Available online at www.sciencedirect.com
ScienceDirect
Energy Reports 6 (2020) 18–23
www.elsevier.com/locate/egyr

7th International Conference on Energy and Environment Research, ICEER 2020, 14–18 September, ISEP, Porto, Portugal

Acid pretreatment of sugarcane biomass to obtain hemicellulosic hydrolisate rich in fermentable sugar

Wilson G. Morais Junior[a,*], Thályta F. Pacheco[b], Priscila S. Corrêa[a], António A. Martins[c], Teresa M. Mata[c], Nídia S. Caetano[a,c]

[a] *CIETI, Department of Chemical Engineering, School of Engineering (ISEP), Polytechnic of Porto (P.Porto), R. Dr. Antonio Bernardino de Almeida 431, 4249-015 Porto, Portugal*

[b] *EMBRAPA Agroenergy, Parque Estação Biológica – PqEB s/n°, 70770-901 Brasília, Federal District, Brazil*

[c] *LEPABE-Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto (FEUP), R. Dr. Roberto Frias S/N, 4200-465 Porto, Portugal*

Received 13 October 2020; accepted 13 October 2020

Abstract

The objective of this work was to find the ideal pretreatment conditions with high efficiency to obtain a hydrolyzate rich in fermentable sugars and low possible inhibitors levels. Thus, it was applied diluted phosphoric acid to pretreat the sugarcane biomass. Through a Central Composite Design, it was evaluated the influence of temperature, operating time and acid concentration. The pretreatment efficiency was verified by the concentration of total monosaccharides in the liquid fraction after the reaction. The phosphoric acid concentration of 4.95% at 80 °C, during 375 min, resulted in a hemicellulosic hydrolyzate with the highest concentration of fermentable sugars (saccharification greater than 99%), with the absence of HMF and furfural, and relatively low amounts of acetic acid.

Peer-review under responsibility of the scientific committee of the 7th International Conference on Energy and Environment Research, ICEER 2020.

Keywords: Acid pretreatment; Fermentable sugars; Lignocellulosic hydrolyzate; Sugarcane biomass

1. Introduction

Nowadays, more and more studies are focusing on the development and production of biofuels, since these are considered a viable alternative to reduce dependence on fossil fuels, helping to alleviate the environmental impacts and reduce the climate change effects, in particular due to the emission of greenhouse gases [1].

The use of lignocellulosic biomass, as nature's main renewable organic matter, is a very promising alternative for the production of bioethanol as liquid fuel, with environmental, social and economic benefits [2]. Lignocellulosic

* Corresponding author.
E-mail address: wgdmj@isep.ipp.pt (W.G. Morais Junior).

https://doi.org/10.1016/j.egyr.2020.10.015

Peer-review under responsibility of the scientific committee of the 7th International Conference on Energy and Environment Research, ICEER 2020.

biomass is composed of cellulose, hemicellulose and lignin polymers, linked through non-covalent and covalent crosslinks to form a complex and recalcitrant structure [1].

Due to structural properties of lignocellulosic biomass, its use for bioethanol production is complex because it requires the use of cellulose and hemicelluloses fractions. Thus, the production process involves several steps, including biomass pretreatment, carbohydrate hydrolysis, fermentation of simple sugars to ethanol, and its distillation for the alcohol recovery [3].

The pretreatment aims to disrupt the association between the main constituents of biomass, increasing its surface area and making cellulose more amenable to enzymatic hydrolysis for its saccharification. Different pretreatment methods act in different ways on lignocellulosic biomass, breaking the lignin seal, separating hemicelluloses and reducing the cellulose crystallinity, while minimizing the chemical destruction of fermentable sugars necessary for the ethanol production [4].

Among the different pretreatment methods developed for lignocellulosic biomass, the one catalyzed by diluted acids is considered the most effective and economically viable [5]. During pretreatment, cellulose and hemicellulose are broken down, releasing, among others sugars, glucose and xylose. However, degradation of lignin (aromatic polymer consisting of phenylpropane) generates inhibitors such as furaldehydes, phenolic compounds (hydroxymethyl furfural — HMF and furfural) and acetic acid that have stressful effects on yeast during alcoholic fermentation [6].

HMF is formed by the glucose dehydration released by cellulose degradation, and of hexoses released by the hemicellulose degradation (galactose, mannose and rhamnose). In turn, furfural is formed by xylose and arabinose dehydration, also released by hydrolysis of hemicellulose [7]. Acetic acid is commonly formed in the hydrolysis stage, by deacetylation of hemicellulose. These compounds inhibit yeast cell growth during alcoholic fermentation, decreasing the productivity and yield of ethanol production [4]. Then, it is essential that the method used in the lignocellulosic material pretreatment be efficient, performs the biomass fiber hydrolysis, releasing the fermentable sugars and generating as less inhibitors as possible.

Research has shown that pretreatment with phosphoric acid diluted in concentrations between 2 and 6% (v/v), at low temperatures and prolonged times, is effective in lignocellulosic biomass saccharification from different materials [8]; Vasconselos et al. 2013; [9].

Thus, this study aims to optimize the acid pretreatment process of sugarcane biomass using diluted phosphoric acid, to obtain a lignocellulosic hydrolyzate with high concentrations of fermentable sugars and the lowest possible concentration of inhibitor. Central Composite Design (CCD) will be applied to assess the effects of acid concentration, temperature variation and operating time.

2. Material and methods

All The sugarcane biomass was kindly provided by sugarcane refinery Jalles Machado S.A, Goias, Brazil. The biomass *in natura* is composed of 39.8 ± 1.0% cellulose, 27.0 ± 1.2% hemicellulose, 23.5 ± 1.1% lignin, 8.4 ± 0.7% extractives and 1.4 ± 1.2% ash. Carbohydrates and lignin content were determined according to NREL procedure [10].

2.1. Central composite design

Central Composite Design (CCD) was used to study the effects of the acid pretreatment method parameters in the sugarcane biomass to obtain a lignocellulosic hydrolyzate with high concentrations of fermentable sugars and low concentration of inhibitors. Thus, a CCD with three variables and three replicates at the center point was applied to optimize the concentration of total monosaccharides in lignocellulosic hydrolyzate.

The independent variables considered were selected as follows: temperature from 63.2 to 96.8 °C (X1), operating time from 5.0 to 375 min (X2) and H_3PO_4 concentration from 1.0 to 6.0% (X3). The alpha for orthogonality used was 1.68. The model used to estimate the response surface was the quadratic polynomial.

2.2. Experimental procedure

The tests were carried out in a 1:15 ratio (biomass (g):acidic solution (mL)). In a 100 mL reagent bottle, 3.3 g of sugarcane biomass were weighed, 50 mL of acid solution were added, followed by heat treatment in a thermostatic bath under the CCD.

After the operating time, the reaction was stopped with an ice bath. The mixture (bagasse + acid hydrolyzate) was filtered in a vacuum system using glass fiber filter. The acid hydrolyzate was stored for analysis by High Performance Liquid Chromatography (HPLC).

2.3. Statistical analysis

The statistical analysis of the results presented in the experiments were made using the software STATISTICA 7.0. A significance level of 95% ($p < 0.05$) was considered [11]. The experiments were carried out under the conditions predicted by models in order to validate them.

2.4. Physical–chemical analysis

The samples were centrifuged at 14,000 rpm for 10 min to separate the biomass residue from the supernatant.

Monosaccharide sugars and inhibitors metabolites were quantified by HPLC (Agilent-1260 Infinity) coupled with an index detector RID 10-A using a reverse-phase column (Aminex® HPX87H, BioRad). Products were eluted using a solution of 5 mM H_2SO_4 as mobile phase at 0.6 mL/min flow rate and 45 °C. Retention times (in min) were approximately: cellobiose 7.3, glucose 9.0, xylose 9.6, arabinose 10.6, acetic acid 15.2, HMF 33.8 and furfural 51.8. The compounds concentrations were determined by using calibration curves and values were calculated from peak areas.

From the results obtained by HPLC analysis, the saccharification percentage was calculated using Eq. (1), with polysaccharide to monosaccharide conversion factor of 0.9, defined by Mandels and Sternberg [12].

$$\%\text{Saccharification} = (\text{Reducing sugar [g/L]} / \text{Initial substrate concentration [g/L]}) \times 0.9 \times 100\% \quad (1)$$

3. Results and discussions

In order to know the optimal acid pretreatment parameters, it was necessary to perform a Central Composite Planning (CCP). Thus, tests were conducted by varying the H_3PO_4 concentration, temperature and operating time. The results of monosaccharides and inhibitors concentration are shown in Table 1. Analysis of the acid hydrolyzates resulting from the experiments carried out showed absence of cellobiose, HMF and furfural in all tested conditions.

Table 1. Results of the monosaccharides and inhibitor obtained from the central composite planning (three replicates at the central point).

Tests	Monosaccharides			Inhibitor
	Glucose [g/L]	Xylose [g/L]	Arabinose [g/L]	Acetic acid [g/L]
1	20.28	0.46	0.21	0.18
2	47.96	0.37	0.26	0.22
3	20.90	0.37	0.49	0.30
4	48.67	0.37	0.53	0.50
5	21.02	0.35	0.17	0.15
6	47.77	0.33	0.20	0.18
7	20.62	0.39	0.71	0.50
8	47.29	0.54	0.91	0.97
9	35.24	0.40	0.29	0.25
10	36.19	0.55	0.89	0.74
11	34.56	0.35	0.03	0.11
12	33.82	0.46	0.94	0.87
13	12.39	0.42	0.54	0.29
14	21.14	0.40	0.68	0.63
15 (C)	34.14	0.36	0.73	0.73
16 (C)	34.06	0.39	0.65	0.52
17 (C)	34.35	0.37	0.73	0.52

The regression equation to monosaccharides concentration (mono), eliminating insignificant terms at 95% confidence level in coded values is given in Eq. (2) ($R^2 = 0.8$).

$$\text{Mono [g/L]} = 34.9 + 0.4X_2 + 18.2X_3 + 3.6{X_1}^2 + 2.4{X_2}^2 - 9.9{X_3}^2 \quad (2)$$

where, X_1 corresponds to temperature, X_2 to operating time and X_3 to H_3PO_4 concentration.

The analysis of variance (ANOVA) for the model is given in Table 2 at 95% confidence level. It can be indicated that the models are statistically significant and the regression could be used for predictive purposes, because F ratio is greater than one [13]. The calculated F value is greater than the tabulated F, indicating that the lack of fit is not significant. It is possible to use response optimization techniques in the evaluated ranges because the pure error value was low for all responses, indicating the good reproducibility of the obtained data.

Table 2. Analysis of CCD regression and variance, considering a significance level of 95%.

	SS[a]	DF[b]	RM[c]	Fcalc[d]	Ftab[e]		Fcalc/Ftab
Regression	1579.78	5	315.96	6.89	**F(5,11)**	3.20	2.15
Residue	504.26	11	45.84				
Lack of fit	504.19	9	56.02	1723.73	**F(9,2)**	19.38	88.94
Pure error	0.07	2	0.03				

[a]SS: sum of squares.

[b]DF: degrees of freedom.

[c]RM: root mean square.

[d]Fcalc: F calculated.

[e]Ftab: F tabulated.

In order to verify the nature of the stationary point, the model adjustments in canonical form and eigenvalues were determined from the model complete equation. In this case, the eigenvalues showed negative and positive values simultaneously, indicating a saddle point, which means that the optimum points of the model cannot be found. Therefore, algorithm optimization was used by a non-linear iterative numerical solution which returns the maximum value that the function assumes in the evaluated range.

For the non-linear iterative numerical solution optimization algorithm, monosaccharides concentration is maximized after acid pretreatment with H_3PO_4 concentration of 4.95%, at 80 °C for 375 min, resulting a predicted monosaccharides concentration value of 50.6 g/L. The samples were centrifuged at 14,000 rpm for 10 min to separate the biomass residue from the supernatant.

To validate the conditions of maximization, pretreatment was carried out, in triplicate, in a 100 mL reagent bottle containing 3.3 g of sugarcane biomass and 50 mL of H_3PO_4 solution 4.95%, and the operation occurred at 80 °C for 375 min. The reaction was stopped with an ice bath. The mixture (bagasse + acid hydrolyzate) was filtered in a vacuum system using filter paper.

The pretreatment produced an acid hydrolyzate with 48.7 ± 0.8 g/L of monosaccharides (98.0% glucose, 1.2% xylose and 0.8% arabinose) and 0.4 ± 0.1 g/L of acetic acid, as shown in Fig. 1. The results obtained experimentally and the small deviation from the predicted value confirm the quality of the model fit.

Applying the parameters optimized by the CCD in the sugarcane biomass acid pretreatment of using diluted H_3PO_4, a 99.3% of saccharification was achieved.

Vasconcelos et al. [14] studied the sugarcane biomass pretreatment in a 20 L batch rotary reactor, with diluted H_3PO_4 with concentration varying from 0.05 to 0.20% (w/w), time from 8 to 24 min and temperature between 144 and 186 °C. The maximum saccharification found was 56.4% using H_3PO_4 0.2%, at 186 °C for 8 min. Another study using diluted H_3PO_4 in the sugarcane biomass pretreatment reached 88% saccharification with acid concentration of 1.0% (w/w) at 180 °C for 60 min [9]. Wang et al. [8] used concentrated H_3PO_4 (80% w/w) and diluted hydrogen peroxide (1.8% w/w) for 300 min, at 50 °C in the wheat straw and softwood (spruce chips) pretreatment achieving a saccharification of 95 and 100%, respectively.

Pretreatment with diluted phosphoric acid generated low levels of inhibitors due to minimal side reactions from sugars, resulting in an acid hydrolyzate with absence of HMF and furfural, and with low and tolerant levels of acetic acid for yeasts, for example, *Saccharomyces cerevisiae* [4].

4. Conclusion

In the present work, diluted phosphoric acid was used in the pretreatment of sugarcane biomass, having achieved a high efficiency in the saccharification of this biomass. Acid hydrolysis with phosphoric acid 4.95% (v/v), at 80 °C

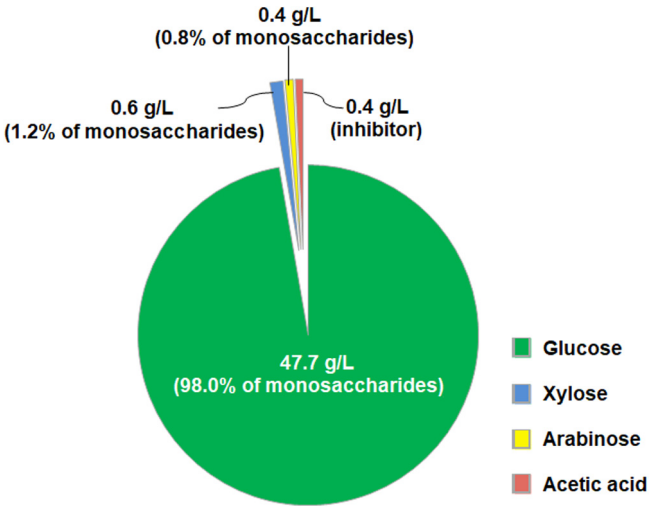

Fig. 1. Concentration of monosaccharides and acetic acid in the hydrolyzate after pre-treatment of sugarcane biomass using phosphoric acid, under conditions optimized by the Central Composite Design.

for 375 min, resulted in a hemicellulose hydrolyzate rich in fermentable sugars (98% glucose) and with low levels of inhibitors. In addition, the phosphoric acid present in the hydrolyzate has adequate concentrations to supply the need for phosphorus by yeast during alcoholic fermentation for ethanol production.

CRediT authorship contribution statement

Wilson G. Morais Junior: Investigation, Formal analysis, Data curation, Validation, Writing - original draft, Project administration. **Thályta F. Pacheco:** Writing, Formal analysis, Data curation, Validation - review & editing. **Priscila S. Corrêa:** Data curation, Writing - review & editing. **António A. Martins:** Review. **Teresa M. Mata:** Writing - review & editing. **Nídia S. Caetano:** Conceptualization, Supervision, Writing - review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank sugarcane refinery Jalles Machado S.A for the sugarcane biomass donation, and to Thályta Fraga Pacheco for the statistical analysis consulting.

Funding

This research is part of the project titled ProEMiBiL supported and financially by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie funding [grant number 867473]; This work was supported by: UID/EQU/00305/2020 – Center for Innovation in Engineering and Industrial Technology — CIETI, Portugal; Base funding – UIDB/00511/2020 of the Laboratory for Process Engineering, Environment, Biotechnology and Energy — LEPABE, Portugal – funded by national funds, Portugal through the FCT/MCTES (PIDDAC).

References

[1] Oliva JM, Negro MJ, Manzanares P, Ballesteros I, Chamorro MA, Sáez F, et al. A sequential steam explosion and reactive extrusion pretreatment for lignocellulosic conversion within a fermentation-based biorrefinery perspective. Ferment 2017;3(2):15. http://dx.doi.org/10.3390/fermentation3020015.

[2] Mata TM, Tavares TF, Meireles S, Caetano NS. Bioethanol from brewers' spent grain: Pentose fermentation. Chem Eng Trans 2015;43:241–6. http://dx.doi.org/10.3303/CET154304.

[3] Caetano NS, Moura RF, Meireles S, Mendes AM, Mata TM. Bioethanol from brewer's spent grains: Acid pretreatment optimization. Chem Eng Trans 2013;35:1021–6. http://dx.doi.org/10.3303/CET1335170.

[4] Almeida JRM, Modig T, Petersson A, Hähn-Hägerdal B, Lidén G, Gorwa-Grauslund MF. Increased tolerance and conversion of inhibitors in lignocellulosic hydrolysates by *Saccharomyces cerevisiae*. J Chem Technol Biotechnol 2007;82:340–9. http://dx.doi.org/10.1002/jctb.1676.

[5] Rezende CA, Lima MA, Maziero P, Azevedo ER, Garcia W, Polikarpov I. Chemical and morphological characterization of sugarcane bagasse submitted to delignification process for enhanced enzymatic digestibility. Biotechnol Biofuel 2011;4:56. http://dx.doi.org/10.1186/1754-6834-4-54.

[6] Kumar AK, Sharma S. Recent updates on different methods of pretreatment of lignocellulosic feedstocks: A review. Bioprocess 2017;4:7. http://dx.doi.org/10.1186/s40643-017-0137-9.

[7] Palmqvist E, Hähn-Hägerdal B. Fermentation of lignocellulosic hydrolysates I: Inhibition and detoxification. Bioresour Technol 2000;74:17–24. http://dx.doi.org/10.1016/S0960-8524(99)00160-1.

[8] Wang Q, Wang Z, Shen F, Hu J, Sun F, Lin L, et al. Pretreating lignocellulosic biomass by the concentrated phosphoric acid plus hydrogen peroxide (PHP) for enzymatic hydrolysis: Evaluating the pretreatment flexibility on feedstocks and particle sizes. Bioresour Technol 2014;166:420–8. http://dx.doi.org/10.1016/j.biortech.2014.05.088.

[9] Geddes CC, Peterson JJ, Roslander C, Zacchi G, Mullinnix MT, Shanmugam KT, et al. Optimizing the saccharification of sugar cane bagasse using dilute phosphoric acid followed by fungal cellulases. Bioresour Technol 2010;101:1851–7. http://dx.doi.org/10.1016/j.biortech.2009.09.070.

[10] Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, et al. NREL/TP-510-42618 analytical procedure – Determination of structural carbohydrates and lignin in biomass. Lab Anal Proced 2012;17. https://doi.org/NREL/TP-510-42618.

[11] Haaland PD. Experimental design in biotechnology. New York: Marcel Dekker Inc; 1989, http://dx.doi.org/10.1002/ep.670110307.

[12] Mandels M, Sternberg D. Recent advances in cellulase technology. J Ferment Technol 1976;54:267–86.

[13] Box GEP, Hunter WG, Hunter JS. Statistics for experimenters. An introduction to design, data analysis and model building. New York: Wiley; 1978.

[14] Vasconcelos SM, Santos AMP, Rocha GJM, Souto-Maior AM. Diluted phosphoric acid pretreatment for production of fermentable sugars in a sugarcane-based biorefinery. Bioresour Technol 2013;135:46–52. http://dx.doi.org/10.1016/j.biortech.2012.10.083.