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Research article

Chemical pretreatment of *Arundo donax* L. for second-generation ethanol production

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ABSTRACT

Background: Pretreatment of lignocellulosic biomass is essential for using it as a raw material for chemical and biofuel production. This study evaluates the effects of variables in the chemical pretreatment of the *Arundo* biomass on the glucose and xylose concentrations in the final enzymatic hydrolysate. Three pretreatments were tested: acid pretreatment, acid pretreatment followed by alkaline pretreatment, and alkaline pretreatment.
Results: The amounts of glucose and xylose released by the enzymatic hydrolysis of the *Arundo* biomass obtained from acid pretreatment ranged from 6.2 to 19.1 g/L and 1.8 to 3.1 g/L, respectively. The addition of alkaline pretreatment led to a higher yield from the enzymatic hydrolysis, with the average glucose concentration 3.5 times that obtained after biomass hydrolysis with an acid pretreatment exclusively. The use of an alkaline pretreatment alone resulted in glucose and xylose concentrations similar to those obtained in the two-step pretreatment: acid pretreatment followed by alkaline pretreatment. There was no significant difference in 5-hydroxymethylfurfural, furfural, or acetic acid concentrations among the pretreatments.
Conclusion: Alkaline pretreatment was essential for obtaining high concentrations of glucose and xylose. The application of an alkaline pretreatment alone resulted in high glucose and xylose concentrations. This result is very significant as it allows a cost reduction by eliminating one step.

1. Introduction

Lignocellulosic biomasses are a promising raw material for bioethanol production as they are the most abundant carbon source on the planet [1,2]. Second-generation bioethanol production uses lignocellulosic biomasses such as sugar feedstock, which are converted into ethanol through a fermentation process. The main advantages of this technology lie in the fact that the raw material is low cost, renewable, and sustainable [3].

However, lignocellulosic biomass has a complex composition that mainly includes cellulose, hemicellulose (carbohydrate polymers), and lignin [4,5,6]. Because of its composition, pretreatment of the biomass is an essential step of the second-generation biofuel process [5,7]. The goal of the pretreatment is to improve the digestibility of the lignocellulosic biomass. Pretreatment processes remove hemicellulose and lignin, increasing the porosity of the biomass and reducing cellulose crystallinity, thus making the cellulose more accessible for conversion into fuels [8].

Pretreatments can be divided into physical, physicochemical, chemical, and biological methods or a combination of any of these [3,5]. The choice of a pretreatment method depends on the biomass characteristics. It should improve the yields of sugars and avoid degradation products that are inhibitory to the subsequent steps of the process [9].

Chemical pretreatments are useful for improving the digestibility of a lignocellulosic biomass. Acid pretreatment is the most commonly employed method. Diluted or concentrated acids can hydrolyze the hemicelluloses of most lignocellulosic raw materials, promoting enzymatic action and increasing the yield from the biomass hydrolysis [2,10,11]. Different acids such as hydrochloric, nitric, and phosphoric acids can be utilized, but sulfuric acid is usually used [5,12].

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Alkaline pretreatment allows the removal of lignin, acetyl groups, and uronic acids by cleavage of the linkage between lignin and hemicellulose. It causes the cellulose to swell, thus decreasing its crystallinity and degree of polymerization and making it more accessible to cellulases [3,4,13].

Because of the characteristics of these chemical pretreatments, a combination of acid and alkaline pretreatments can increase the enzymatic hydrolysis yield compared to an individual acid pretreatment or alkaline pretreatment. Guo et al. [14] evaluated the two-stage acid–alkaline hydrothermal pretreatment conditions of *Miscanthus* biomass and compared them with single-stage acid and alkaline pretreatments. The results showed higher glucose and xylose concentrations after the two-stage pretreatment compared to the single-stage pretreatments. Similarly, Wang et al. [15] investigated corn stover pretreatments and obtained a higher glucose yield for an acid–alkaline two-stage pretreatment than for either acid or alkaline pretreatment. However, Guilherme et al. [16] applied different pretreatments to sugar cane bagasse and found higher glucose and xylose concentrations after alkaline pretreatment alone compared with a combined acid and alkaline pretreatment.

Arundo donax L., also known as giant reed, is a perennial grass belonging to the Poaceae family. This plant presents advantages as a raw material for ethanol production such as high biomass production, rapid growth, low agronomic input, low production costs, and the ability to grow in different kinds of environments [17,18].

The goal of this study was to evaluate the effects of chemical pretreatments on glucose and xylose production from *A. donax* L. biomass aiming for ethanol production.

2. Materials and methods

2.1. Raw material

Arundo biomass was harvested in the São Gonçalo watercourse in Pelotas, Brazil (latitude 31°46′33″ south and longitude 52°21′34″ west). The biomass was milled and air-dried. Then it was milled again to reduce the particle size. The particle size distribution was determined by sieving through 1.00, 0.50, 0.25, 0.105, and 0.053 mm sieves.

2.2. Compositional analysis

The biomass was analyzed for extractives [19], cellulose, hemicellulose, lignin, and ash [20], following the National Renewable Energy Laboratory (NREL) methods.

2.3. Biomass pretreatment

Arundo biomass was first subjected to acid pretreatment, and the liquid and solid fractions were separated. The pretreated biomass, named acid cellulignin (ACCL), was used for the second alkaline pretreatment step. The *Arundo* integral biomass was also subjected to alkaline pretreatment only. The biomasses resulting from the three pretreatments were then hydrolyzed enzymatically (Fig. 1), and the released sugars were quantified using high-performance liquid chromatography (HPLC).

2.3.1. Acid pretreatment

Arundo biomass (100 g DM) was treated with sulfuric acid and autoclaved at 120°C. Variations in the sulfuric acid concentration (x_1), exposure time (x_2), and solid-to-liquid ratio (S:L ratio) (x_3) followed a central composite rotational design.

After pretreatment, the biomass (solid fraction) was separated from the liquid fraction. The ACCL was repeatedly washed with water to remove the excess acid until the pH was 4.5–5.0. It was then dried in an oven at 65°C.

2.3.2. Alkaline pretreatment after acid pretreatment

The second step of the pretreatment was the alkaline treatment. A solution of sodium hydroxide (0.5 M) at a S:L ratio of 1:20 g/mL was added to 50 g DM of ACCL, obtained from acid pretreatment under the conditions in Section 2.3.1, and allowed to react for 30 min at 127°C. Afterward, the liquid and biomass fractions were separated, and

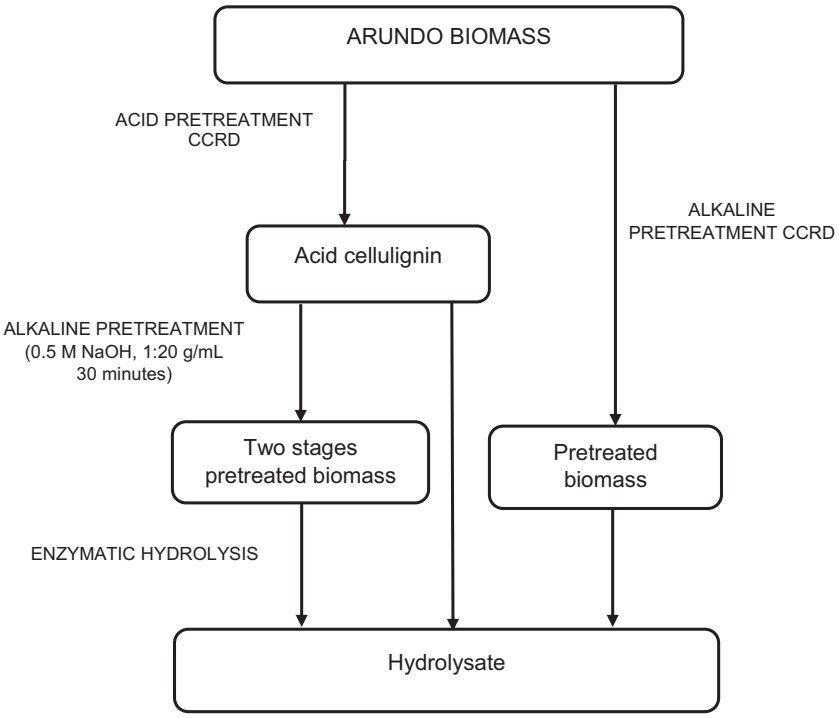

Fig. 1. Flow chart for pretreatment of *Arundo* biomass.

the biomass fraction was washed several times until the pH reached 4.5–5.0.

2.3.3. Comparison between biomass pretreatments

To compare the different pretreatments, a solution of sodium hydroxide (0.5 M) at a S:L ratio of 1:20 g/mL was added to the integral biomass (without acid pretreatment), the ACCL (0.11% H_2SO_4, 5 min, 1:2 g/mL S:L ratio), and the biomass resulting from the hydrothermal pretreatment (5 min, 1:2 g/mL S:L ratio). The resulting system, in each case, was autoclaved for 30 min at 127°C. The experiment involving the hydrothermally pretreated biomass aimed to evaluate the effects of the physical conditions of the acid pretreatment. For the hydrothermal pretreatment, the biomass was moistened with water and subjected to the same conditions as the acid pretreatment prior to the alkaline pretreatment (30 min at 127°C). The liquid and biomass fractions were separated, and the biomass fraction was washed several times until the pH settled at 4.5–5.0.

2.3.4. Alkaline pretreatment

A solution of sodium hydroxide was added to 100 g DM of the integral biomass and autoclaved at 127°C for 30 min. Variations in the sodium hydroxide concentration (x_1) and S:L ratio (x_2) followed a central composite rotational design. The liquid and biomass fractions were separated, and the biomass fraction was washed several times until the pH reached 4.5–5.0. The response variables used to evaluate the pretreatment conditions were the glucose and xylose concentrations released upon enzymatic hydrolysis.

2.4. Enzymatic hydrolysis

Ten grams DM of pretreated biomass was subjected to hydrolysis with 10 FPU/g Cellic CTec 3 (Novozymes) of solids suspended (100 g/L) in 50-mM citrate buffer (pH 5.0) at 50°C, 200 rpm for 48 h.

2.5. High-performance liquid chromatography analysis

All the solvents used were of HPLC grade. The solutions were made using ultrapure-grade water from a Milliq system (Academic). Glucose and xylose (Supelco, USA), acetic acid (Sigma-Aldrich, USA), furfural (Fluka, USA), and 5-hydroxymethylfurfural (Fluka, China) were used as standards.

The glucose, xylose, and acetic acid concentrations were determined by HPLC, using an HPX87H column (300 × 7.8 mm and 9 μm particle size - BioRad), 5 mM H_2SO_4 as eluent at a flow rate of 0.6 mL/min, and a refractive index detector (LC-20A Prominence, Shimadzu). Furfural and 5-hydroxymethylfurfural concentrations were determined by a Shim-pack CLC-ODS(M) C18 column (150 × 4.6 mm and 5 μm particle size - Shimadzu) with a water:acetonitrile:acetic acid (79:20:1) eluent at a flow rate of 1.1 mL/min with a diode array detector in the same equipment.

2.6. Statistical analysis

A central composite rotational design (CCRD) was used to evaluate the acid and alkaline pretreatments. The dependent variables, glucose and xylose concentrations, were analyzed using STATISTICA 12.0 (StatSoft, Inc.) software. To verify the differences between biomass pretreatments, analysis of variance (ANOVA) and Tukey's test were used, at a significance level of 0.95 ($P \leq 0.05$).

3. Results and discussion

3.1. Raw material composition

A physical treatment to reduce the particle size of the biomass is normally the first step in the pretreatment of a lignocellulosic biomass and aims to increase its superficial area and reduce its degree of polymerization [3]. Most of the biomass was characterized by particle sizes greater than 0.50 mm. Particles smaller than 0.50 mm represented 28.5% of the total average mass. Lignocellulosic biomasses are basically composed of cellulose, hemicellulose, and lignin [2,3,21,22]. Table 1 shows the chemical composition of the *Arundo* used in the present work: cellulose, hemicellulose, lignin, and ashes were 33.0, 14.6, 30.4, and 3.6 (% m/m), respectively. Corno et al. [17] reported that the chemical composition of *A. donax* obtained by different authors was 11.2 to 21.6% extractives, 29.2 to 39.1% cellulose, 14.5 to 32.0% hemicellulose, 19.2 to 24.3% of lignin, and 4.2 to 6.1% ash. Lemons e Silva et al. [23] found 31.1% cellulose, 35.3% hemicellulose, and 18.5% lignin in the *Arundo* biomass harvested in Pelotas, Brazil. Sun and Cheng [10] and Anwar et al. [11] reported lignin content in grasses ranging from 10 to 30%. In the present work (Table 1), extractives, cellulose and hemicellulose contents are in accordance with these reported ranges. The amount of total lignin (30.4%) was higher, and the percentage of ashes was lower than literature data.

3.2. Acid pretreatment

3.2.1. Effects of acid pretreatment on the solid fraction

The mass loss in acid pretreatment assays of the *Arundo* biomass ranged from 18.1 to 49.6% (Table 2). The lowest mass loss was obtained for assays with the lowest sulfuric acid concentration (0.11% w/w), and the highest mass loss was obtained for assay 7 involving 4% sulfuric acid for 45 min and a S:L ratio of 1:4 g/mL.

Similar to the mass losses, the highest glucose concentration (19.1 g/L) was obtained with 4% sulfuric acid treatment for 45 min, and the lowest concentration (6.2 g/L) was obtained with the lowest concentration of sulfuric acid (0.11% w/w) (Table 2).

For the xylose concentrations, the same behavior was observed in relation to mass loss and glucose concentration. However, the highest xylose concentration (2.9 g/L) was obtained with 4% sulfuric acid treatment for 15 min and a S:L ratio of 1:2 g/mL (Table 2).

The average concentrations of glucose and xylose released during enzymatic hydrolyses were 12.8 g/L and 2.5 g/L, respectively. The total average concentration of sugars available for fermentation was 15.4 g/L. Lemons e Silva et al. [23] obtained 13.9 g/L glucose from sulfuric acid-pretreated *Arundo* biomass (1.1% w/w), treated with a S:L ratio of 1:2.8 g/mL for 30 min at 120°C.

Degradation products such as HMF, furfural, and acetic acid in the hydrolysates were also determined as these substances are fermentation process inhibitors. HMF and furfural concentrations were 0.01 g/L in all tests. Acid acetic was not detected in assays 7 to 14 and 17, and in the remaining trials, the concentration ranged from 0.4 to 0.5 g/L (Table 2).

Scordia et al. [24] found higher concentrations of HMF and furfural in hydrolysates of *Arundo* after pretreatment with oxalic acid vapor. The furfural concentrations ranged from 1.84 to 6.96 g/L. It should be noted that in this study, the HMF and furfural concentrations were determined in the enzymatic hydrolysate, whereas in the cited

Table 1
Chemical composition of the *Arundo* biomass.

Compounds	*Arundo* biomass (% w/w DM)		
	This study	[17]	[23]
Moisture	1.7 ± 0.3	–	3.0
Extractives	14.7 ± 0.5	11.2–21.6	–
Cellulose	33.3 ± 4.4	29.2–39.1	31.1
Hemicellulose	14.6 ± 2.3	14.5–32.0	35.3
Acid-soluble Lignin	10.8 ± 0.3	–	–
Klason Lignin	19.6 ± 0.4	19.2–24.3	18.5
Ash	3.6 ± 0.3	4.2–6.1	–

– not available.

Table 2
Experimental design and results: mass loss (%w/w DM) and glucose, xylose, and acetic acid concentrations (g/L) obtained on central composite design CCRD for the acid pretreatment of the *Arundo* biomass.

Run	Coded values and real values			Acid pretreatment			Acid pretreatment + Alkaline pretreatment			
	x_1 H_2SO_4 (% w/w)	x_2 Time (min)	x_3 S:L ratio (g/mL)	Mass loss	Glucose	Xylose	Acetic acid	Mass loss	Glucose	Xylose
1	−1 (1.1)	−1 (15)	−1 (1:4)	30.8	10.4	2.7	0.5	53.8	52.6	8.4
2	−1 (1.1)	−1 (15)	1 (1:2)	24.9	8.3	2.8	0.5	52.0	51.1	13.0
3	−1 (1.1)	1 (45)	−1 (1:4)	36.2	12.5	2.8	0.5	53.4	56.8	4.8
4	−1 (1.1)	1 (45)	1 (1:2)	27.2	10.5	3.1	0.5	52.7	53.1	9.5
5	1 (4.0)	−1 (15)	−1 (1:4)	42.0	15.4	2.8	0.4	51.6	41.1	2.5
6	1 (4.0)	−1 (15)	1 (1:2)	39.6	13.3	2.9	0.4	54.6	45.4	3.1
7	1 (4.0)	1 (45)	−1 (1:4)	49.6	19.1	2.2	nd	55.1	33.8	0.8
8	1 (4.0)	1 (45)	1 (1:2)	47.0	16.6	2.4	nd	50.4	36.2	1.4
9	−1.68 (0.11)	0 (30)	0 (1:3)	18.1	6.2	1.8	nd	50.4	49.1	17.1
10	1.68 (5.0)	0 (30)	0 (1:3)	45.9	13.3	1.9	nd	50.7	33.2	1.5
11	0 (2.55)	−1.68 (4.8)	0 (1:3)	22.8	15.4	2.5	nd	53.8	53.8	14.2
12	0 (2.55)	1.68 (55.2)	0 (1:3)	45.6	15.5	2.6	nd	50.9	38.9	1.7
13	0 (2.55)	0 (30)	−1.68 (1:1.67)	40.0	10.8	2.5	nd	52.2	53.8	3.5
14	0 (2.55)	0 (30)	1.68 (1:6.6)	45.9	13.7	2.2	nd	51.0	38.0	1.6
15	0 (2.55)	0 (30)	0 (1:3)	40.8	11.8	2.6	0.4	52.2	49.7	3.0
16	0 (2.55)	0 (30)	0 (1:3)	41.5	12.7	2.8	0.4	53.4	47.2	3.0
17	0	0 (30)	0 (1:3)	41.4	12.6	2.6	nd	50.8	44.8	2.8

nd = not detected; LOD = 0.007 g/L; LOQ = 0.022 g/L.

study [24], they were determined in the oxalic acid hydrolysate (water-soluble fraction).

3.2.2. Effects of alkaline pretreatment after the acid pretreatment

The lower concentrations of glucose and xylose obtained from the biomass hydrolysis after pretreatment with sulfuric acid alone, presented in Table 2, indicate that an additional pretreatment step is needed. This hypothesis is based on the fact that a better result was obtained with an additional pretreatment step in a previous study by Lemons e Silva et al. [23]. In their study, 42 g/L of glucose was obtained from the *Arundo* biomass after pretreatment with 1.1% H_2SO_4, S:L ratio of 1:2.8 g/mL, for 30 min followed by an alkaline pretreatment. The addition of the alkaline pretreatment led to a higher yield from the enzymatic hydrolysis (Table 2), with glucose concentrations ranging from 33.2 to 56.8 g/L. The average glucose concentration was 3.5 times higher than the average glucose concentration obtained from the biomass hydrolysis after acid pretreatment alone (12.8 g/L).

The average mass loss after the addition of the alkaline pretreatment was 52.3%, with a range of 50.4 to 55.1% (Table 2). This behavior can be attributed to the use of bases such as sodium hydroxide, which act to partially remove the lignin, increasing the accessibility of the cellulose to the enzymes and consequently increasing the yields of the hydrolysis [11,13].

Although the glucose concentration was high in all assays with the addition of the second stage of pretreatment, the same behavior was not detected for the xylose concentration, which ranged from 0.8 to 17.1 g/L., when two stages of biomass pretreatment were used.

Fig. 2 presents the response surface for the glucose concentration. Regarding the concentration of sulfuric acid in the acid pretreatment, it was observed that it had a significant negative effect on the glucose concentration when alkaline pretreatment was applied following acid treatment ($P = 0.0019$). The other variables and their interactions did not produce a significant effect on the glucose concentration ($P > 0.05$).

The concentration of sulfuric acid ($P = 0.0005$) and its exposure time ($P = 0.0099$) had significant negative effects on the xylose

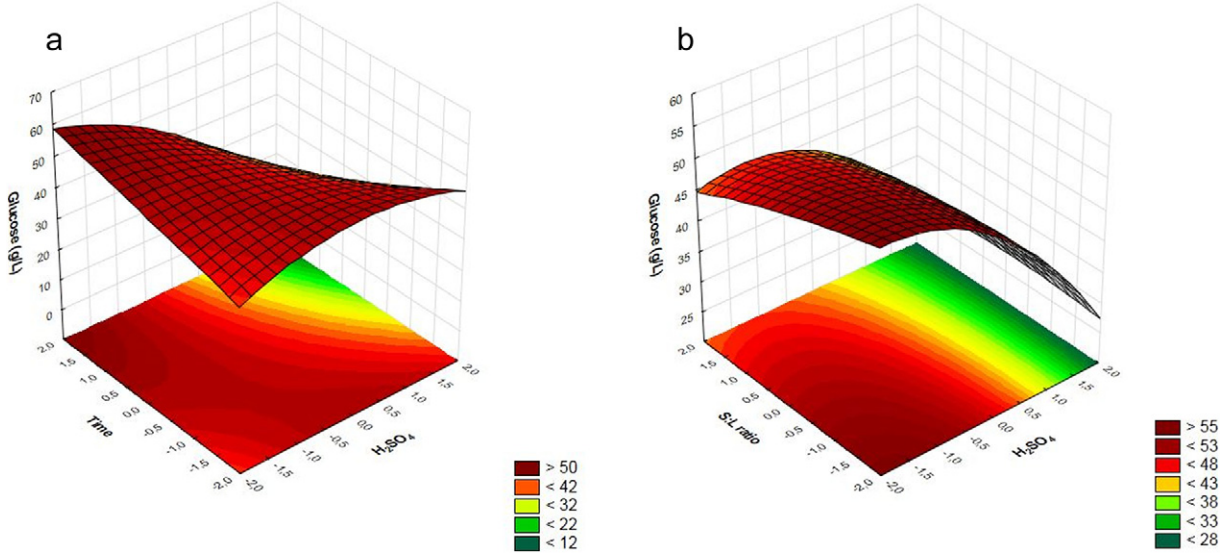

Fig. 2. Response surface for the glucose concentration in the hydrolysates from the solid fraction obtained from the alkaline pretreatment after the acid pretreatment as functions of the following variables: (a) sulfuric acid concentration and exposure time and (b) sulfuric acid concentration and solid-to-liquid ratio.

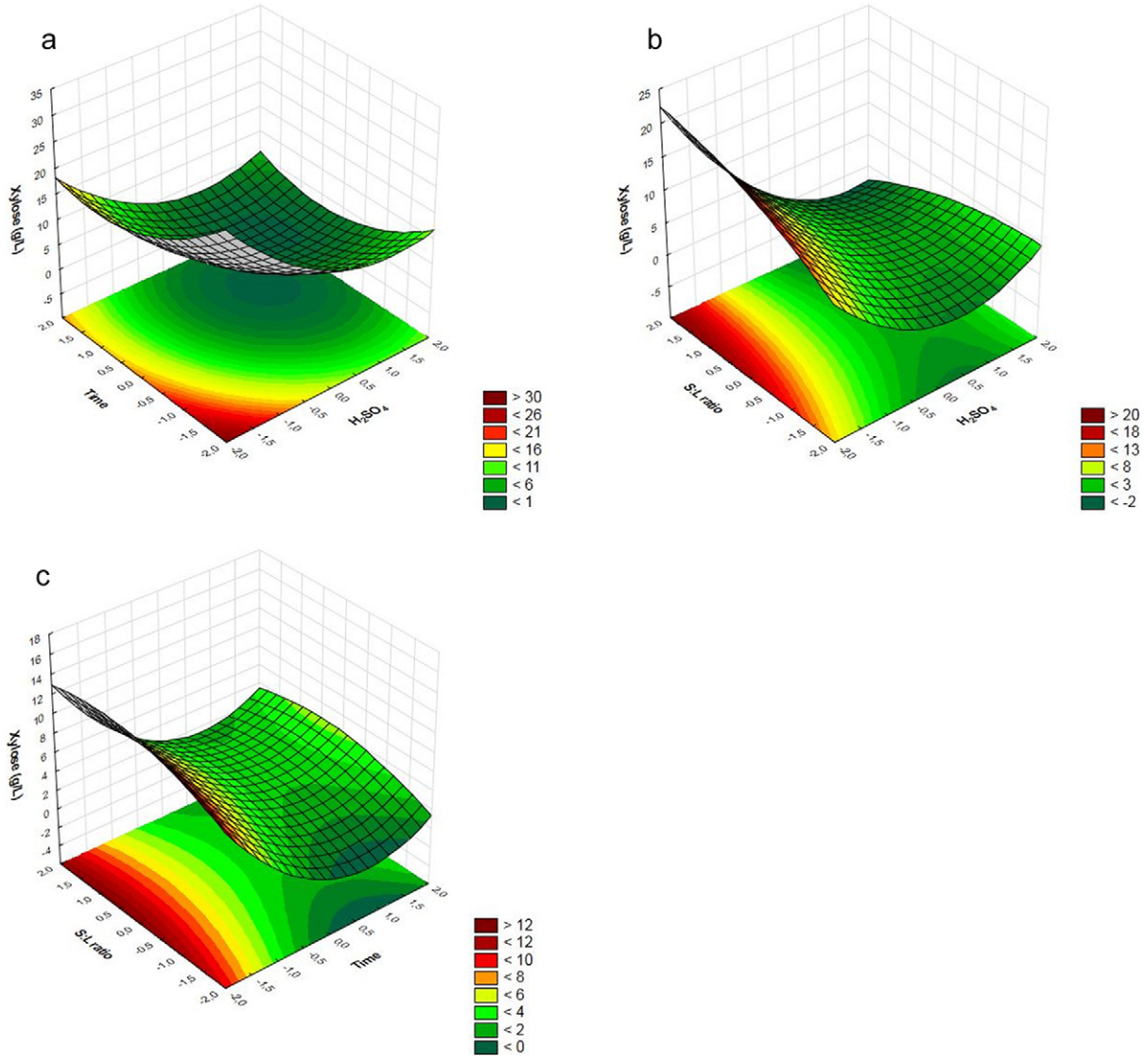

Fig. 3. Response surface for the xylose concentration in the hydrolysates from the solid fraction obtained from the alkaline pretreatment after the acid pretreatment as functions of the following variables: (a) sulfuric acid concentration and exposure time and (b) sulfuric acid concentration and solid-to-liquid ratio (c) exposure time and solid-to-liquid ratio.

concentration after the two stages of *Arundo* biomass pretreatment. The S:L ratio and the interactions among the variables did not exhibit significant effects on hydrolysis yields (Fig. 3).

The sulfuric acid concentration and the pretreatment time alter the biomass structure, making it necessary to optimize the pretreatment conditions to obtain lower losses of sugars during the pretreatment and higher concentrations of sugars released during the enzymatic hydrolysis. The formation of secondary products, which are inhibitors of the fermentation, is the main disadvantage of using acids in the pretreatment. Acids decrease the yield of pentoses and hexoses through degradation, leading to the formation of furfural, HMF, and acetic acid. Determination of the degradation products that can be formed during pretreatment is critical to prevent losses in fermentation yields. The concentrations of HMF and furfural in the hydrolysates, obtained after the two pretreatment steps, were evaluated. No HMF was detected in assay 2, and in the remaining assays, the HMF concentration was 0.01 g/L. Furfural was detected in all assays at concentrations of 0.01 g/L. Such low concentrations of HMF and furfural indicate that these variables had no effect on the conditions tested.

3.2.3. Comparison between biomass pretreatments

The results obtained from the pretreatment trials carried out to verify the negative effect of the use of sulfuric acid on sugar concentration are presented in Table 3. It can be observed that the highest average concentrations of sugars were obtained when an acid

Table 3
Mass loss (%w/w DM) and glucose, xylose, HMF, and furfural concentrations (g/L) in the hydrolysates from the pretreated biomass and the sugar yield (g/g).

Pretreatment	Mass loss	Glucose	Xylose	HMF	Furfural	Sugar yield (pretreated biomass)	Sugar yield (raw biomass)
Acid + Alkaline	63.8 ± 1.1	62.0 ± 14.8 a	19.7 ± 3.9 a	0.01 ± 0.01	0.01 ± 0.00	0.83 ± 0.26	0.30 ± 0.09
Water + Alkaline	65.6 ± 1.7	62.4 ± 11.1 a	20.0 ± 3.7 a	nd	0.01 ± 0.00	0.82 ± 0.15	0.28 ± 0.06
Alkaline	51.4 ± 0.7	53.2 ± 6.2 a	17.3 ± 1.6 a	0.01 ± 0.01	0.01 ± 0.00	0.74 ± 0.08	0.38 ± 0.04

Means followed by the same letters in the column do not differ significantly by the Tukey's test at 5%.

Table 4
Experimental design and results: mass loss (%w/w DM) and glucose, xylose, HMF, and furfural concentrations (g/L) obtained and the sugar yield (g/g raw biomass) on CCRD for the alkaline pretreatment of the *Arundo* biomass.

Run	Coded and real values		Mass loss	Glucose	Xylose	HMF	Furfural	Sugar yield
	x_1 NaOH (M)	x_2 S:L ratio(g/mL)						
1	−1 (0.5)	−1 (1:20)	51.4	48.3	15.7	0.01	0.01	0.31
2	1 (1.5)	−1 (1:20)	59.0	74.8	10.7	nd	0.01	0.35
3	−1 (0.5)	1 (1:10)	48.9	65.7	23.8	0.01	0.01	0.46
4	1 (1.5)	1 (1:10)	56.7	51.3	8.8	0.02	0.01	0.26
5	−1.41 (0.3)	0 (1:15)	47.9	45.2	17.9	nd	0.01	0.33
6	1.41 (1.7)	0 (1:15)	57.9	58.0	8.0	nd	0.01	0.28
7	0 (1.0)	−1.41 (1:22)	55.5	59.2	12.5	nd	0.01	0.32
8	0 (1.0)	1.41 (1:7.9)	54.1	50.9	13.7	nd	0.01	0.30
9	0 (1.0)	0 (1:15)	55.8	73.3	15.4	nd	0.01	0.40
10	0 (1.0)	0 (1:15)	55.0	85.5	17.7	nd	0.01	0.46
11	0 (1.0)	0 (1:15)	55.8	68.4	14.6	nd	0.01	0.37

nd = not detected; LOD = 0.000035 g/L; LOQ = 0.00011 g/L.

pretreatment was followed by an alkaline pretreatment and when the pretreatment with water was followed by an alkaline pretreatment. However, the use of an alkaline pretreatment alone allowed the glucose and xylose sugars to be released at concentrations that did not differ significantly from those in the other pretreatments.

The sugar yield (g sugar/g pretreated biomass) was higher with the acid pretreatment, as were the glucose and xylose concentrations (Table 3). However, the sugar yield (g sugar/g raw biomass), taking into account the mass loss, ranged from 0.28 to 0.38 g/g. The highest sugar yield was obtained from the alkaline pretreatment.

There were no significant differences in HMF or furfural concentrations among the pretreatments, which is a good result. The low concentrations of HMF and furfural obtained may be due to washing of the pretreated biomass after the pretreatment step. This procedure can remove inhibitory compounds such as acetic acid, HMF, and furfural. Gurram et al. [25] observed removals of 14, 26, and 42% of acetic acid, HMF, and furfural, respectively, after washing the pretreated solids of Ponderosa pine wood [25].

The use of an alkaline pretreatment alone is advantageous for the process because, besides the cost reduction resulting from the elimination of one step, there is no need to neutralize the acid used, and the quantity of water required to wash the biomass after the process is also reduced. In addition, the mass loss for the acid pretreatment was 37.6% on average, requiring a higher initial amount of biomass in previous steps and enzymatic hydrolysis.

3.3. Alkaline pretreatment

The alkaline pretreatment conditions of the *Arundo* biomass without previous acid pretreatment were evaluated. The variables analyzed were the concentration of sodium hydroxide (x_1) and the S:L ratio (x_2). The mass loss and the concentrations of glucose, xylose, HMF, and furfural in the hydrolysates of the pretreated biomass are shown in Table 4.

The lowest mass loss (47.9%) was obtained using the lowest sodium hydroxide concentration (0.3 M), whereas the highest mass loss (57.9%) was obtained with the highest concentration of sodium hydroxide (1.7 M). The average mass loss for the pretreatment conditions evaluated was 54.37 ± 3.54%.

Glucose concentrations ranged from 45.2 to 85.5 g/L. The lowest concentration was obtained with the lowest concentration of sodium hydroxide (0.3 M, 1:15 g/mL), and the highest concentration of glucose was obtained at the central point of the experimental design (1.0 M, 1:15 g/mL). The average glucose concentration at the central point was 75.7 ± 8.8 g/L.

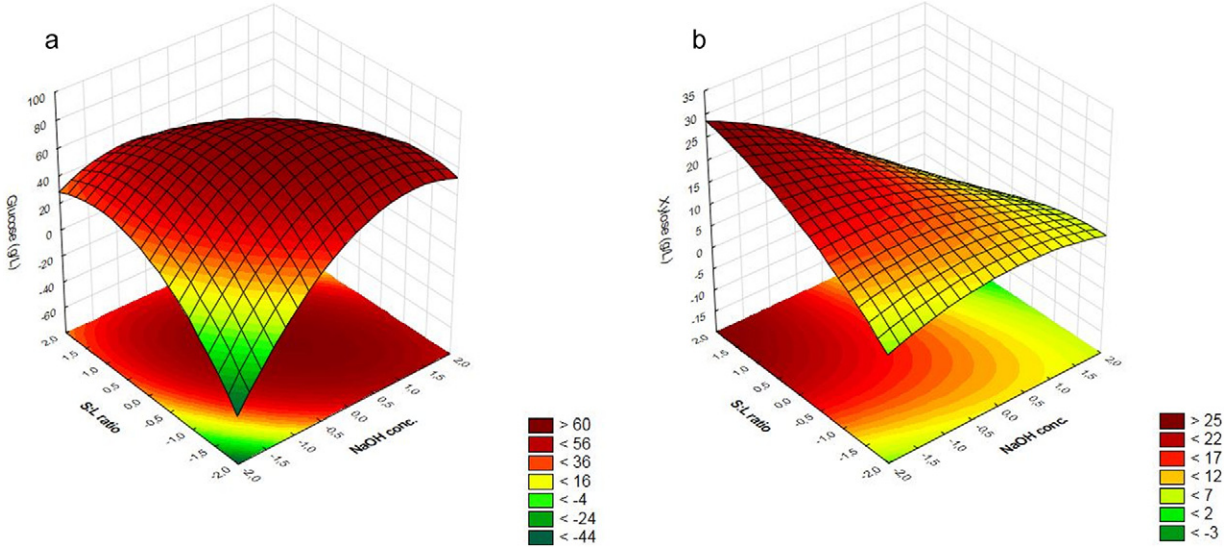

Fig. 4. Response surface for the glucose (a) and xylose (b) concentration in the hydrolysates from the solid fraction obtained from the alkaline pretreatment as functions of variables sodium hydroxide concentration and solid-to-liquid ratio.

Table 5
ANOVA for the regression model of the alkaline pretreatment for glucose and xylose concentrations.

	Glucose				Xylose			
	SS	DF	MS	F-value	SS	DF	MS	F-value
Model	1373.2	5	274.6	5.35	186.2	5	37.2	10.25
(1) NaOH (L)	114.0	1	114.0	2.22	144.6	1	144.6	39.81
NaOH (Q)	606.6	1	606.6	11.82	6.1	1	6.1	1.68
(2)S:L ratio(L)	39.7	1	39.74	0.77	7.8	1	7.8	2.15
S:L ratio(Q)	421.2	1	421.2	8.21	5.2	1	5.2	1.44
1 L by 2 L	418.2	1	418.2	8.15	25.0	1	25.0	6.88
Error	256.5	5	51.30		18.2	5	3.6	
Total SS	1629.7	10			204.3	10		

SS = sum of square; DF = degree free; MS = mean square.

The xylose concentration varied from 8.0 to 17.9 g/L. The lowest concentration of xylose was obtained with the highest concentration of sodium hydroxide, and the highest concentration of xylose was obtained with the lowest concentration of sodium hydroxide, indicating a negative effect of sodium hydroxide on the xylose concentration. The average concentration of xylose at the center point was 15.9 ± 1.6 g/L.

The sugar yield ranged from 0.26 to 0.46 g/g. The lowest sugar yields were obtained in the trials carried out with the highest NaOH concentrations. The highest sugar yields were obtained in trial 3 (1.0 M, 1:10 g/mL) and the center point (1.0 M, 1:15 g/mL). Zhao et al. [26] obtained sugar yields ranging from 0.30 to 0.52 g/g in experiments designed to optimize liquid ammonia pretreatments of *Arundo* biomass.

HMF was detected in only three trials of the experimental design for alkaline pretreatment. The furfural concentration was 0.01 g/L and did not differ significantly from the concentrations obtained for the acid pretreatment followed by the alkaline pretreatment.

The quadratic effects of the two variables and the interaction between the two variables showed a negative effect on glucose concentration. The model equation for glucose concentration was as follows:

$$\text{Glucose} = 75.71 + 3.78x_1 - 10.41x_1^2 + 2.23x_2 - 8.76x_2^2 - 10.22x_1x_2$$

Fig. 4a shows the response surface for the glucose concentration as a function of the variables studied. The reference points for assay 2 (1.5 M, 1:20 g/mL) and the center point (1.0 M, 1:15 g/mL) are in the region of highest glucose concentration. In this case, the use of conditions relating to the center point is more advantageous as the concentration of sodium hydroxide and the volume of black liquor generated during the pretreatment are lower and the glucose yields are equivalent.

The concentration of sodium hydroxide (x_1) had a significant negative effect on the concentration of xylose ($P = 0.0015$), as did the interaction between the variables studied ($P = 0.0469$). Fig. 4b shows the response surface for the xylose concentration as a function of the variables studied. The model equation for xylose concentration was as follows:

$$\text{Xylose} = 15.89 - 4.25x_1 - 1.04x_1^2 - 0.99x_2 - 0.97x_2^2 - 2.50x_1x_2$$

The *P*-values ($P = 0.000009$ for glucose and $P = 0.000029$ for xylose) showed that the models are significant. Table 5 presents the ANOVA for the regression model of the alkaline pretreatment for the glucose and xylose concentrations. For both glucose and xylose models, the calculated F statistic was greater than the table F value (5.05), confirming that the model is valid at a 95% confidence interval. In addition, the coefficients of determination (R^2) were 0.8426 for the glucose concentration and 0.9111 for the xylose concentration, indicating a good correlation between the observed and predicted data.

4. Conclusions

An alkaline pretreatment was essential for obtaining high concentrations of glucose and xylose. The application of an alkaline pretreatment alone yielded concentrations of glucose and xylose similar to those obtained in the two-step pretreatment: acid pretreatment followed by alkaline pretreatment. This result is very significant as it allows cost reduction by eliminating one step. In addition, there were no significant differences in HMF, furfural, and acetic acid concentrations among the applied pretreatments.

Conflict of interest

The authors declare that they have no conflict of interest.

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