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XYLOSE PRODUCTION BY DILUTE ACID PRETREATMENT OF SUGARCANE LEAVES WITH MINIMUM SUGAR DEGRADATION PRODUCTS

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ABSTRACT: Xylose is a monosaccharide produced from biomass by acid pretreatment that can be converted into several by-products of value-added. Using response surface methodology, this study evaluated the acid pretreatment of sugarcane leaves to obtain a high xylose yield with a minimum generation of furfural (degradation product). The studied variables were: sulfuric acid concentration, reaction time, and solid/liquid rate in a study region 1 (10-50% m/m of acid, 20-60 min, 32-8% m/v), and a study region 2 (50-70% m/m of acid, 20-50 min, 3-7% m/v). The sugarcane leaves present 37.47% of cellulose, 29.69% of hemicellulose, and 12.24% of lignin. Maximum xylose production (52.82%) was observed with a minimum generation of furfural (1.7%), applying specific conditions of pretreatment containing acid (50% mass/mass - m/m, or 1% mass/vol - m/v), reaction time (60 min) and solid rate/liquid (8% m/v). The highest xylose yields (above 40%) occurred with the application of higher acid concentrations (above 1% m/v or 50% m/m), the most important variable observed in this study. This study pointed to process conditions for xylose production, and such parameters that can be used in the process of obtaining high added-value products by xylose conversion.

Keywords: sugarcane, biomass, pretreatment, xylose, chemical composition, fermentable sugar.

PRODUÇÃO DE AÇÚCARES FERMENTÁVEIS POR PRÉ-TRATAMENTO ÁCIDO DILUTO DE FOLHAS DE CANA-DE-AÇÚCAR COM MÍNIMA DEGRADAÇÃO DE AÇÚCAR

RESUMO: A xilose é um monossacarídeo produzido a partir da biomassa por pré-tratamento ácido que pode ser convertido em diversos subprodutos de valor agregado. Utilizando a metodologia de superfície de resposta, este estudo avaliou o pré-tratamento ácido de folhas de cana-de-açúcar para obter um alto rendimento de xilose com mínima geração de furfural (produto de degradação). As variáveis estudadas foram: concentração de ácido sulfúrico, tempo de reação e taxa sólido/líquido em uma região de estudo 1 (10-50% m/m de ácido, 20-60 min, 32-8% m/v), e região de estudo 2 (50-70% m/m de ácido, 20-50 min, 3-7% m/v). As folhas da cana-de-açúcar apresentam 37,47% de celulose, 29,69% de hemicelulose e 12,24% de lignina. Produção máxima de xilose (52,82%) foi observada com geração mínima de furfural (1,7%), aplicando condições específicas de pré-tratamento contendo ácido (50% m/m massa/massa ou 1% m/v massa/volume), tempo de reação (60 min) e taxa de sólidos/líquido (8% m/v). Os maiores rendimentos de xilose (acima de 40%) ocorreram com a aplicação de maiores concentrações de ácido (acima de 1% m/v ou 50% m/m), a variável mais importante observada neste estudo. Este estudo apontou condições de processo para produção de xilose e parâmetros que podem ser utilizados no processo de obtenção de produtos de valor agregado pela conversão de xilose.

Palavras-chave: cana-de-açúcar, biomassa, pré-tratamento, xilose, composição química, açúcar fermentável.

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1 INTRODUCTION

Sugarcane leaves are left in the plantation fields after harvesting. This biomass is an important source due to its chemical composition rich in polysaccharides and presented a high calorific value (3500-4196 Kcal/Kg) (PATIL, 2017). Usually, leaves are burnt in sugarcane crops before sugarcane harvest, which causes damage to soil microbiota. However, in Brazil, the Law number 11.241/2002 regulates the controlled burning of sugarcane in the plantation fields, making this residue a huge amount. The use of sugarcane straw for the production of several by-products instead of being burned presents itself as better use of this natural resource.

Dry leaves, fresh leaves, and cane tops are described as sugarcane straw since their chemical composition is similar to the sugarcane bagasse (SZCZERBOWSKI *et al*., 2014). Between 2017/2018, only in Brazil were generated 90.7 million tons of sugarcane straw in plantation fields (BATISTA *et al*., 2019). Due to its composition and the amount of residue available, sugarcane leaves represent a significant source to obtaining products derived from xylose, since this biomass has 20-22% of xylan (FORSAN *et al*., 2021b; MENANDRO *et al*., 2017). Moreover, xylan is a polysaccharide focused on several studies such as biopolymer (MELATI *et al*., 2023; MARTINS *et al*., 2021; MELATI *et al*., 2021; FELIPUCI *et al*., 2021; ALVES *et al*., 2021), xylose production (FERNANDES *et al*., 2020), xylooligosaccharides (FREITAS *et al*., 2021), and xylan applied for biomaterials production (ABE *et al*., 2022; ABE, BRANCIFORTI, BRIENZO, 2021).

Xylose can be converted into by-products through microbial fermentation, such as ethanol, butanol, lactic acid, xylonic acid, succinic acid. In addition to xylose fermentation, other products can be obtained by different chemical processes such as xylitol, furfural, and levulinic acid. Therefore, xylose is a feedstock to obtain various products of industrial interest beyond biofuels, solvents, and drugs. Lignocellulosic biomass pretreatments can be chemicals, physicals, physicochemical, and biologicals (MELATI *et al*., 2019). Between chemical pretreatments, dilute acid pretreatment is widely used due to less cost and easy applicability. The diluted acid pretreatment has been studied among different sugarcane biomasses (MIYAMOTO *et al*., 2018; MESA *et al*., 2017).

Diluted acid pretreatment is an efficient method for hemicellulose solubilization with rich xylose yield (BRIENZO *et al*., 2017; BETANCUR *et al*., 2010). With optimized conditions, acid pretreatment can be applied for xylooligosaccharides production (FORSAN et al., 2021a). However, pretreatment acid conditions of lignocellulosic material can cause the formation of fermentation inhibitors, such as furfural, hydroxymethylfurfural, and acetic acid (SCHMATZ; TYHODA; BRIENZO, 2020; SANT'ANNA; SOUZA; BRIENZO, 2014). In this context, acid pretreatment is recognized as less attractive if generates a large amount of furfural, an important inhibitor of fermentable processes (CANDIDO *et al*., 2020; KAPOOR *et al*., 2017). Despite that, it is possible to look for conditions where there is minimal furfural formation with high xylose yield applying diluted acid pretreatment.

For an industrial application is necessary a high xylose yield with a minimum of degradation products to have a feasible microbial process. Studies involving anatomical regions of sugarcane biomass generally use stem, external fraction, and straw as raw material. However, in this study, the sugarcane leaves biomass was evaluated as a raw material for obtaining xylose, avoiding the generation of furfural. Therefore, different diluted acid pretreatment conditions were applied, evaluating variables such as sulfuric acid concentration, solid/liquid ratio, and reaction time. The results obtained demonstrate high xylose yield and low furfural generation, which possibly indicates that the sugarcane leaves biomass is a viable option for future studies involving the conversion of this biomass to obtain products with high added value. The process was evaluated based on variables such as a solid/liquid ratio (m/v), reaction time (min), and acid concentration (% m/m or m/v).

2 MATERIAL AND METHODS

2.1 Biomass and sample preparation

The sugarcane biomass used in this study comes from the city of Duque de Caxias, State of Rio de Janeiro. To obtain the biomass, the leaves were manually removed from the sugarcane culm and cut into small pieces (approximately 0.5 cm). The leaves pieces were dried in an oven at 45 °C for 48 h, ground and selected material passed through a 20 mesh sieve for particle size reduction using a Willey Knife Mill Macro (Marconi) (FERNANDES *et al*., 2020). The milled material was stored in screw-capped plastic tubes.

2.2 Chemical characterization

In duplicates, 1 g of the sample was weighed into sachets made of filter paper and then placed in a Soxhlet extraction tube and extractor flask, 190 mL of 95% ethanol was added. The heating was adjusted to 4 to 5 siphonings/h for 8 h. The sample was subjected to a new extraction, using deionized water for 8 h. At the end of the process, the sample was oven-dried at 105 °C for 12 h. For chemical characterization, 0.3 g of ground leaves extractive-free were placed in borosilicate reaction tubes. The tubes were heated in a 30 °C water bath by adding 3 mL of 72% (m/m) sulfuric acid in each tube and mixed every 10 minutes for 1 h. The reaction was diluted by adding 84 mL of distilled water. The tubes were closed and autoclaved at 121 ºC for 1 h. The hydrolysate was filtered through a previously tared porous plate crucible. To insoluble lignin, the filter-retained material was washed with distilled water and dried at 105 °C until constant mass, to lignin content determination. Additionally, it was possible to quantify the soluble lignin by filtration from acid hydrolysis. The samples were diluted in 1:6 ratio and analyzed at 240 nm wave on the TECAN Infinite 200 Pro Spectrophotometer. Carbohydrate quantifications were elaborated using a High Performance Liquid Chromatography (HPLC - Shimadzu) with a refractive index detector (RID) and column Aminex Biorad HPX 87H. The mobile phase used was a solution of sulfuric acid 5 mmol/L, with a flow of 0.6 mL/min and oven temperature of 60 ºC.

2.3 Acid pretreatment

Diluted acid pretreatment reactions were elaborated using a Schott flask in an autoclave at 121 °C/1 atm. After the reaction time, the hydrolysates were filtered with filter paper and collected approximately 10 mL for further analysis. The solid materials retained on the filter paper were washed thoroughly with distilled water until reached a pH close to 5. The solid materials were oven-dried at 45 °C for 48 h and then weighed. The liquid fractions were filtered using 5 mL syringes and 0.22 µm filters (Millex) for analysis of sugars and potential fermentation inhibitors by (HPLC).

The variables such as sulfuric acid concentration (%, mass per mass m/m), reaction time (minutes), and solid/liquid ratio (%, mass per volume m/v) in the pretreatment were analyzed by a factorial design 2^3 with central points in the study region 1, varying the sulfuric acid concentration (10% to 50% m/m, mass of acid per mass of material; or 0.2 to 1% m/v), reaction time (20 min to 60 min) and mass/volume relation (2% to 8%, m/v), and as a variable response the percentage of hydrolyzed hemicellulose (xylose released). The study region 2 was investigated with a factorial design 2^3 with star points with variables value of sulfuric acid concentration (50% to 70% m/m, mass of acid per mass of material, or 1% to 1.4% m/v), reaction time (10 to 70 min) and mass/volume relation (3% to 8% m/v). These acid concentrations range and were based on a study dedicated to bioethanol production (MESA *et al*., 2017). However, with lower temperature and long reaction time to avoid degradation products.

The xylose yield was calculated based on the amount of xylose released in relation to the hemicellulose content in the material. All statistical analyses of this study were performed using the "Statistica 8" software, considering 95% of confidence level.

2.4 Furfural and sugars quantifications

Sugars were quantified by liquid chromatography (Shimadzu) (HPLC) using a refractive index detector (RID) with the Aminex Biorad HPX 87H column. The mobile phase was composed of sulfuric acid 5 mmol/L, with a flow rate of 0.6 mL/min at 60 °C.

Furfural was quantified using an HPLC (Shimadzu) with diode array detector (DAD), and the column used was C18. The mobile phase used a water/methanol (8:1) solution, with a flow rate of 0.8 mL/min, injection volume of 10 μL, oven temperature 30 ºC for 10 min.

3 RESULTS AND DISCUSSION

3.1 Chemical composition

Xylose is the second most abundant sugar in nature after glucose (LEE *et al*. 2015) and can be used as a carbon source in microbial fermentation. In this way, xylose can be converted into several by-products, among which are arabitol, erythritol, 2,3-butanediol, isobutanol, citric acid, lactic acid, isoprenoids, carotenoids, and fatty alcohol (JAGTAP; RAO, 2018). All of these products from xylose are of great importance to the industrial sector. Due to this context of the generation of high added value by-products, this study was dedicated to maximum xylose yield through a low-cost process that is easy to apply and reproducible.

Biomass leaves showed 37.47% of cellulose, 29.69% of hemicellulose and 12.24% of lignin, and 11.89% of extractives (Table 1). Compared to other anatomic regions of sugarcane biomass, the hemicellulose composition of sugarcane biomass leaves (29.69%) is close to node (10.82 - 27.67%), internode (9.46 - 29.88%), and the external fraction of the culm (9.71 - 16.07%) (BRIENZO *et al*., 2015). However, cellulose composition observed in this study (37.47 %) is bellow observed in the literature to node (42.26 - 46%), internode (44.67 - 51.49 %), and epidermis (50.12 - 55.01%), as well as the composition of lignin which is also below that observed for the node (22.10 - 32.21%), although is between the results related to the external fraction (9.71 - 13.7%) and internode regions (9.46 - 29.88%) of sugarcane biomass (BRIENZO *et al*., 2014).

Sugarcane leaves were reported with a chemical composition containing 36 - 37 % of cellulose, 28 - 30% of hemicellulose, and 20 - 22% of lignin (MENANDRO *et al*., 2017). However, sugarcane straw presents 36.91% of cellulose, 19.73% of hemicellulose, and 13.51% of lignin. Both have values close to cellulose, but the hemicellulose and lignin content is greater in sugarcane leaves than in sugarcane straw. In this study, the chemical composition of sugarcane leaves is close to the data described in the literature, but with variations due to influences of climate, soil, planting, and harvesting method, among others.

Table 1. Chemical composition (%) of sugarcane leaves

Chemical Composition (%)	Dry leaves biomass
Cellulose	37.47 ± 0.23
Hemicellulose	29.69 ± 1.80
Total lignin	12.24 ± 0.59
Extractives	11.89 ± 0.80
Total	91.29

3.2 Xylose yield

Pretreatment of sugarcane leaves resulted in greater xylose yield (52.82%) with assay 8, where more severe conditions such as acid concentration 50% (m/m), a reaction time of 60 min, and solid/liquid ratio equal to 8% (Table 2). The effect of sulfuric acid concentration demonstrated that the use of higher concentrations was able to increase or decrease xylose. Likewise, xylose extraction was higher in cases with longer reaction time (60 min) and higher solid/liquid ratio (8%) were used, compared with the results obtained with shorter reaction time (20 min) and lowest solid/liquid ratio (2%) (Table 2). Xylose is the main product of the dilute acid hydrolysis, however, it can be found in minor amount arabinose, glucose, and cellobiose.

Table 2. Xylose yield and pretreatment conditions to sugarcane leaves biomass in the study region 1

Experiment	H_2SO_4 concentration (% m/m or m/v) #	Reaction time (min)	Solid/Liquid (% m/v)	Xylose yield (%)	Furfural (%)
1	10 (0.2)#	20	2	5.10	0.00
2	50 (1)	20	2	28.07	0.21
3	10 (0.2)	60	2	8.94	0.07
4	50 (1)	60	2	33.24	0.20
5	10 (0.2)	20	8	10.51	0.03
6	50 (1)	20	8	35.31	1.39
7	10 (0.2)	60	8	18.11	0.05
8	50 (1)	60	8	52.82	1.70
9*	30 (0.6)	40	5	15.10	0.06
9*	30 (0.6)	40	5	19.37	0.08
9*	30 (0.6)	40	5	17.66	0.06

* Central points performed in triplicate; other experiments in duplicate; (#) number in parenthesis represents the acid percentage considering mass of acid per volume of reaction (%, m/v).

The study region was characterized by different experimental condition, region 1 (Figure 1) and region 2 (Figure 2). The highest xylose yield in the acid pretreatment of the leaves occurred under maximum level conditions (50% m/m acid concentration, 60 min reaction time, and 8% solid/liquid ratio) (Figure 2). The response surface for xylose percentage through the interaction between acid concentration and reaction time (Figure 1-A and 2-A) showed that at higher the acid concentrations and longer reaction times, the greater the xylose yield. The interaction between acid concentration and solid/liquid ratio (Figure 1-B and 2-B) showed that both higher solid-liquid ratios and acid concentrations promoted high xylose yields. The interaction between the reaction time and the solid/liquid ratio (Figure 1-C and 2-C) demonstrated that better xylose yields are obtained when the highest values for these two variables were used to both 1 and 2 study regions.

The concentration levels of sulfuric acid were increased from 10, 30, and 50% to 50, 60, and 70% m/m, in the minimum level, central point, and maximum level, successively. Considering that the acid concentration had the values increased, it was decided to decrease the reaction time to avoid the degradation of the material. The solid/liquid ratio was readjusted, with its minimum, central and maximum levels adjusted from 2, 5, and 8% to 3, 5, and 7% (Table 3). The xylose yields were higher than those found in study region 1.

The most expressive result obtained in the optimization was observed in assay 4 (49.83%), conducted with the maximum values of acid concentration (50% m/m) and reaction time (40 min) and, the minimum value for the solid/liquid ratio (3%). This result was slightly below that observed in study region 1 (52.82%). The lowest yield was observed in assay 1 (20.71%), where was used minimum values of all variables: acid concentration 50% m/m, reaction time of 20 min, and solid/liquid ratio equal to 3% (Table 3).

Table 3. Xylose yield and pretreatment conditions to sugarcane leaves biomass in the study region 2

Experiment	H_2SO_4 concentration (% m/m or m/v)#	Reaction time (min)	Solid/Liquid (% m/v)	Xylose yield (%)	Furfural (%)
1	50 (1)#	20	3	20.71	0.13
2	70 (1.4)	20	3	48.08	0.56
3	50 (1)	50	3	40.86	0.45
4	70 (1.4)	50	3	49.83	0.93
5	50 (1)	20	7	37.04	0.39
6	70 (1.4)	20	7	42.00	0.96
7	50 (1)	50	7	45.25	0.78
8	70 (1.4)	50	7	42.88	2.00
9	70 (1.4)	35	5	37.01	0.00
10	50 (1)	35	5	30.09	0.00
11	60 (1.2)	50	5	35.02	0.00
12	60 (1.2)	20	5	33.82	0.00
13	60 (1.2)	35	7	40.63	0.19
14	60 (1.2)	35	3	32.38	0.00
15*	60 (1.2)	35	5	43.87	1.21
16*	60 (1.2)	35	5	42.72	1.24
17*	60 (1.2)	35	5	41.56	1.23

* Central points performed in triplicate; other experiments in duplicate; (#) number in parenthesis represents the acid percentage considering mass of acid per volume of reaction (%, m/v).

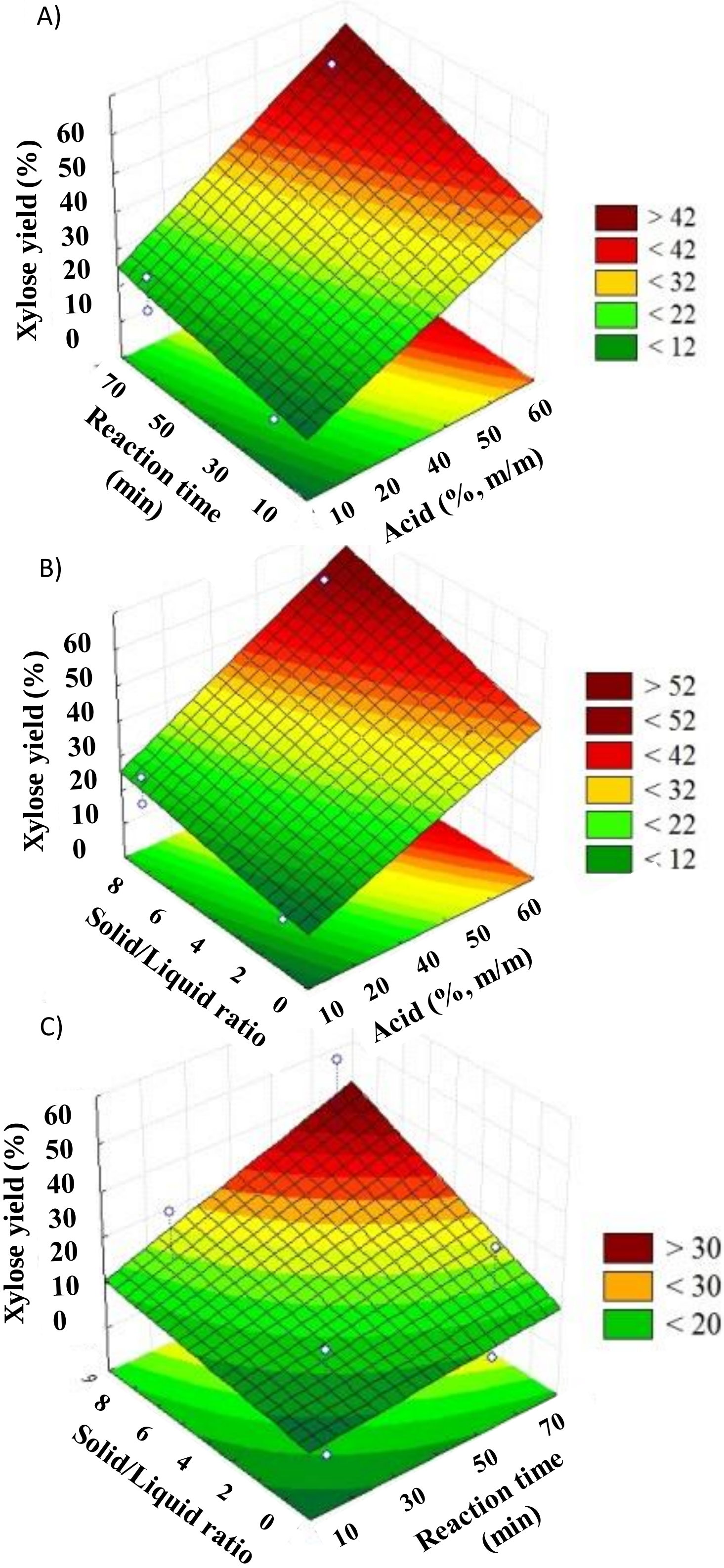
To sugarcane leaves, the optimal conditions to xylose yield described in the literature were 30 min of reaction time, 2.9% (m/v) of sulfuric acid and 1:4 solid/liquid ratio at 130 ºC (MOUTTA *et al*., 2011), and 0.5–5.0% (v/v) of acid concentration, 60–100 ºC of reaction temperature, 30–50 % (m/v) of solid/liquid ration and 60–240 min of reaction time (MOODLEY; KANA, 2018), whereas in this study were observed conditions such as 60 min of reaction time, 50% (m/m) of sulfuric acid and 8% of solid/liquid ratio in the study region 1, and 50 min of reaction time, 70% (m/m) of sulfuric acid and 3 % of solid/liquid ratio, both at 121 ºC.

The highest xylose yield presented in the literature corresponds to 56.8%, since sugarcane straw biomass pretreated by ball milling was the raw material, and also, about 44.9% xylose when the same biomass was pretreated with wet disk milling (SILVA *et al*., 2010). Despite the application of a different pretreatment for the production of xylose from sugarcane leaves, the results obtained in this study were close to those described in the literature.

There are different approaches to lignocellulosic pretreatments, such as chemicals, physicals, physicochemicals, and biologicals (ROBAK; BALCEREK, 2019). In general, there are more than 20 different biomass pretreatment methods, and the dilute acid method is one of the most commonly used chemical pretreatment. However, it is recognized as less attractive to xylose recovery due to the generation of large amounts of inhibitors, such as furfural (KUMAR *et al*., 2017). However, in this study, different conditions were applied to achieve the maximum xylose yield with a minimum generation of furfural through acid dilute pretreatment, taking into account its low cost, which is one of the advantages of more conventional pretreatments.

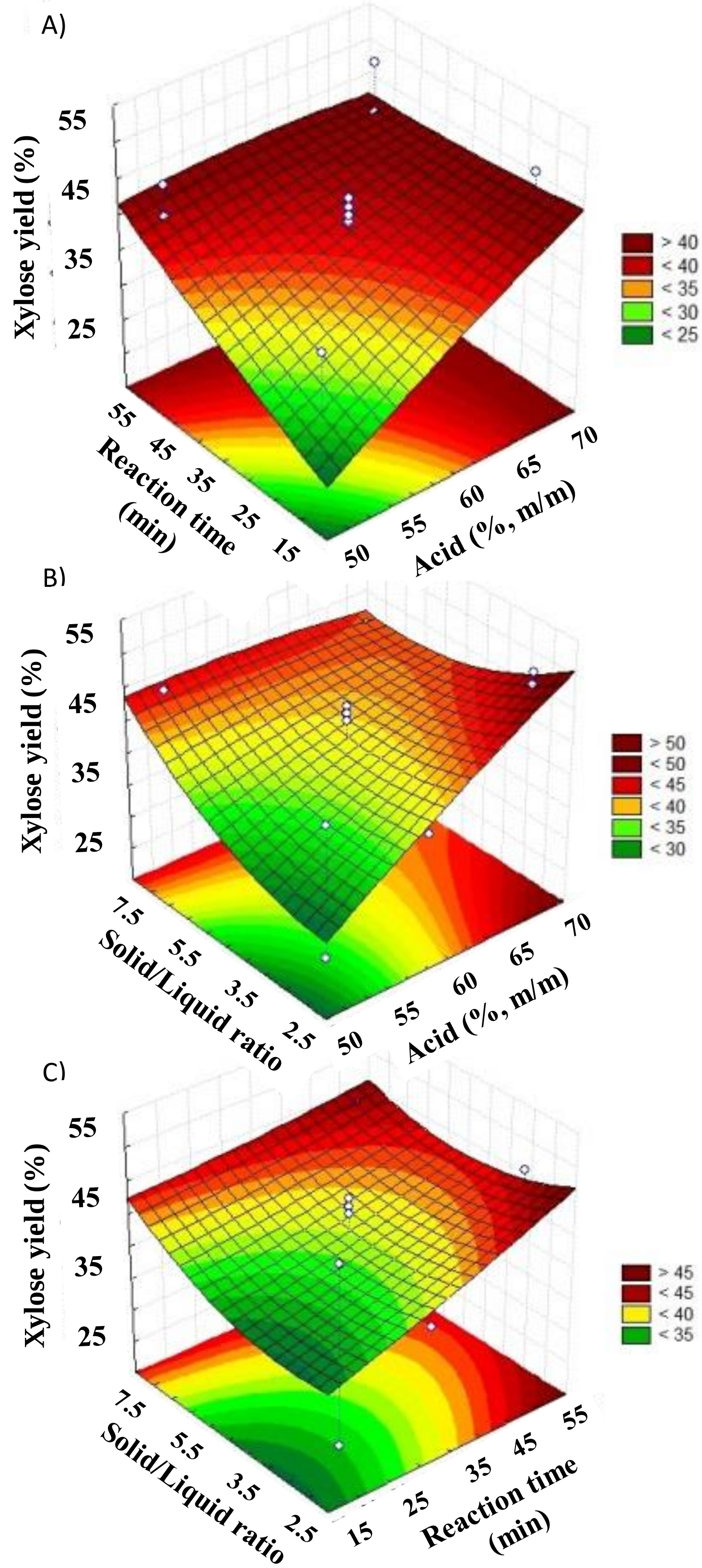
Maximum xylose production (52.82%) observed in this study with a minimum generation of furfural (1.7%), was obtained when applied specific conditions of pretreatment, such as acid (50% m/m or 1% m/v), reaction time (60 min) and solid rate/liquid (8% m/v) at 121 ºC. Therefore, lower temperature and long reaction time avoided the formation of large amounts of degradation products, such as furfural, as previously described in the literature (MESA *et al*., 2017). These experimental conditions possibly contribute to the enzymatic hydrolysis of the pretreated material and subsequent conversion to bioethanol due to the low amounts of inhibitors.

Figure 1. Response surface for xylose percentage after pretreatment of leaves in study region 1



(A) Sulfuric acid concentration and reaction time; (B) Sulfuric acid concentration and solid/liquid ratio; (C) Time and solid/liquid ratio.

Figure 2. Response surface for xylose percentage after pretreatment of leaves in study region 2



(A) Sulfuric acid concentration and reaction time; (B) Sulfuric acid concentration and solid/liquid ratio; (C) Time and solid/liquid ratio.

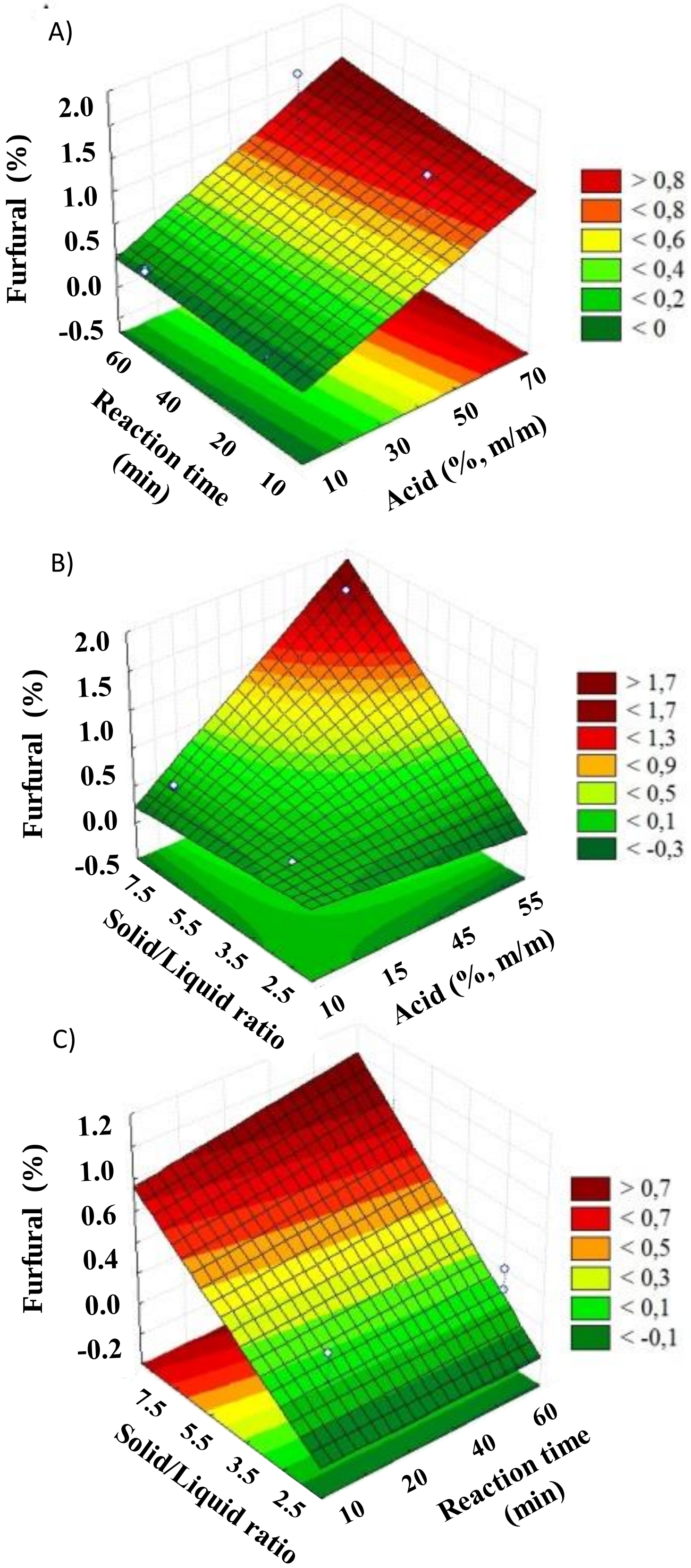
3.3 Furfural production

The assay 8 performed with the maximum severity showed the highest production of furfural from sugarcane leaves (1.70%). The severity applied to the experiment is directly associated with the generation of degradation products. On the other hand, assay 1 did not generate furfural (Table 2).

The interaction between acid concentration and reaction time generated a response surface in which the highest furfural production was observed when higher concentrations of acid and longer reaction time were used (Figure 3-A and 4-A). The highest furfural production was given both in higher reaction times and solid/liquid ratios (Figure 3-B and 4-B). Finally, the highest furfural production was obtained as the experiments were performed in higher reaction times and higher solid/liquid ratios (Figure 3-C and 4-C). In general, the smallest furfural formations were observed in the central region, while the highest in the maximum levels of the studied variables to both 1 and 2 study regions.

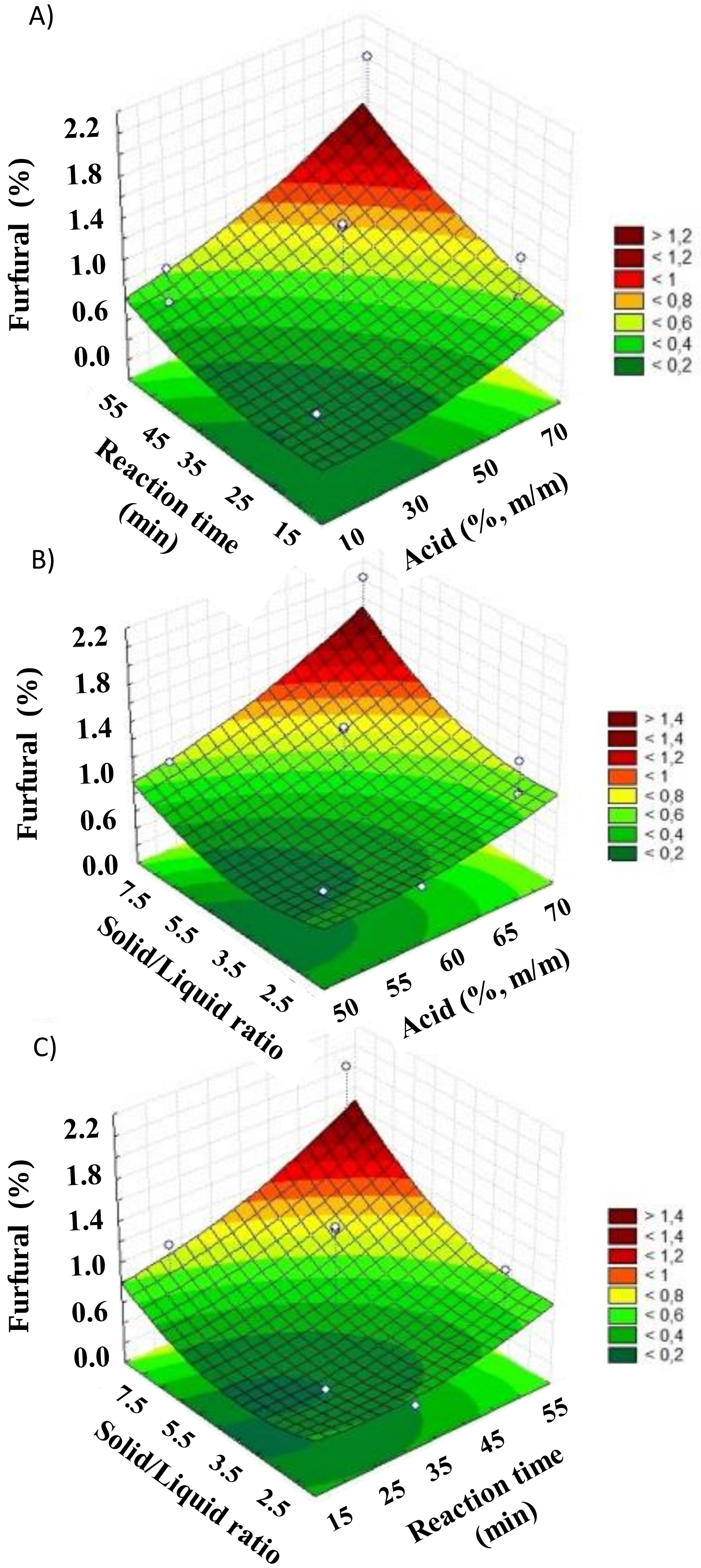
The acid pretreatment conditions of this study led to the formation of low amounts of furfural, different from that observed in the literature. In the best condition to obtain xylose yield (52.82%), 1% m/v of acid concentration was applied to the pretreatment, which led to the formation of 1.7% of furfural, while in the literature, when this same concentration was used of acid in the pretreatment, 22.8 ± 1.1 mg/L of furfural was generated, reaching 36.9 ± 1.8 mg/L when 2% (m/v) of acid concentration was used (HASSAN *et al*., 2021).

Figure 3. Response surface to furfural production after pretreatment of leaves in study region 1.



(A) Sulfuric acid concentration and reaction time; (B) Sulfuric acid concentration and solid/liquid ratio; (C) Time and solid/liquid ratio.

Figure 4. Response surface to furfural production after pretreatment of leaves in study region 2.



(A) Sulfuric acid concentration and reaction time; (B) Sulfuric acid concentration and solid/liquid ratio; (C) Time and solid/liquid ratio.

4 CONCLUSIONS

The state of the novelty of this study is the highest amounts of xylose observed applying optimal conditions of acid concentration, reaction time, and solid/liquid ratio by dilute acid pretreatment of sugarcane leaves (50% m/m or 1% m/v, 60 min, and 8% m/v, respectively). Moreover, a minimal formation of furfural was observed. New perspectives evaluating the sugarcane leaves as biomass are necessary for the production of fermentable sugars, such as xylose, which can contribute to the reduction of the use of high enzymatic charges for the conversion of cellulose and hemicellulose, further reducing the use of chemical compounds applied in different pretreatments for this biomass. In addition, such perspectives are essential for the search for high-quality and low-cost production of value-added products from xylose as feedstock.

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6 REFERENCES

ABE, M. M.; BRANCIFORTI, M. C.; BRIENZO, M. Biodegradation of hemicellulose-cellulose-starch-based bioplastics and microbial polyesters. **Recycling**, Basel, v. 6, n. 1, p. 1-20, 2021. DOI: https://doi.org/10.3390/RECYCLING6010022 . Available at: https://www.mdpi.com/journal/recycling. Accessed on: 22 Jan. 2024.

ABE, M. M.; BRANCIFORTI, M. C.; NALLIN MONTAGNOLLI, R.; MARIN MORALES, M. A.; JACOBUS, A. P.; BRIENZO, M. Production and assessment of the biodegradation and ecotoxicity of xylan- and starch-based bioplastics. **Chemosphere**, Oxford, v. 287, p. 1-21, 2022. DOI: https://doi.org/10.1016/j.chemosphere.2021.132290. Available at: https://www.mdpi.com/2313-4321/6/1/22. Accessed on: 22 Jan. 2024.

ALVES, R. C.; MELATI, R. B.; CASAGRANDE, G. M. S.; CONTIERO, J.; PAGNOCCA, F. C.; BRIENZO, M. Sieving process selects sugarcane bagasse with lower recalcitrance to xylan solubilization. **Journal of Chemical Technology and Biotechnology**, Oxford, v. 96, n. 2, p. 327-334, 2021. DOI: https://doi.org/10.1002/jctb.6541. Available at: https://onlinelibrary.wiley.com/doi/epdf/10.1002/jctb.6541. Accessed on: 22 Jan. 2024.

BATISTA, G.; SOUZA, R. B. A.; PRATTO, B.; SANTOS-ROCHA, M. S. R. dos; CRUZ, A. J. G. Effect of severity factor on the hydrothermal pretreatment of sugarcane straw. **Bioresource technology**, Essex, v. 275, p. 321-327, 2019. DOI: 10.1016/J.BIORTECH.2018.12.073. Available at: ccn.ibict.br/busca.jsf. Accessed on: 22 Jan. 2024.

BETANCUR, G. J. V.; PEREIRA, N. Sugar cane bagasse as feedstock for second generation ethanol production. Part I: Diluted acid pretreatment optimization. Electronic Journal of Biotechnology, Chile, v. 13, n. 3, p. 1-9, 2010. DOI: 10.2225/vol13-issue3-fulltext-3. Available at: https://www.scielo.cl/scielo.php?pid=S0717-34582010000300010&script=sci_arttext&tlng=en. Accessed on: 22 Jan. 2024.

BRIENZO, M.; ABUD, Y.; FERREIRA, S.; CORRALES, R. C. N. R.; LEITÃO, V. S. F.; SOUZA, W.; SANT'ANNA, C. Characterization of anatomy, lignin distribution, and response to pretreatments of sugarcane culm node and internode. **Industrial Crops and Products**, Netherlands, v. 84, p. 305-131, 2014. DOI: https://doi.org/10.1016/j.indcrop.2016.01.039. Available at: https://www.sciencedirect.com/science/article/pii/S0926669016300395. Accessed on: 22 Jan. 2024.

BRIENZO, M.; FIKIZOLO, S.; BENJAMIN, Y.; TYHODA, L.; GÖRGENS, J. Influence of pretreatment severity on structural changes, lignin content and enzymatic hydrolysis of sugarcane bagasse samples. **Renewable Energy**, Oxford, v. 104, p. 271-280, 2017. DOI: https://doi.org/10.1016/j.renene.2016.12.037. Available at: https://www.sciencedirect.com/science/article/pii/S096014811631093X?via%3Dihub. Accessed on: 22 Jan. 2024.

BRIENZO, M.; TYHODA, L.; BENJAMIN, Y.; GÖRGENS, J. Relationship between physicochemical properties and enzymatic hydrolysis of sugarcane bagasse varieties for bioethanol production. **New Biotechnology**, Netherlands, v. 32, n. 2, p. 253-262, 2015. DOI: https://doi.org/10.1016/j.nbt.2014.12.007. Available at: https://www.sciencedirect.com/science/article/pii/S1871678414022079?via%3Dihub. Accessed on: 22 Jan. 2024.

CANDIDO, J. P.; CLARO, E. M. T.; PAULA, C. B. C. de; SHIMIZU, F. L.; LEITE, D. A. N. de O.; BRIENZO, M.; ANGELIS, D. F. de. Detoxification of sugarcane bagasse hydrolysate with different adsorbents to improve the fermentative process. **World Journal of Microbiology and Biotechnology**, Dordrecht, v. 36, n. 3, p. 1-12, 2020. DOI: https://doi.org/10.1007/s11274-020-02820-7. Available at: https://link.springer.com/article/10.1007/s11274-020-02820-7. Accessed on: 22 Jan. 2024.

FELIPUCI, J. P.; SCHMATZ, A. A.; ANGELIS, D. A. de; BRIENZO, M. Biological pretreatment improved subsequent xylan chemical solubilization. **Biomass Conversion and Biorefinery**, Germany, v. 13, p. 5317-5324, 2021. DOI: https://doi.org/10.1007/s13399-021-01506-w. Available at: https://link.springer.com/article/10.1007/s13399-021-01506-w. Accessed on: 22 Jan. 2024.

FERNANDES, É. S.; BUENO, D.; PAGNOCCA, F. C.; BRIENZO, M. Minor Biomass Particle Size for an Efficient Cellulose Accessibility and Enzymatic Hydrolysis. **ChemistrySelect**, Germany, v. 5, n. 25, p. 7627-7631, 2020. DOI: https://doi.org/10.1002/slct.202001008. Available at: https://chemistry-europe.onlinelibrary.wiley.com/doi/10.1002/slct.202001008. Accessed on: 22 Jan. 2024.

FORSAN, C. F.; FREITAS, C.; MASARIN, F.; BRIENZO, M. Xylooligosaccharide production from sugarcane bagasse and leaf using Aspergillus versicolor endoxylanase and diluted acid. **Biomass Conversion and Biorefinery 2021**, Heidelberg, v. 13, p. 3375-3390, 2021a. DOI: 10.1007/S13399-021-01403-2. Available at: https://link.springer.com/article/10.1007/s13399-021-01403-2. Accessed on: 22 Jan. 2024.

FORSAN, C. F.; PAZ CEDEÑO, F. R.; MASARIN, F.; BRIENZO, M. Xylooligosaccharides production by optimized autohydrolysis, sulfuric and acetic acid hydrolysis for minimum sugar degradation production. **Bioactive Carbohydrates and Dietary Fibre**, Amsterdam, v. 26, p. 1-8, 2021b. DOI: https://doi.org/10.1016/j.bcdf.2021.100268. Available at: https://www.sciencedirect.com/science/article/pii/S2212619821000085?via%3Dihub. Accessed on: 22 Jan. 2024.

FREITAS, C.; TERRONE, C.C.; MASARIN, F.; CARMONA, E.C.; BRIENZO, M. In vitro study of the effect of xylooligosaccharides obtained from banana pseudostem xylan by enzymatic hydrolysis on probiotic bacteria. **Biocatalysis and Agricultural Biotechnology**, Amsterdam, v. 33, p. 1-7, 2021. DOI: https://doi.org/10.1016/j.bcab.2021.101973. Available at: https://www.sciencedirect.com/science/article/pii/S1878818121000694. Accessed on: 22 Jan. 2024.

HASSAN, M. K.; CHOWDHURY, R.; GHOSH, S.; MANNA, D.; PAPPINEN, A.; KUITTINEN, S. Energy and environmental impact assessment of Indian rice straw for the production of second-generation bioethanol. **Sustainable Energy Technologies and Assessments**, Amsterdam, v. 47, p. 1-12, 2021. DOI: https://doi.org/10.1016/j.seta.2021.101546. Available at: https://www.sciencedirect.com/science/article/pii/S2213138821005580. Accessed on: 22 Jan. 2024.

JAGTAP, S. S.; RAO, C. V. Microbial conversion of xylose into useful bioproducts. **Applied Microbiology and Biotechnology**, Berlin, v. 102, p. 9015-9036, 2018. DOI: https://doi.org/10.1007/s00253-018-9294-9. Available at: https://link.springer.com/article/10.1007/s00253-018-9294-9. Accessed on: 22 Jan. 2024.

KAPOOR, M.; SOAM, S.; AGRAWAL, R.; GUPTA, R. P.; TULI, D. K.; KUMAR, R. Pilot scale dilute acid pretreatment of rice straw and fermentable sugar recovery at high solid loadings. **Bioresource Technology**, Essex, v. 224, p. 688-693, 2017. DOI: https://doi.org/10.1016/j.biortech.2016.11.032. Available at: https://www.sciencedirect.com/science/article/pii/S0960852416315371. Accessed on: 22 Jan. 2024.

MARTINS, R. P.; SCHMATZ, A. A.; FREITA, L. A.; MUTTON, M. J. R.; BRIENZO, M. Solubilization of hemicellulose and fermentable sugars from bagasse, stalks, and leaves of sweet sorghum. **Industrial Crops and Products**, Amsterdam, v. 170, p. 1-10, 2021. DOI: https://doi.org/10.1016/j.indcrop.2021.113813. Available at: https://www.sciencedirect.com/science/article/pii/S092666902100577X?via%3Dihub. Accessed on: 22 Jan. 2024.

MELATI, R. B.; SASS, D. C.; PAGNOCCA, F. C.; BRIENZO, M. Anatomic influence of sugarcane biomass on xylan solubilization. **Industrial Crops and Products**, Amsterdam, v. 164, p. 1-6, 2021. DOI: https://doi.org/10.1016/j.indcrop.2021.113357. Available at: https://www.sciencedirect.com/science/article/pii/S0926669021001217. Accessed on: 22 Jan 2024.

MELATI, R. B.; SHIMIZU, F. L.; OLIVEIRA, G.; PAGNOCCA, F. C.; DE SOUZA, W.; SANT'ANNA, C.; BRIENZO, M. Key Factors Affecting the Recalcitrance and Conversion Process of Biomass. **Bioenergy Research**, New York, v. 12, n. 1, p. 1-20, 2019. DOI: https://doi.org/10.1007/s12155-018-9941-0. Available at: https://link.springer.com/article/10.1007/s12155-018-9941-0. Accessed on: 22 Jan. 2024.

MELATI, R. B.; SASS, D. C.; CONTIERO, J.; BRIENZO, M. Xylan Solubilization from Partially Delignified Biomass, and Residual Lignin Removal from Solubilized Xylan. **Polysaccharides**, Swiss, v. 4, p. 176-188, 2023. DOI: https://doi.org/10.3390/polysaccharides4020013. Available at: https://www.mdpi.com/2673-4176/4/2/13. Accessed on: 22 Jan. 2024.

MENANDRO, L. M. S.; CANTARELLA, H.; FRANCO, H. C. J.; KÖLLN, O. T.; PIMENTA, M. T. B.; SANCHES, G. M.; RABELO, S. C.; CARVALHO, J. L. N. Comprehensive assessment of sugarcane straw: implications for biomass and bioenergy production. **Biofuels, Bioproducts and Biorefining**, Chichester, v. 11, n. 3, p. 488-504, 2017. DOI: 10.1002/BBB.1760. https://onlinelibrary.wiley.com/doi/full/10.1002/bbb.1760. Accessed on: 22 Jan. 2024.

MESA, L.; MARTÍNEZ, Y.; BARRIO, E.; GONZÁLEZ, E. Desirability function for optimization of Dilute Acid pretreatment of sugarcane straw for ethanol production and preliminary economic analysis based in three fermentation configurations. **Applied Energy**, London, v. 198, p. 299-311, 2017. DOI: https://doi.org/10.1016/J.APENERGY.2017.03.018. Available at:

https://www.sciencedirect.com/science/article/pii/S0306261917302362?via%3Dihub. Accessed on: 22 Jan. 2024.

MIYAMOTO, T.; MIHASHI, A.; YAMAMURA, M.; TOBIMATSU, Y.; SUZUKI, S.; TAKADA, R.; KOBAYASHI, Y.; UMEZAWA, T. Comparative analysis of lignin chemical structures of sugarcane bagasse pretreated by alkaline, hydrothermal, and dilute sulfuric acid methods. **Industrial Crops and Products**, Amsterdam, v. 121, p. 124-131, 2018. DOI: https://doi.org/10.1016/J.INDCROP.2018.04.077. Available at: https://www.sciencedirect.com/science/article/pii/S0926669018304060?via%3Dihub. Accessed on: 22 Jan. 2024.

MOODLEY, P.; GUEGUIM KANA, E. B. Comparative study of three optimized acid-based pretreatments for sugar recovery from sugarcane leaf waste: A sustainable feedstock for biohydrogen production. **Engineering Science and Technology, an International Journal**, Amsterdam, v. 21, n. 1, p. 107-116, 2018. DOI: https://doi.org/10.1016/J.JESTCH.2017.11.010. Available at: https://www.sciencedirect.com/science/article/pii/S221509861730695X?via%3Dihub. Accessed on: 22 Jan. 2024.

MOUTTA, R. O.; CHANDEL, A. K.; RODRIGUES, R. C. L. B.; SILVA, M. B.; ROCHA, G. J. M.; SILVA, S. S. Statistical Optimization of Sugarcane Leaves Hydrolysis into Simple Sugars by Dilute Sulfuric Acid Catalyzed Process. **Sugar Tech,** India, v. 14, n, 1, p. 53–60, 2011. DOI: 10.1007/S12355-011-0116-Y. Available at: https://link.springer.com/article/10.1007/s12355-011-0116-y. Accessed on: 22 Jan. 2024.

PATIL, R. A. Dry Sugarcane Leaves: Renewable Biomass resources for Making Briquettes. **International Journal of Engineering Research and Technology**, New Delhi, v. 10, n. 1, p. 232-235, 2017.

ROBAK, K.; BALCEREK, M. Current state-of-the-art in ethanol production from lignocellulosic feedstocks. **Microbiological Research**, Jena, v. 240, p. 1-16, 2019. DOI: https://doi.org/10.1016/j.micres.2020.126534. Available at: https://www.sciencedirect.com/science/article/pii/S094450132030402X. Accessed on: 22 Jan. 2024.

SANT'ANNA, C.; SOUZA, W.; BRIENZO, M. The influence of the heterogeneity, physicochemical and structural properties on the recalcitrance and conversion of sugarcane bagasse. *In*: WEBB, E. **Sugarcane**: Production, Consumption and Agricultural Management Systems. Hauppauge: Nova Science Publishers, 2014. p. 1-34.

SCHMATZ, A. A.; TYHODA, L.; BRIENZO, M. Sugarcane biomass conversion influenced by lignin. **Biofuels, Bioproducts and Biorefining**, Chichester, v. 14, n. 2, p. 469-480, 2020. DOI: https://doi.org/10.1002/bbb.2070. Available at: https://onlinelibrary.wiley.com/doi/10.1002/bbb.2070. Accessed on: 22 Jan. 2024.

SILVA, A.S.; INOUE, H.; ENDO, T.; YANO, S.; BON, E.P.S. Milling pretreatment of sugarcane bagasse and straw for enzymatic hydrolysis and ethanol fermentation. **Bioresource Technology**, Essex, v. 101, n. 19, 7402-7409, 2010. DOI: https://doi.org/10.1016/j.biortech.2010.05.008. Available at: https://www.sciencedirect.com/science/article/pii/S0960852410008199. Accessed on: 22 Jan. 2024.

SZCZERBOWSKI, D.; PITARELO, A. P.; ZANDONÁ FILHO, A.; RAMOS, L. P. Sugarcane biomass for biorefineries: comparative composition of carbohydrate and non-carbohydrate components of bagasse and straw. **Carbohydrate polymers**, Barking, v. 114, p. 95-101, 2014. DOI: 10.1016/J.CARBPOL.2014.07.052. Available at: https://pubmed.ncbi.nlm.nih.gov/25263869/. Accessed on: 22 Jan. 2024.