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ORIGINAL ARTICLE

Acid pretreatment optimization for xylose production from *Agave tequilana* Weber var. *azul*, *Agave americana* var. *oaxacensis*, *Agave karwinskii*, and *Agave potatorum* bagasses using a Box-Behnken design

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Abstract
There is a growing interest in the use of lignocellulosic biomass for biofuel production leading to by-products with high added value such as xylitol, a sweetener obtained from chemical synthesis or fermentative pathways, characterized by high degree of sweetness, recommended for people with diabetes, making it a product of great interest in the industrial market. The objective of this study was to use the bagasse of four agave plants from Mexico: *Agave tequilana* Weber var. *azul* (AT), *Agave Americana* var. *oaxacensis* (AA), *Agave karwinskii* (AK), and *Agave potatorum* (AP) to obtain xylose as an alternative for xylitol or ethanol production. The dilute acid pretreatment was optimized to obtain the maximum concentration of xylose and the minimum concentration of acetic acid, using a Box-Behnken design, where H_2SO_4 concentration, hydrolysis time, and liquid-to-solid ratio were evaluated. Xylose concentrations of 23.18 g/L (AT), 27.63 g/L (AA), 31.8 g/L (AK), and 24.42 g/L (AP) were achieved. It was also observed that by using more concentrated H_2SO_4 solutions, higher concentrations of xylose were obtained, but also high concentrations of acetic acid (1.02 g/L in AT, 2.3 g/L in AA, 3.46 g/L in AK, and 2.01 g/L in AP). This study shows that AT, AA, AK, and AP are a promising alternative feedstock for bioethanol or xylitol production using xylose as substrate.

Keywords Optimization · Pretreatment · Agave bagasse · Xylose · Acetic acid

1 Introduction

Due to the increase in environmental pollution, there has been growing interest in the use of biomass for the production of biofuels from renewable sources [1]. The generation of these biofuels offers ecological and economic advantages, since their combustion generates a lower amount of carbon monoxide, nitrogen oxides, and total hydrocarbon emissions. It is estimated that by 2020, biofuel production will exceed 125 billion liters, making bioethanol an important object of study in the biotechnology area, since it is obtained from carbohydrate-rich raw materials among which corn, sorghum, and sugarcane stand out [2].

Currently the USA and Brazil are the countries that stand out worldwide in bioethanol production from cornstarch and sugarcane, respectively [3, 4]; however, the crops used for ethanol production are also used for food processing, causing direct competition among the users of these crops. Therefore, a viable alternative for biofuel production is to use raw materials that are agricultural and agroindustrial wastes as a source of carbohydrates [5].

Among the most abundant agricultural and agroindustrial wastes in Mexico are those obtained from the production of tequila, mezcal, bacanora, and pulque from the *Agave* plant [6]. It has been estimated that there is an overproduction of the plant and about 100,000 tons are wasted annually [7, 8]. Agave plants have been attractive because of their specific

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characteristics such as high sugar content, their ability to grow in arid lands, and high productivity [6]. Additionally, the biofuel produced from agave plants has extraordinarily low CO_2 emissions at only 35 g/J; in contrast, the CO_2 emission of corn-based biofuel production can be as high as 85 g/J [2]. *Agave tequilana* Weber var. *azul* has been reported as the agave species with the greatest economic importance, with an annual consumption in 2018 in Mexico of around 1.13×10^6 t [9]. About 40% of the harvested plant is residual fiber, and at the moment, just a small fraction is being used for soil composting or as plywood, while the rest is accumulated in landfills [10–12].

The ethanol production process using lignocellulosic materials may not be economically viable if only the glucose present in the hydrolysate is converted to ethanol [13, 14], but also due to the cost at the recovery stage [15]; however, obtaining in parallel another important metabolite with high added value, such as xylitol, which increases the economic viability of the process from lignocellulosic residues rich in hemicellulose has been proposed [13, 16–19].

Xylitol is a sweetener obtained from chemical synthesis or fermentative pathways, its precursor being xylose. It is characterized by its high degree of sweetness, providing 40% fewer calories than sucrose and affording an anticariogenic effect. Its use is recommended for people with diabetes, making it a product of great interest in the industrial market.

The composition of the lignocellulosic material comprises from 30 to 45% cellulose and 20–35% hemicellulose which, after pretreatments and enzymatic hydrolysis, can be converted to glucose and xylose, respectively [2, 20–22].

Hemicellulose is a heterogeneous polymer consisting of different types of monosaccharides usually pentoses and hexoses, and is more easily hydrolyzed than cellulose [2]. In order to achieve this conversion, it is necessary to apply physical or physicochemical pretreatments to favor cellulose and hemicellulose degradation.

A physicochemical pretreatment used to hydrolyze hemicellulose is hydrothermal processing, which uses hot water as the hydrolysis medium. It displays favorable features in terms of environmental impact, limitation of chemicals and sludge, selectivity of component separation, limitation of equipment corrosion, and reduced capital, operational costs, and also, the production of inhibitors is minimized [23].

Hydrothermal processing provides potential advantages compared with chemical methods as no chemicals beyond water are required. Hemicelluloses are converted into soluble compounds, and the treated solid may present high susceptibility toward enzyme hydrolysis. Process residence times and temperatures are similar; however, the formation of degradation products is lower [23]. Another physicochemical pretreatment used to hydrolyze hemicellulose is acid pretreatment where the hemicellulose is degraded to its corresponding monomers in order to facilitate the use of these sugars for xylitol production [2, 20–22]. Complete hemicellulose removal from agroindustrial residue during acid pretreatment is a necessary requirement for efficient enzymatic hydrolysis of the cellulose fraction, in turn increasing the porosity of the lignocellulosic substrate, favoring accessibility to cellulose. Dilute acid is a viable pretreatment for all types of lignocellulosic substrates such as corn stover, wheat, barley, sugarcane, agave and sorghum bagasses, and rice husk to obtain high xylose yields in the hydrolysate. In this process, sulfuric, hydrochloric, or nitric acid can be used in dilute amounts [22].

It uses strong acids at concentrations of 1–5% for the case of dilute hydrolysis, or up to 30% acid for concentrated hydrolysis [24]. During acid hydrolysis, large amounts of inhibitors are produced such as furfural, 5-hydroxymethylfurfural, and acetic acid, and the pretreatment conditions strongly affect the amounts of inhibitors with the resulting toxicity, for example at the end of sugarcane bagasse acid hydrolysis with 2% H_2SO_4 where they obtained 0.5 g/L furfural and 4.6 g/L acetic acid [17] or, on the other hand, where up to 0.23 g/L furfural, 0.15 g/L 5-HMF, and 1.37 g/L acetic acid in corn stover hydrolysis were obtained [24].

Pretreatments using dilute acid result in the hydrolysis of a significant amount of the hemicellulosic fraction of the biomass, obtaining high yields of soluble sugars: up to 30 g/L xylose in sugarcane bagasse hydrolysates and obtaining 26.64 g/L xylose in corn stover hydrolysate [17, 24]. The objective of this work was to optimize the acid hydrolysis process for the four most abundant *Agave* varieties in Mexico in order to evaluate the potential of their residues in xylose production, which can be later transformed into ethanol or xylitol.

2 Materials and methods

2.1 Raw materials

The materials used for this study were the bagasses from four *Agave* varieties: 3-year-old *Agave tequilana* Weber var. *azul* from the State of Jalisco (AT), as well as *Agave americana* var. *oaxacensis* (AA), *Agave karwinskii* (AK), and *Agave potatorum* (AP) from the region of Villa Sola de Vega, Oaxaca (15, 3, and 5 years old, respectively).

2.2 Pretreatment of raw materials

In order to obtain xylose from the lignocellulosic material, it is necessary to pretreat it. All four bagasse varieties were subjected to an acid pretreatment where the variables to be studied were H_2SO_4 concentration, hydrolysis time (t), and liquid-to-solid ratio (LSR). This pretreatment was carried out in an autoclave (AESA brand, model CV-250, Mexico) at 121 °C

and 1.1 atm absolute pressure, according to the conditions indicated in the Box-Behnken design matrix (Table 1).

2.3 Analysis of lignocellulosic composition

For the analysis of the lignocellulosic composition of the four *Agave* bagasses, lignocellulosic material is subjected to a double acid hydrolysis [25]; the first hydrolysis was carried out for 60 min using a 72% H_2SO_4 solution at 30 °C (Thermo Haake C10-B3 water bath, Germany), and the second hydrolysis was carried out using a 4% H_2SO_4 solution in an autoclave (AESA, CV-250, Mexico) at 121 °C and 1.1 atm for 60 min. Glucose, xylose, acetic acid, furfural, and 5-hydroxymethylfurfural (5-HMF) were analyzed by HPLC (Waters 600 TSP Spectra System, Waters, Milford, MA, USA) using a Shodex SH1011 8 Å—300-mm column (H203153, Japan), at 50 °C, a 5-mM sulfuric acid mobile phase, a 0.6 mL/min flow rate, and a refractive index detector (Waters 2414, TSP Refracto Monitor V, Waters, Milford, MA, USA). Acid-soluble lignin was analyzed in a UV-Vis spectrophotometer with a diode array detector (Hewlett-Packard 8452 A, Germany), insoluble lignin moisture was determined according to the AOCS methodology (Ab 2-49) using a vacuum oven (Thermo Scientific Lab-Line, 3608-1CE, USA), and ash determination was carried out using the AOCS methodology (Ba 5^a-49) in a furnace (Thermo Scientific Thermoline, model FB1310M-33, USA).

2.4 Experimental design

A three-factor, three-level Box-Behnken design was used to optimize the maximum xylose concentration and minimum acetic acid concentration in acid hydrolysis by deriving a second-order polynomial equation and constructing response surface plots to predict result outcomes. The evaluated variables were sulfuric acid concentration (X_1), pretreatment time (X_2), and liquid/solid ratio (X_3), and the response variables were xylose and acetic acid concentrations, obtained through twelve combinations with three central points, total of fifteen experiments. The matrix studied for the pretreatment is shown in Table 2.

2.4.1 Optimization of acid pretreatment

At the end of the Box-Behnken design experiments, the results were analyzed in order to obtain higher xylose concentrations and minimum acetic acid concentrations during acid pretreatment in each of the lignocellulosic materials and thus perform experimental validation.

Table 1 Levels of the experimental design of the variables studied

	Factors	Levels		
Variable (units)	X	(− 1)	(0)	(+ 1)
[H_2SO_4] (% v/v)	X_1	0.5	1	1.5
t (min)	X_2	15	30	45
LSR (mL/g)	X_3	6:1	7:1	8:1

Table 2 The Box-Behnken design of acid pretreatment

Treatment	[H_2SO_4] (% v/v)	t (min)	LSR (mL/g)
1	1.5	45	7:1
2	1.5	15	7:1
3	0.5	45	7:1
4	0.5	15	7:1
5	1.5	30	8:1
6	1.5	30	6:1
7	0.5	30	8:1
8	0.5	30	6:1
9	1	45	8:1
10	1	45	6:1
11	1	15	8:1
12	1	15	6:1
13	1	30	7:1
14	1	30	7:1
15	1	30	7:1

2.4.2 Statistical analysis

NCSS Data Analysis Version 11 software (Kaysville, UT, USA) was used to evaluate the results from the Box-Behnken design experiments to pinpoint the best conditions ($p < 0.05$) to investigate optimization of acid pretreatment. Subsequently, using the same software, an ANOVA analysis was used on the results obtained from the treatments carried out in duplicate.

3 Results and discussion

3.1 Lignocellulosic characterization

The results obtained from the lignocellulosic characterization are shown in Table 3 where it can be seen that of the four bagasses studied, AT bagasse is the material richest in cellulose, representing 50% of the total weight, followed by AK bagasse, where cellulose represents 30% of the total weight, AA bagasse with 28% cellulose, and AP bagasse with 24% cellulose; this is important if this material is to be used for the second-generation ethanol production. In turn, AT bagasse possesses 22% hemicellulose, AP bagasse 20%, AA bagasse 16%, and AK bagasse 15%.

Hemicellulose is the most important fraction in the development of this work because it contains xylose, the sugar of interest, which will be converted by the fermentative route into xylitol. The percentage of lignin is present in these

Table 3 Lignocellulosic characterization of *Agave* bagasses

Lignocellulosic material	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Moisture (%)	Ash (%)
Agave tequilana Weber var. *azul*	50 ± 2.3	22 ± 2	17 ± 1.1	8 ± 0.5	3 ± 0.07
Agave americana var. *oaxacensis*	28 ± 1.5	16 ± 0.5	15 ± 1.1	11 ± 0.1	2 ± 0.05
Agave karwinskii	30 ± 2	20 ± 1	14 ± 0.5	12 ± 0.3	5 ± 0.1
Agave potatorum	24 ± 1.2	15 ± 0.7	14 ± 0.9	14 ± 1.1	4 ± 0.2

bagasses, although in the reported range can generate problems for achieving high alkaline pretreatment efficiency since the bagasse contains 14% lignin in the case of AP and AK bagasses, 15% in AA bagasse, and 17% in AT bagasse [2, 26, 27]. A higher xylose concentration in the acid hydrolysate obtained from AT bagasse would be expected, followed by the hydrolyzed bagasses of AK, AA, and AP.

3.2 Acid pretreatment

Acid pretreatment carried out on the *Agave* bagasses yielded the results shown in Fig. 1, where the combination of pretreatments was as explained in Table 2, Section 2.4. For AT, there was no formation of inhibitors such as furfural and 5-HMF during the hydrolysis process; nevertheless, a maximum concentration of acetic acid of 3.41 g/L was obtained when evaluating a 0.5% solution of H_2SO_4, 15 min hydrolysis time, and LSR 7:1 (treatment 4). It is important to mention that growth of the yeast *Candida tropicalis* IEC5-ITV can be inhibited due to the presence of different inhibitors, among which acetic acid, furfural, and 5-HMF stand out [15, 28]. The presence of glucose was observed in 3 out of 15 treatments (0.5% acid, 30 min, and LSR 8:1) with the highest concentration of 3.54 g/L obtained in treatment 7. A low glucose concentration can be related with a high lignin content which can generate a barrier preventing cellulose hydrolysis. In the same way, the maximum xylose concentration was obtained with 1.5% acid, 30 min hydrolysis time, and LSR 8:1, obtaining up 19.82 g/L xylose (treatment 5), the best treatment in terms of xylose obtained; however, acetic acid concentration was 3.31 g/L.

In the case of AA bagasse, the best treatment for maximum xylose concentration in the Box-Behnken design was observed with a 1.5% solution of H_2SO_4, 30 min, and LSR 6:1, obtaining 2.02 g/L glucose, 12.96 g/L xylose, and 2.32 g/L acetic acid (treatment 6). No furfural was detected in this treatment, although in treatment 7 only, a value of 0.51 ± 0.004 g/L was detected; however, acetic acid concentration is a great growth inhibitor, affecting the performance of the yeast during fermentation because it only tolerates a maximum of 2 g/L acetic acid in the culture medium [20]. The treatment with a 0.5% solution of H_2SO_4, 30 min, and LSR 8:1 yielded the lowest acetic acid concentration; however, the concentration of xylose was 7.5 g/L (treatment 7).

At the end of acid pretreatment on bagasse of AK, it was possible to obtain xylose concentrations of 17.7 g/L (treatment 6), and the glucose concentration in this treatment was 1.91 g/L and 1.85 g/L acetic acid, this being the best treatment in terms of xylose concentration, which was carried out with a 1.5% H_2SO_4 solution, 30 min hydrolysis time, and LSR 6:1. It is important to mention that the minimum concentrations of acetic acid were obtained when treatments with the minimum concentration of sulfuric acid were used.

With acid pretreatment on AP bagasse, 16.9 g/L xylose, 1.8 g/L glucose, and 1.18 g/L acetic acid were obtained, this being the best treatment carried out with a 1.5 solution % of H_2SO_4, 45 min hydrolysis time, and LSR 7:1 (treatment 2).

In general, it was observed that for the four *Agave* bagasses studied, when solutions with a higher concentration of H_2SO_4 are used, an increase in the concentration of acetic acid is observed at the end of the acid pretreatment.

Using a significance level of $p < 0.05$, xylose concentration was greatest with an $[H_2SO_4] \times$ hydrolysis time interaction for AT, a time × LSR interaction in AA, and $[H_2SO_4]$ and hydrolysis time alone for AK and AP, respectively.

In the case of the acetic acid concentration present in the hydrolysate, the variable that has the most effect in AA is the interaction of concentration with LSR, while in AT, AK, and AP, it is the H_2SO_4 concentration; the difference between these variables can be related with lignocellulosic material characteristics, mainly humidity, porosity, and lignin content.

3.3 Optimization

By entering the values obtained from each of the treatments carried out on the four bagasses studied into the NCSS 11 Data Analysis software, it was possible to obtain the conditions with which the maximum xylose concentration (XYL) and the minimum acetic acid concentration (ACET) can be obtained. The conditions, together with the models, are shown in Table 4.

3.3.1 Maximum xylose concentration

A quadratic model was chosen to fit the data for maximum xylose concentration optimization. Model equations were

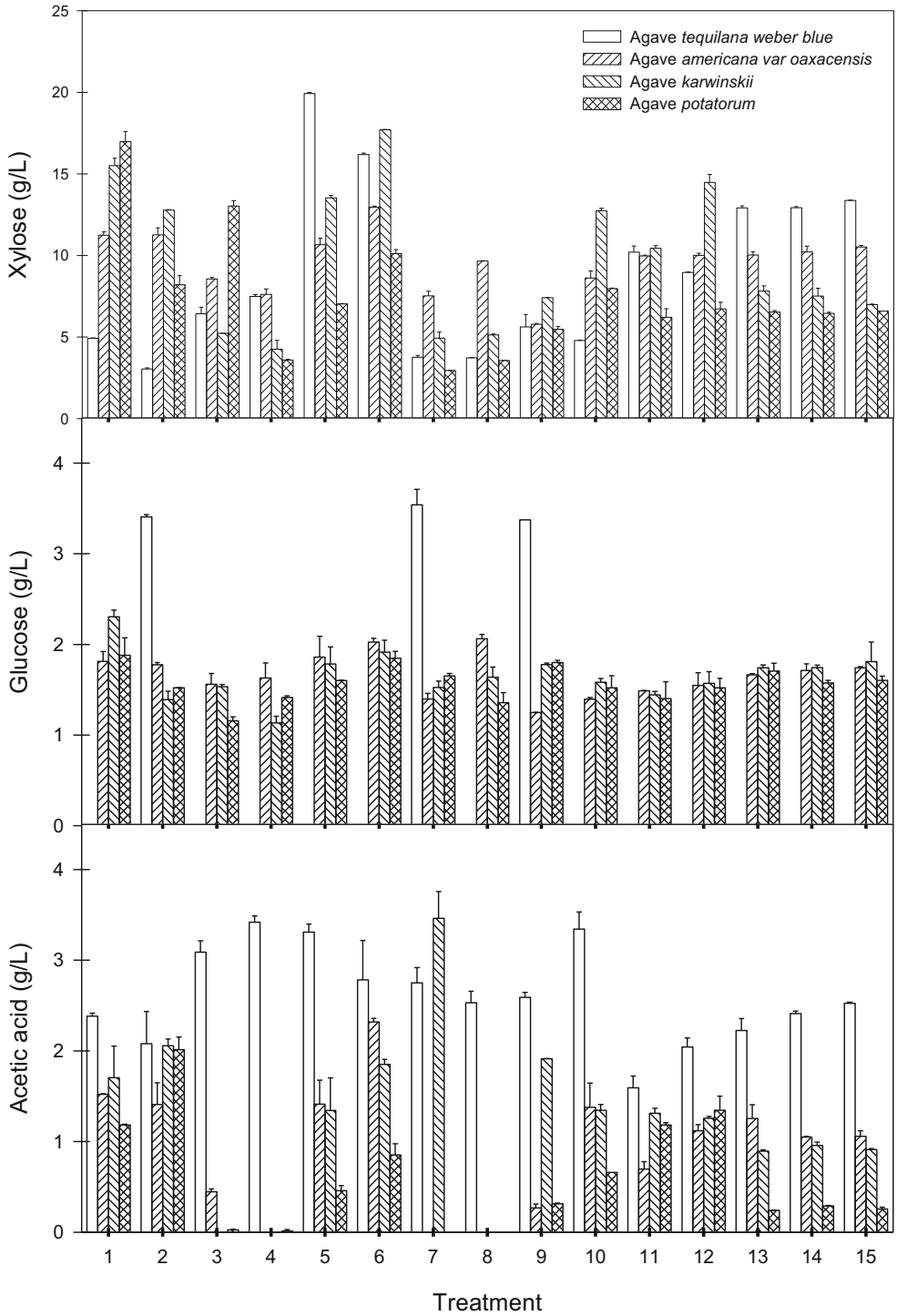

Fig. 1 Concentrations of xylose, glucose, and acetic acid in the acid hydrolysate of *Agave tequilana* Weber var. *azul* bagasse, *Agave Americana* var. *oaxacensis* bagasse, *Agave karwinskii* bagasse, and *Agave potatorum* bagasse

obtained for AT, AA, AK, and AP bagasses (Eqs. 3.1, 3.2, 3.3, and 3.4, respectively), where H_2SO_4 is expressed in % v/v, LSR in mL/g, and time in minutes. The relationship between the three factors studied, their interactions, and xylose concentration can be observed in Eqs. 3.1–3.4.

$$\begin{aligned}[\mathbf{XYL\ AT}] = {} & 278.75 - (29.18 \times [H_2SO_4]) - (11.53 \times t) - (2216.15 \times LSR) \\ & - \left(8.19 \times [H_2SO_4]^2\right) + \left(0.053 \times t^2\right) + \left(11547.20 \times LSR^2\right) \\ & + (4.76 \times [H_2SO_4] \times t) - (90.04 \times [H_2SO_4] \times LSR) \\ & + (113.25 \times t \times LSR) - \left(0.078 \times [H_2SO_4] \times t^2\right) \\ & - \left(387.99 \times t \times LSR^2\right). \end{aligned} \tag{3.1}$$

Table 4 Optimization conditions for acid hydrolysis

Lignocellulosic material	Maximum xylose concentration	Minimum acetic acid concentration	Xylose (g/L)	Acetic acid (g/L)	Eq. model	R^2
Agave tequilana Weber *var. azul*	$[H_2SO_4]$ (% v/v) 2 *t* (min) 31 LSR 6.93:1	$[H_2SO_4]$ (% v/v) 1 *t* (min) 28 LSR 7:1	19.26	2.24	3.1 3.5	0.998635 0.864617
Agave americana var. *oaxacensis*	$[H_2SO_4]$ (% v/v) 3 *t* (min) 32 LSR 6.93:1	$[H_2SO_4]$ (% v/v) 1 *t* (min) 28 LSR 5:1	16.99	2.83	3.2 3.6	0.963527 0.953427
Agave karwinskii	$[H_2SO_4]$ (% v/v) 3 *t* (min) 32 LSR 7:1	$[H_2SO_4]$ (% v/v) 1 *t* (min) 32 LSR 7:1	27.75	9.907	3.3 3.7	0.928051 0.919756
Agave potatorum	$[H_2SO_4]$ (% v/v) 3 *t* (min) 28 LSR 6.08:1	$[H_2SO_4]$ (% v/v) 1 *t* (min) 28 LSR 5:1	18.80	0.875	3.4 3.8	0.996950 0.984571

$$[\mathbf{XYL\ AA}] = 141.96 + (5.11 \times [H_2SO_4]) - (5.83 \times t) - (1891.51 \times \mathrm{LSR}) - (0.005 \times t^2) + (6403.06 \times \mathrm{LSR}^2) - (30.89 \times [H_2SO_4] \times \mathrm{LSR}) + (82.31 \times t \times \mathrm{LSR}) + (120.79 \times [H_2SO_4] \times \mathrm{LSR}^2) - (274.99 \times t \times \mathrm{LSR}^2) \quad (3.2)$$

$$[\mathbf{XYL\ AK}] = 129.38 + (9.99 \times [H_2SO_4]) - (0.39 \times t) - (34.17 \times \mathrm{LSR}) + (0.006 \times t^2) + (2.318 \times \mathrm{LSR}^2) \quad (3.3)$$

$$[\mathbf{XYL\ AP}] = -584.30 + (59.97203 \times [H_2SO_4]) + (13.36 \times t) + (83.53 \times \mathrm{LSR}) + (0.009 \times t^2) - (2.88 \times \mathrm{LSR}^2) - (8.26 \times [H_2SO_4] \times \mathrm{LSR}) - (1.96 \times t \times \mathrm{LSR}) + (0.303 \times [H_2SO_4] \times \mathrm{LSR}^2) + (0.067 \times t \times \mathrm{LSR}^2) \quad (3.4)$$

Table 3 shows the optimal conditions obtained for maximum xylose concentration from the optimization performed on acid pretreatment for each of the *Agave* bagasses. This shows the coefficient of determination (R^2) defined as the ratio of explained variation to total variation, and is a measure of the degree of fit; a good model fit should yield an R^2 of at least 0.8 [29]. The model evaluated in this study explains the reaction well, with R^2 values of 0.9986, 0.9635, 0.9280, and 0.9969 for AT, AA, AK, and AP, respectively.

3.3.2 Minimum acetic acid

As for maximum xylose concentration, inhibitor concentration in the acid hydrolysate is very important as it generates growth problems in the yeasts during fermentation processes and also, *Candida tropicalis* IEC5-ITV yeast is inhibited by acetic acid at concentrations of 2 g/L, 1 g/L 5-HMF, and 2 g/L furfural [20, 30]. Therefore, an optimization evaluation was performed to obtain the minimum acetic acid concentration (ACET), although a decrease in this also entails a decrease in xylose concentration. In the same way as for xylose, the equations of the mathematical models were obtained to optimize the minimum acetic acid concentration (Eqs. 3.5, 3.6, 3.7, and 3.8 for AT, AA, AK, and AP bagasses, respectively), where H_2SO_4 is expressed in % v/v, LSR in mL/g, and time in minutes. Equally, a quadratic model was chosen to fit the data. The relationship between the three factors studied and acetic acid concentration can be observed in Eqs. 3.5–3.8.

$$[\mathbf{ACET\ AT}] = 118.14 - (4.15 \times [H_2SO_4]) - (3.44 \times t) - (1567.42 \times \mathrm{LSR}) + (0.32 \times [H_2SO_4]^2) + (0.002 \times t^2) + (5284.56 \times \mathrm{LSR}^2) + (0.33 \times [H_2SO_4] \times t) - (7.44 \times [H_2SO_4] \times \mathrm{LSR}) + (45.10 \times t \times \mathrm{LSR}) - (0.005 \times [H_2SO_4] \times t^2) + (0.00 \times [H_2SO_4] \times \mathrm{LSR}^2) - (149.33 \times t \times \mathrm{LSR}^2) \quad (3.5)$$

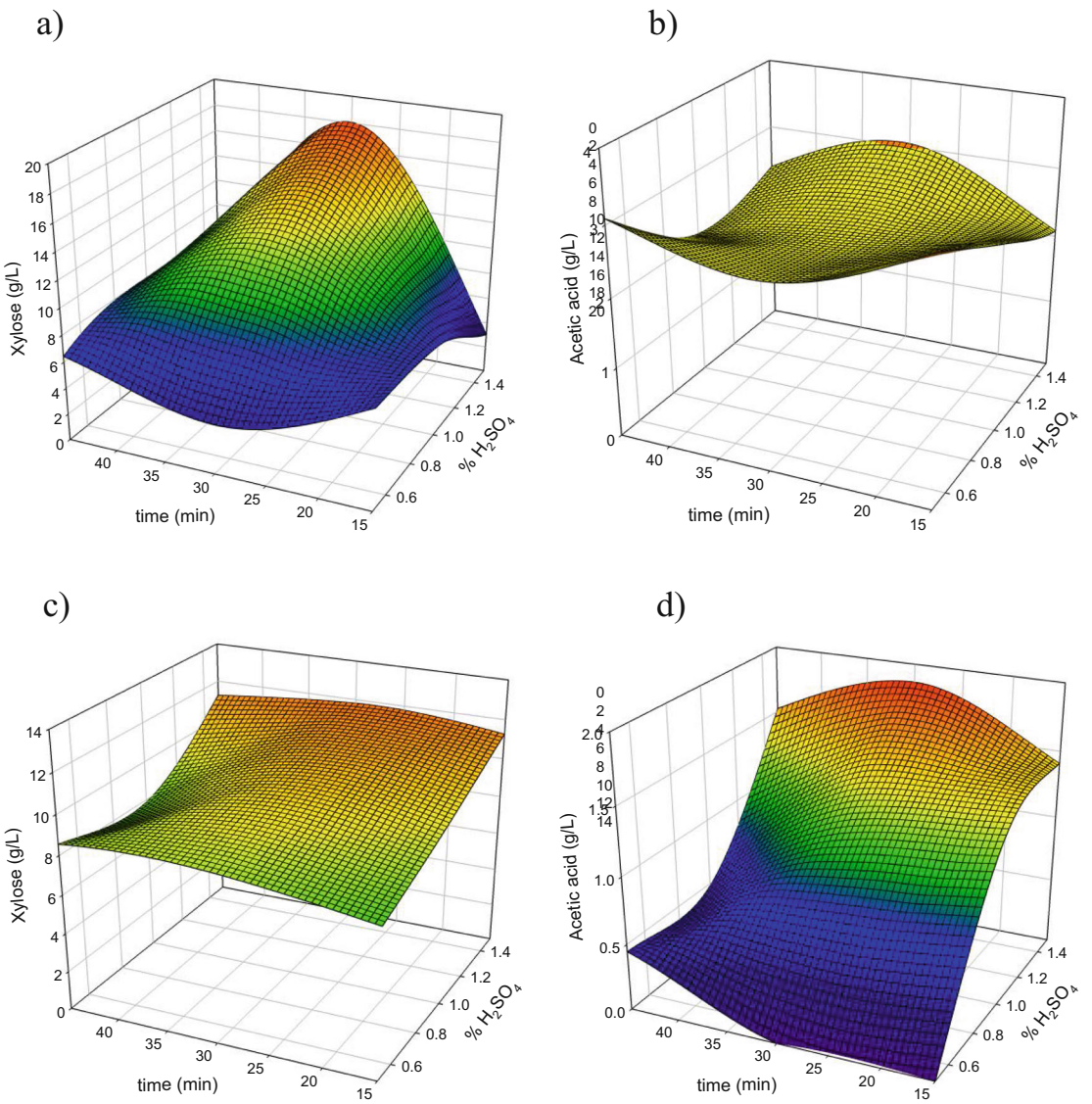

Fig. 2 Response surfaces obtained from agave bagasse optimization where **a** and **c** correspond to the optimization for obtaining maximum xylose concentration in *Agave tequilana* Weber var. *azul* bagasse and *Agave Americana* var. *oaxacensis* bagasse, respectively; **b** and **d** correspond to the optimization for minimum acetic acid concentration in acid pretreatment of *Agave tequilana* Weber var. *azul* bagasse and *Agave Americana* var. *oaxacensis* bagasse, respectively

$$
\begin{aligned}
[\mathbf{ACETAA}] = {} & -10.40 + (3.71 \times [H_2SO_4]) - (0.622 \times t) - (3.45 \times \mathrm{LSR}) \\
& - \left(0.415 \times [H_2SO_4]^2\right) + \left(0.002 \times t^2\right) + \left(0.282 \times \mathrm{LSR}^2\right) \\
& + (0.155 \times [H_2SO_4] \times t) - (0.453 \times [H_2SO_4] \times \mathrm{LSR}) \\
& + (0.158 \times t \times \mathrm{LSR}) - \left(0.003 \times [H_2SO_4] \times t^2\right) \\
& + \left(0.00 \times [H_2SO_4] \times \mathrm{LSR}^2\right) - \left(0.012 \times t \times \mathrm{LSR}^2\right)
\end{aligned} \tag{3.6}
$$

$$
\begin{aligned}
[\mathbf{ACETAK}] = {} & -17.02 + (21.26 \times [H_2SO_4]) + (1.36 \times t) + (0.66 \times \mathrm{LSR}) \\
& + \left(0.45 \times [H_2SO_4]^2\right) - \left(0.009 \times t^2\right) + \left(0.108 \times \mathrm{LSR}^2\right) \\
& - (0.549 \times C \times t) - (1.98 \times C \times \mathrm{LSR}) - (0.234 \times t \times \mathrm{LSR}) \\
& + \left(0.009 \times C \times t^2\right) + \left(0.00 \times C \times \mathrm{LSR}^2\right) \\
& + \left(0.017 \times t \times \mathrm{LSR}^2\right)
\end{aligned} \tag{3.7}
$$

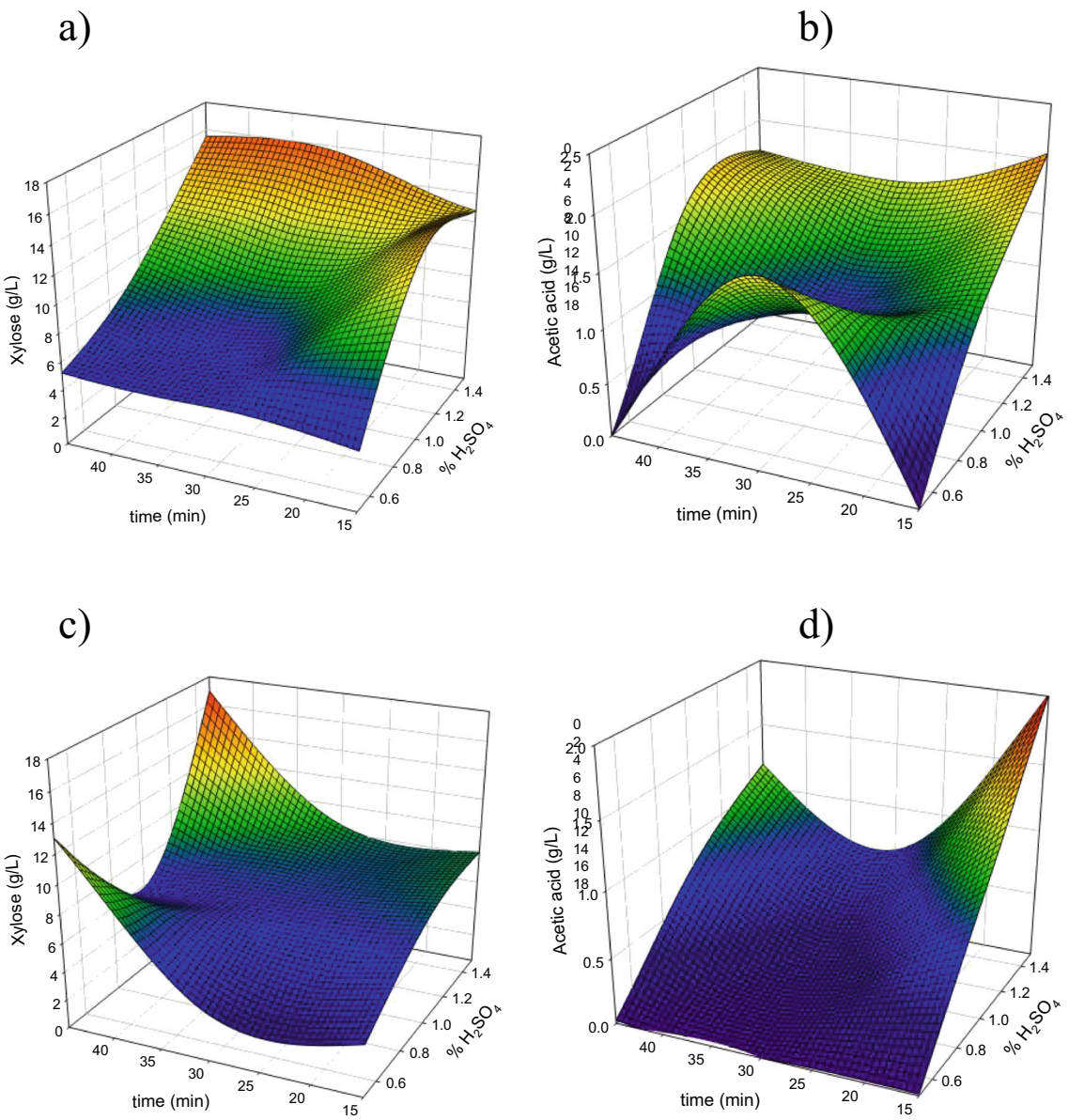

Fig. 3 Response surfaces obtained from *Agave* bagasse optimization where **a** and **c** correspond to the optimization for obtaining maximum xylose concentration in *Agave karwinskii* bagasse and *Agave potatorum* bagasse, respectively; **b** and **d** correspond to the optimization for minimum acetic acid concentration in acid pretreatment of *Agave karwinskii* bagasse and *Agave potatorum* bagasse, respectively

$$
\begin{aligned}
[\mathbf{ACETAP}] = {} & 17.71 + (6.58 \times [H_2SO_4]) - (0.46 \times t) - (5.90 \times \mathrm{LSR}) \\
& - \left(0.002 \times [H_2SO_4]^2\right) - (0.002 \times t^2) + (0.432 \times \mathrm{LSR}^2) \\
& - (0.274 \times [H_2SO_4] \times t) - (0.198 \times [H_2SO_4] \times \mathrm{LSR}) \\
& + (0.169 \times t \times \mathrm{LSR}) + \left(0.004 \times [H_2SO_4] \times t^2\right) \\
& + \left(0.00 \times [H_2SO_4] \times \mathrm{LSR}^2\right) - \left(0.0122 \times t \times LSR^2\right)
\end{aligned} \tag{3.8}
$$

In the same way, Table 4 shows the optimal conditions obtained in relation to the minimum acetic acid concentration from the optimization performed on acid pretreatment for each of the *Agave* bagasses. The coefficient of determination (R^2) in this study explains the reaction well, with R^2 values of 0.8646, 0.9534, 0.9197, and 0.9845 for AT, AA, AK, and AP, respectively.

Response surface graphs were obtained for each of the points evaluated in the studied bagasses (see Figs. 2 and 3), where it is possible to observe the effect of hydrolysis time and H_2SO_4 concentration on the xylose (a) and acetic acid (b) concentrations present in the hydrolysate. In the case of AT bagasse, it is observed that there is a greater effect on xylose concentration with long hydrolysis times and high H_2SO_4 concentrations to obtain the optimum point (Fig. 2a); however, when H_2SO_4 concentration decreases but hydrolysis time is maintained, xylose concentration also decreases. The same

Table 5 Experimental validation of acid pretreatment optimization by maximum xylose concentration (model Eqs. 3.1, 3.2, 3.3, and 3.4) and minimum acetic acid concentration (model Eqs. 3.5, 3.6, 3.7, and 3.8)

Treatment		Glucose (g/L)	Xylose (g/L)	Acetic acid (g/L)	5-HMF (g/L)	Furfural (g/L)
AT	Eq. model 3.1	2.424 ± 0.258	23.175 ± 1.642	3.794 ± 0.147	0.000 ± 0.000	0.308 ± 0.004
	Eq. model 3.5	2.159 ± 0.094	21.066 ± 1.600	1.001 ± 0.305	0.059 ± 0.083	0.057 ± 0.080
AA	Eq. model 3.2	2.587 ± 0.337	27.632 ± 0.590	1.533 ± 0.028	0.000 ± 0.000	0.242 ± 0.001
	Eq. model 3.6	4.587 ± 0.218	22.289 ± 0.336	1.26 ± 0.149	2.209 ± 0.025	0.206 ± 0.065
AK	Eq. model 3.3	2.951 ± 1.214	31.800 ± 0.831	1.853 ± 0.325	0.000 ± 0.000	0.209 ± 0.023
	Eq. model 3.7	2.532 ± 0.154	27.665 ± 1.443	1.477 ± 0.082	1.653 ± 0.018	0.000 ± 0.000
AP	Eq. model 3.4	4.043 ± 0.196	24.423 ± 0.747	1.758 ± 0.269	1.289 ± 0.222	0.222 ± 0.003
	Eq. model 3.8	3.502 ± 0.873	16.731 ± 0.330	0.936 ± 0.171	0.544 ± 0.060	0.000 ± 0.000

effect is observed in acetic acid concentration when H_2SO_4 concentration and hydrolysis time increase (Fig. 2b) the concentration of this inhibitor; this effect can be observed in the four bagasses studied, an unfavorable condition in this process. As for AA bagasse, when solutions with a low H_2SO_4 concentration and low hydrolysis times are used, low xylose concentrations are obtained, an effect opposite to that obtained when both the H_2SO_4 concentration and the hydrolysis time are increased, but lower compared with AT (Fig. 2c). As for acetic acid production, high concentrations of H_2SO_4 and prolonged hydrolysis times generate high concentrations of acetic acid; however, concentration does not exceed 2 g/L (Fig. 2d). Due to the nature of AK and AP (mezcal-producing *Agaves*), in order to obtain high xylose concentrations, it is necessary to treat them more aggressively than AT, as can be seen in Fig. 3, where it can be observed that it is necessary to use a higher sulfuric acid concentration to increase xylose concentration and to be able to observe a stronger red-orange color in the response surface graph. It is necessary to use hydrolysis times greater than 30 min and maximum H_2SO_4 concentrations to obtain high xylose concentrations; however, as the treatments are aggressive, the acetic acid concentration obtained is greater compared with treatments with lower sulfuric acid concentrations.

Table 4 shows the results obtained from the experimental validation of the optimization process both for maximum xylose concentration and for minimum acetic acid concentration in each of the *Agave* bagasses studied: AT, AA, AK, and AP. In the case of xylose and acetic acid optimizations, experimental results are indicated in Table 5, where it can be seen that with optimization, it was possible to increase xylose concentration to 3.25 g/L for AT, to 14.6 g/L for AA, to 14.09 g/L for AK, and to 7.44 g/L for AP, but also to increase acetic acid concentration compared with the data obtained in the Box-Behnken design. This is due to the fact that in xylose optimization, solutions with sulfuric acid concentrations higher than those evaluated in the experimental design were used, and as mentioned previously, with an increase in acid concentration, there is an increase in the concentration of an inhibitor, that is, acetic acid.

Comparing xylose optimization with acetic acid optimization in AT, 73% less acetic acid was formed in acetic acid optimization, an acceptable value because these concentrations are tolerated by *C. tropicalis* IEC5-ITV.

Comparing the results obtained from optimization with AT bagasse, a reduction in acetic acid in the acid hydrolysate of up to 74% was obtained, whereas for AA, AK, and AP bagasses, decreases of 17%, 20%, and 46% were observed, respectively.

4 Conclusions

The Box-Behnken design is an effective tool in the optimization of hemicellulose hydrolysis conditions in order to obtain maximum xylose concentration with minimal acetic acid concentration, that is, maximum xylose production in the hydrolysate medium with minimum inhibitors present. The four bagasse samples from different *Agave* varieties did not present relevant differences in their optimization conditions (see Table 3).

Effective hemicellulose removal is necessary to obtain xylose, and acid pretreatments were proven to be effective to this end, yielding concentrations between 23.3 and 31.8 g/L xylose. This sugar is the principle building block for renewable fuels and xylitol production in biorefineries in a sustainable manner.

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