolysis. Within the experimental design, furfural concentration increases approximately fivefold as compared to the autohydrolysis liquor, reaching 1.07 g/L (trial D). The highest furfural concentration was 1.16 g/L, for the harsher condition tested, the standard posthydrolysis condition (Z). HMF also increases, but its concentration was always below

Table 3 Sugars and byproducts concentration obtained after posthydrolysis according to the Doehlert design (A–G), additional validation trials (V–Y) and standard posthydrolysis condition (Z).

	Trial											
	A	B	C	D	E	F	G	V	W	X	Y	Z
Glucose	1.11±0.02	1.31±0.04	0.37±0.08	1.45±0.04	0.53±0.04	0.92±0.01	0.94±0.04	1.45±0.07	0.94±0.02	1.26±0.02	0.73±0.00	1.43±0.05
Xylose	10.27±0.05	10.14±0.11	0.65±0.05	10.00±0.02	4.24±0.30	9.97±0.01	10.08±0.07	10.43±0.12	10.35±0.19	10.25±0.25	9.80±0.01	10.10±0.03
Arabinose	1.96±0.08	1.98±0.01	0.88±0.01	1.97±0.05	1.74±0.01	2.04±0.04	2.02±0.09	2.00±0.09	2.06±0.04	1.95±0.03	2.07±0.03	2.05±0.04
Mannose	1.02±0.09	0.99±0.04	0.88±0.01	1.16±0.05	0.87±0.04	0.89±0.08	1.13±0.10	1.28±0.01	1.02±0.06	1.08±0.05	1.11±0.02	1.04±0.01
Galactose	1.42±0.25	1.45±0.03	0.23±0.02	1.06±0.07	0.90±0.08	1.19±0.13	1.24±0.02	0.92±0.02	0.75±0.00	1.61±0.08	0.69±0.09	0.92±0.06
Total sugars (TS)	15.78±0.48	15.86±0.17	3.02±0.08	15.63±0.05	8.29±0.40	15.02±0.01	15.41±0.14	16.09±0.01	15.12±0.20	16.14±0.32	14.40±0.09	15.53±0.16
Formic acid	1.56±0.10	1.41±0.04	1.53±0.02	1.52±0.16	1.53±0.05	1.51±0.00	1.20±0.08	1.45±0.04	1.44±0.09	1.50±0.07	1.49±0.09	1.40±0.07
Acetic acid	2.43±0.11	2.24±0.02	2.06±0.04	2.36±0.03	2.21±0.04	2.37±0.02	2.14±0.08	2.27±0.02	2.32±0.06	2.33±0.05	2.34±0.07	2.32±0.03
HMF	0.06±0.00	0.07±0.00	0.02±0.00	0.07±0.00	0.02±0.00	0.04±0.00	0.05±0.00	0.06±0.00	0.04±0.00	0.07±0.00	0.04±0.00	0.06±0.00
Furfural	0.68±0.04	0.99±0.00	0.22±0.03	1.07±0.00	0.30±0.02	0.48±0.00	0.55±0.00	0.79±0.02	0.51±0.02	0.83±0.06	0.43±0.01	1.16±0.00
Phenolics	1.59±0.01	1.49±0.03	2.26±0.01	1.73±0.14	1.65±0.03	1.69±0.01	1.57±0.03	1.48±0.03	1.46±0.03	1.67±0.00	1.78±0.03	1.35±0.02
Total inhibitors (TI)	6.32±0.19	6.19±0.03	6.08±0.02	6.75±0.01	5.71±0.14	6.09±0.04	5.51±0.12	6.05±0.02	5.77±0.11	6.40±0.17	6.08±0.11	6.29±0.13
TS−TI	9.46±0.29	9.67±0.13	-3.06±0.09	8.89±0.05	2.57±0.26	8.93±0.02	9.90±0.25	10.04±0.03	9.35±0.31	9.74±0.50	8.33±0.20	9.24±0.04

V (X_1=1, X_2=−0.5); *W* (X_1=0.5, X_2=0); *X* (X_1=0.5, X_2=0); *Y* (X_1=−0.5, X_2=0); *Z* (X_1=1, X_2=1), standard posthydrolysis condition

0.1 g/L. The concentration of total phenolic compounds was always similar or lower than the quantified in the autohydrolysis liquor.

Posthydrolysis Modeling

The modeling of the posthydrolysis process of hemicellulosic liquors can be a useful tool to identify the condition(s) that maximize sugar recovery, minimize byproduct formation, acid/alkalis spending, and energy requirements. Previous approaches include the use of a combined severity parameter [14] and a more complex approach using explicit kinetic models [12, 13]. In this work, we used an intermediate approach, an empirical model, to capture the advantages of both approaches. Actually, the empirical model allows exploiting the relations between the relevant factors (namely, catalyst concentration and reaction time) to the final posthydrolyzate composition in a still straightforward manner.

Equation 1 was initially fitted to the different studied responses based on the data from the Doehlert proposed experimental trials (trials A–G). To further established the validity of the proposed models and the estimation for the optimal operational range, some additional trials were carried out, namely, the standard posthydrolysis condition (Z) and four additional conditions (V–Y) chosen at specific relevant model regions (suboptimal, optimal, and over-optimal conditions; for definition of operational conditions, please see Table 3). The new estimates for the model coefficients are comparable with the initial, as only minimal qualitative or quantitative differences were observed between the two different set of estimates, and therefore, only one set of estimates are presented and discussed.

Table 4 presents the regression coefficients estimates for the polynomial model based on the extended data set, together with the coefficient of determination (R^2) for the different responses analyzed. All compounds could be effectively correlated to the studied variables by the proposed equation, giving statistically significant regressions at p value<0.01, except for mannose for which the regression is only significant at p value<0.05 (data not shown). Mannose also presents the lowest R^2.

Acid concentration linear coefficient is statistically significant for all studied responses, clearly demonstrating its relevance for posthydrolysis performance. Acid concentration not only favored sugar recovery but also acetic acid and furan derivatives production. The acid concentration quadratic effect is also statistically significant for all these responses, except for mannose. The high negative values for the both pentose sugars and galactose recovery mean that under the studied range, high acid concentrations lead to increased sugar decomposition, thus diminishing sugar recovery. Conversely, for glucose and mannose, the balance favors production. The furfural pattern is similar to the described for glucose and mannose and the inverse of the pentoses profile, as this is the main degradation product of these sugars. Acetic acid exhibits a similar trend to the pentoses.

The effect of acid concentration on phenolic compounds recovery is quite different of the other compounds. Only the linear coefficient is statistically significant, and conversely to the other compounds, it has a negative effect. This can be explained both by the low solubility, at low pH, of the phenolic compounds present in the autohydrolysis liquor, and hence their selective precipitation upon acid addition and subsequent removal by filtration before analysis, together with the putative low phenolic substitution of the oligosaccharides. Therefore, it can be stated that, concerning phenolic compounds, acid concentration has a positive consequence on subsequent bioprocesses performance as it decreases posthydrolyzate toxicity toward microbial metabolism, given that phenolic compounds are usually considered important inhibitors [9, 26–28].

Table 4 Regression coefficients estimates for the polynomial model for the different responses analyzed.

	β_0	β_1	β_2	β_{12}	β_{11}	β_{22}	R^2
Glucose	**1.07±0.04 (0.00)**	**0.51±0.03 (0.00)**	**0.23±0.03 (0.00)**	−0.08±0.05 (0.14)	**−0.18±0.05 (0.00)**	−0.09±0.05 (0.13)	0.95
Xylose	**10.60±0.51 (0.00)**	**3.99±0.42 (0.00)**	**1.84±0.47 (0.00)**	**−2.21±0.73 (0.01)**	**−4.57±0.71 (0.00)**	−0.58±0.76 (0.45)	0.87
Arabinose	**2.03±0.08 (0.00)**	**0.38±0.06 (0.00)**	0.07±0.07 (0.31)	−0.12±0.11 (0.31)	**−0.52±0.11 (0.00)**	0.10±0.12 (0.38)	0.73
Mannose	**1.06±0.04 (0.00)**	**0.08±0.04 (0.04)**	**0.12±0.04 (0.01)**	**−0.13±0.06 (0.05)**	**−0.04±0.06 (0.49)**	−0.04±0.06 (0.55)	0.46
Galactose	**1.26±0.11 (0.00)**	**0.42±0.09 (0.00)**	0.15±0.10 (0.18)	−0.20±0.16 (0.24)	**−0.52±0.16 (0.00)**	−0.13±0.17 (0.44)	0.58
Total Sugars (TS)	**16.03±0.58 (0.00)**	**5.37±0.48 (0.00)**	**2.41±0.53 (0.00)**	**−2.74±0.83 (0.00)**	**−5.83±0.81 (0.00)**	−0.73±0.86 (0.41)	0.90
Acetic acid	**2.38±0.03 (0.00)**	**0.12±0.02 (0.00)**	−0.01±0.03 (0.75)	0.06±0.04 (0.15)	**−0.20±0.04 (0.00)**	−0.06±0.04 (0.17)	0.68
HMF	**0.06±0.00 (0.00)**	**0.02±0.00 (0.00)**	**0.01±0.00 (0.00)**	**−0.01±0.00 (0.02)**	**−0.02±0.00 (0.00)**	**−0.02±0.00 (0.00)**	0.96
Furfural	**0.67±0.02 (0.00)**	**0.37±0.01 (0.00)**	**0.23±0.02 (0.00)**	**0.11±0.03 (0.00)**	**−0.08±0.02 (0.00)**	**−0.09±0.03 (0.00)**	0.98
Phenolics	**1.64±0.07 (0.00)**	**−0.25±0.06 (0.00)**	−0.01±0.06 (0.92)	−0.01±0.10 (0.88)	0.19±0.09 (0.06)	−0.10±0.10 (0.32)	0.59
Total Inhibitors (TI)	**4.75±0.08 (0.00)**	**0.26±0.07 (0.00)**	**0.23±0.07 (0.01)**	0.16±0.11 (0.18)	−0.11±0.11 (0.32)	**−0.27±0.12 (0.03)**	0.65
TS−TI	**11.28±0.63 (0.00)**	**5.11±0.52 (0.00)**	**2.18±0.58 (0.00)**	**−2.90±0.91 (0.00)**	**−5.72±0.88 (0.00)**	−0.46±0.94 (0.63)	0.87

All values are presented in the form "Coefficient ± standard error (p-value)". Coefficients statistically significant at p value < 0.05 are in bold

Reaction time not only positively influenced sugar recovery but also byproducts formation, especially furfural. The reaction time quadratic coefficient was not statistically significant for sugar recovery, meaning that under the studied range, longer reaction times do not lead to increased sugar decomposition *per se*. Furthermore, longer reaction times negatively effect furan derivatives concentration, probably by enabling the settling of furan decomposition reactions. This can be beneficial, as the aliphatic acids produced are better tolerated by the microorganisms than the furan derivatives [27, 29, 30].

The interaction coefficient for acid concentration and reaction time is statistically significant for the most relevant studied compounds, namely, xylose and furfural and, to a less extent, mannose and HMF. Actually, the estimated interaction coefficients imply that these variables act synergistically to increase sugar degradation into furfural.

Based on the above discussion, it is clear that this simple modeling approach can be a useful alternative to more complex models, as it completely identifies and quantifies the main features of the posthydrolysis process. The proposed model and its estimated coefficients can now be used for the definition of the optimal operational conditions and to study its robustness for a given purpose/criteria.

Numerical Optimization

A constrained optimization model was implemented in order to find the best hydrolysis conditions. Among other possibilities, the desired criterion chosen was to maximize the direct difference between sugars and inhibitors concentration (TS−TI, total sugars minus total inhibitors), i.e., no weighting is introduced to favor sugar production or minimize inhibitors. A sensitivity analysis was then carried out on the optimum conditions, yielding that the optimal conditions are rather flexible, i.e., there is an operational range for catalyst concentration and reaction time for which the outcome is rather stable. Figure 1 presents the contour plot for TS−TI in relation to acid concentration and reaction time and clearly demonstrates that in the range defined by −0.43 (1.21% H_2SO_4 *w*/*w*)<X_1<0.975 (3.95%),

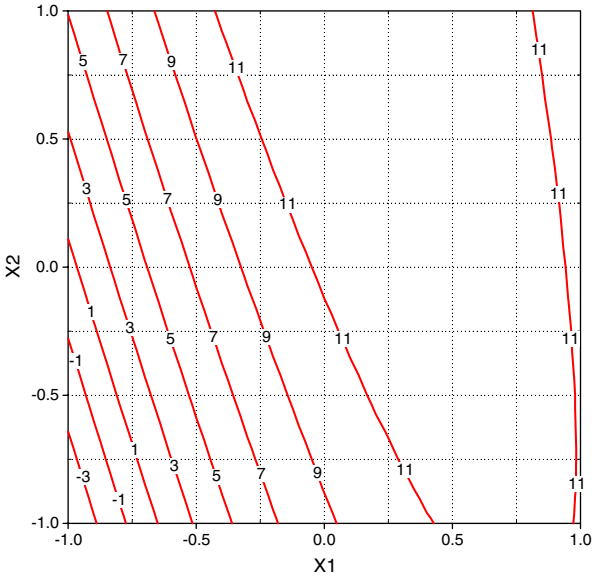

Fig. 1 Response surface for the concentration of total sugars minus concentration of total inhibitors in relation to acid concentration (X_1) and reaction time (X_2)

there are many combinations with reaction time for which it is possible to obtain the optimal values with minor variation. This corresponds to a 70% less acid spending or, alternatively, lesser reaction times, as compared to standard posthydrolysis conditions. The choice of the more convenient operational condition is now a function of cost analysis and a trade-off between the cost of acid (and alkalis for neutralization) and energy.

The composition of the hydrolyzates produced in the optimal range (e.g., A, X) compared to wheat straw hydrolyzates obtained by acid hydrolysis have a xylose content similar to hydrolyzates produced under milder conditions [31], but lower than the obtained for a more severe acid hydrolysis [32]. No significant differences exist for acetic acid content, but furan derivatives vary, with this work exhibiting lower HMF but higher furfural content than the published data. Mannose and galactose are usually not reported, as they are accounted as xylose by the more common chromatographic methods, which can explain the lower xylose content reported in this work. Glucose content is similar to the one reported in [31] but far less than the obtained in [32], which can be explained by the higher selectivity of autohydrolysis/mild acid hydrolysis processes toward hemicellulose solubilization, over the direct acid hydrolysis. Wheat straw hydrolyzates obtained by wet oxidation treatments [33] contain far less pentoses and glucose than all the hydrolyzates previously discussed, although no furan derivatives are present. Comparing to hydrolyzates obtained from other feedstocks using this two-stage (autohydrolysis-dilute acid hydrolysis) approach, it has a lower total sugar content than corn cobs [10, 13], *Eucalyptus* wood [12], and brewery's spent grain [28]. Aliphatic acids and furan derivatives are lower than for corn cobs and *Eucalyptus* wood but, together with the phenolic content, slightly higher than that reported for brewery's spent grain [28].

Nevertheless, this hydrolyzate could be readily fermented by the yeast *Debaryomyces hansenii* to produce xylitol [34], an attractive added value-product that can be used to upgrade the hemicellulosic sugar stream in a biorefinery framework.

Conclusions

Based on the data from a Doehlert experimental design, it was possible to develop an empirical model that identifies and quantifies the main features of the posthydrolysis process. Furthermore, it was possible to define a region for the optimal operational conditions for wheat straw oligosaccharides hydrolysis. In this region, it was possible to obtain a slight increase in monosaccharides content together with a slight decrease in inhibitors content, as compared to the standard acid hydrolysis treatment. This is achieved with 70% acid savings and potential significant reductions on reaction time.

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