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Autohydrolysis of sugarcane bagasse with water reuse: Impacts on residues' composition and enzymatic hydrolysis

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ABSTRACT

This work presents a new sequential approach to the sugarcane bagasse autohydrolysis process in a way that the liquor from the previous reaction was reused in the next one, and the makeup water for the next batch was used to wash the solid fraction before being added to the liquor in the next batch. This approach was suggested first as a way of reducing the water usage in the process, and second as a way of concentrating the liquor in any interesting component, working with the possibility of losing efficiency towards the glucose production through a cellulose loss to the liquor or enzymatic efficiency loss from a higher inhibitor concentration. Two sets of five sequential batches were performed: one washing the solids with the makeup water prior to the enzymatic step (Set 2), and the other one without the washing step (Set 1), looking forward to the effects of the water reuse over the glucose production and the liquor composition. Autohydrolysis pretreatment removed most of the hemicellulose (~94 %), with liquor recycling improving its removal until the third batch and stabilizing after that. Although the washing step showed little impact on the composition of the solids, it was determinant to the success of the enzymatic hydrolysis, since it was possible to maintain the cellulose to glucose yield around 53% throughout the batches. There was a 64 % reduction in the water used in the sequential reactions without interfering in the glucose production, which indicates that the proposed strategy could be successfully used to reduce costs and environmental impacts associated with the pretreatment of lignocellulosic biomass.

1. Introduction

Today, the world is changing from a fossil-based economy to a more sustainable bio-based economy, mostly by the pressure for a more environmentally sound approach to daily-used products. This scenario brings lignocellulosic biomass to attention, since it is vastly available worldwide, both in its natural shape (e.g., energetic forests) and as agro-industrial residues (e.g., sugarcane bagasse), and its use to produce valuable products tends to reduce costs [1,2]. Taking a closer look at the Brazilian scenario, sugarcane bagasse brings out the immediate advantage of integration with the existent sugar and alcohol industries, since it is already present in large amounts within them [2–9].

The continuous progress of the development of studies focusing on lignocellulosic biorefineries over the years has paved an avenue toward the sustainable transformation of biomass into biochemicals of high value-added and reduced carbon footprints, which can significantly minimize environmental issues, narrowing down the dependency on petroleum sources. The bioeconomy is closely related to an efficient and sustainable utilization of natural resources to produce biofuels and a plethora of bio-based chemicals and materials of high commercial interest. In this challenging bioeconomy-based scenario, the successful use of biomass feedstock is strongly dependent on the developing of commercially reliable transformation processes in order to allow for the sustainable production of bio-based products to meet market requirements from technical, economic, and environmental points of view [10–13].

Lignocellulosic materials are composed mainly of three macromolecules: hemicellulose, cellulose, and lignin, organized in a recalcitrant matrix that is difficult to access and transform into other products [1]. The composition of these macromolecules makes the raw material extremely versatile in terms of application, as long as it is possible to isolate, totally or partially, each of these fractions. For that matter, it is necessary to submit the material to pretreatment [14].

The integral use of lignocellulosic biomass is an actual challenge

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despite the past efforts to make it economically viable over time, as the processes needed to convert the polymeric components into valuable products have issues, such as low specificity and efficiency. In this scenario, autohydrolysis process can be successfully used as an efficient hydrothermal pretreatment to fractionate the biomass into a liquid fraction rich in hemicellulose and/or lignin products (liquor), and a solid fraction rich in cellulose. This cellulose-rich fraction goes through an enzymatic hydrolysis to be converted to glucose, and from there to any other component through fermentation, as the well-known second-generation ethanol.

Autohydrolysis is a pretreatment that utilizes water at a high temperature (between 150 and 230 °C) and vapor pressure to promote partial biomass fractionation. Furthermore, autohydrolysis is a selective treatment for hemicellulose removal, causing low cellulose and lignin degradation, which reduces the formation of inhibitors. This way, the need for water during the washing step is reduced, improving the technical and economic feasibility of the technology [15–18]. The absence of chemical catalysts is the great advantage of autohydrolysis pretreatment, as it eliminates the need for recovery steps. This reduces costs associated with residue management, decreases equipment corrosion, and avoids the necessity of using special metal alloys in construction. On the other hand, chemical pretreatments typically require the addition of at least one washing step at the end to reduce inhibitor concentrations. They also involve adjusting the pH of the medium, which, if left unaddressed, can significantly reduce enzymatic hydrolysis efficiency and, consequently, the overall yield of the process [19].

Lignin, as it is originally linked to the extracted hemicellulose, suffers rearrangement at the macrostructure left in the solid phase [17], and in the process, can be partially solubilized. This rearrangement tends to increase the mean pore size of the obtained solid, which increases the enzymes' accessibility to the cellulose, improving its efficiency [20]. Lignin may play an important role in the enzymatic hydrolysis of cellulose, ultimately affecting the overall process efficiency, and under certain pretreatment conditions, lignin can exhibit an undesirable. As observed by Selig and collaborators [21], the redeposition of lignin droplets onto the pretreated biomass residuals, under dilute acid conditions, can negatively affect the enzymatic saccharification of pretreated cellulose, resulting in a decrease of hydrolysis process efficiency, which will consequently affect operational cost associated with the overall biorefinery process.

The autohydrolysis process, as well as other pretreatment processes, occurs under mechanical stirring, so there is a need for water not only to let the chemical reactions happen, but to decrease the viscosity of the reaction medium and the energy needed for stirring, or even to be able to stir the medium in the case of laboratory scale reactors. Larger reacting volumes require larger and more robust equipment for their handling (e.g., heating, stirring, cooling, and pumping), and larger volumes of residues are generated, increasing capital and operational costs. This represents a big problem at the industrial scale, and, in addition, the indiscriminate use of water has serious environmental impacts [17,19].

Strategies like decreasing the liquid-to-solid ratio (LSR) at pretreatment and adopting a fed-batch system at the enzymatic hydrolysis have been considered as alternatives to minimize water consumption [19,22], but stirring and mass transfer issues are commonly reported when working with high-solid content [23], and feedback inhibition is a problem when the glucose concentration starts to increase at the hydrolysis medium [1].

In the study conducted by Vallejos et al. [2], a xylan-rich liquor, composed of a high content of xylooligosaccharides (XOS), was obtained using a low liquid-to-solid ratio (LSR) of approximately 3 g/g during the autohydrolysis of sugarcane bagasse. However, the yield relative to the raw material composition was approximately 60 %. Kim et al. [24] used a liquid-to-solid ratio of 5 g/g in their study for a comparative analysis of different autohydrolysis conditions (temperature and reaction time) and treatment with diluted acid using corn stover at 20 % solids content. The results obtained in terms of xylose conversion were very similar to those obtained by Vallejos and collaborators, within the range of 60 % conversion for autohydrolysis. In the same study, the glucose yield was also analyzed, and for the best condition achieved, at 180 °C for 4 min, the conversion was around 50 %. Gütsch et al. [18] explored the effects of water autohydrolysis and acid-catalyzed hydrolysis on Eucalyptus globulus wood chips and achieved similar results to the other studies mentioned, using a low liquid-to-solid ratio of 5 g/g.

Although the expectation was that the low dilution of solids would result in a more concentrated product medium, the results highlight one of the difficulties when working with a high solid load, which is the loss of homogenization efficiency and mass transfer in the reaction medium due to the reduction in the amount of free water and the consequent increase in the apparent viscosity of the medium [22,23]. The poor homogenization of the reaction content hinders its diffusion throughout the raw material, leading to the observed decrease in yield.

These difficulties observed in the pretreatment stage become even more evident in the enzymatic saccharification step, as the low amount of free water and increased agitation challenge the access of catalysts to the substrate. The presence of a limited amount of water also promotes the concentration of inhibitors generated in the preceding pretreatment steps. Furthermore, the concentrated medium accentuates the presence of product inhibition, as the diffusion of catalytic products away from the enzymes is hindered [22,23].

Martins et al. [23] compared batch and fed-batch (FB) systems for the enzymatic hydrolysis of pretreated sugarcane bagasse. In the batch system, increasing the solid load (10, 15, and 20 %) resulted in a higher final glucose concentration in the less diluted condition (reaching up to 78 g/L for the alkali-pretreated solid). However, cellulose conversion to glucose was reduced by 12 % when compared to the less-concentrated medium. This pattern was also observed for solids pretreated with diluted acid (13 % reduction in conversion) and hypochlorite-peroxide (20 % reduction). On the other hand, the fed-batch system managed to mitigate the negative effects of a high solid load. In this case, for example, the enzymatic hydrolysis of an acid-treated solid increased its final concentration from 50 to 57 g/L when increasing the solid load from 15 % to 17 %, while maintaining a conversion rate of 53 %. In comparison, batch hydrolysis at a 15 % solid load yielded 45 g/L of glucose with a lower conversion of 47 %.

The objective of this work was to minimize water consumption during autohydrolysis through water reuse and to analyze its impact on liquor composition and glucose yield after enzymatic hydrolysis. To achieve this aim, a new sequential approach to the sugarcane bagasse autohydrolysis process is proposed in the present work, where the liquor from the previous reaction stage is reused in the next one, and the makeup water for the next batch is used to wash the solid fraction before being added to the liquor in the next batch. To the best of our knowledge, the present work illustrates, for the first time, an original approach that allows for the reduction of water usage in the process and concentration of the liquor in any interesting component. Several sequential batches were carried out to evaluate the effect of the water reuse on the glucose production, and the liquor composition and the hydrolysis products were analyzed through HPLC measurements.

2. Materials and methods

2.1. Raw materials

Sugarcane bagasse, kindly provided by the Ethanol Industry Jalles Machado S.A. (Goianésia, GO, Brazil), was dried for 48 h at 50 °C and then reduced to a particle size of 3 mm using a knife mill. The bagasse contains 21.38 % ± 0.25 % lignin, 29.94 % ± 0.50 % hemicellulose, 36.01 % ± 0.30 % cellulose, 1.75 % ± 0.24 % ashes, 4.69 % ± 0.41 % extractives, and nearly 6 % water content [25]. Fresh water was used as the makeup water and deionized water used as the mobile phase for sugar characterization by HPLC. Commercial Enzyme Complex Cellic®

CTec3 (Novozymes Latin America Ltda., Araucária, PR, Brazil) was used for the hydrolysis of sugarcane bagasse. Cellobiose (99.0 %), glucose (99.5 %), xylose (99.0 %), Arabinose (99.0 %), 5-hydroxymethylfurfural (99.0 %), furfural (99.0 %), citric acid (≥99.5 %), sodium citrate (≥99.0 %), acetic acid (≥99.7 %), and sulfuric acid (95.0–98.0 %) were supplied by Sigma-Aldrich Brasil Ltda., São Paulo, SP, Brazil. All reagents were used as received, without further purification.

2.2. Autohydrolysis of sugarcane bagasse and water reuse

Autohydrolysis reactions were carried out in a 5 L Büchi reactor (Büchi AG, KILOCLAVE TYPE4, Uster, Switzerland), using an LSR of 10 g of water/g of dry biomass, under a stirring speed of 600 rpm. Around 350 g of sugarcane bagasse was mixed with 3.5 L of water and heated to reach the target temperature of 180 °C. After 40 min, the medium was cooled by circulating cold liquid through the vessel jacket. Solid fraction and liquor were separated by a single filtration step, weighed, and the recovered liquor quantified.

It is worth mentioning that the operating conditions for the autohydrolysis of sugarcane bagasse in a batch reactor were set up based on previous studies by our research group (unpublished data), which indicated that (i) at least 10 g of water per gram of dry biomass is required to ensure the homogenization of the mixture and better heat distribution during the heating stage; (ii) 180 °C is a temperature at which lignocellulosic biomass undergoes significant chemical transformations, increasing enzyme access to cellulose without a high amount of inhibitors being produced; and (iii) the use of a solid load of 10 mL/g of biomass and a stirring speed of 150 rpm are experimental conditions that facilitate proper homogenization of the reaction medium and enzyme action during enzymatic hydrolysis.

There were two sets of experiments (Table 1). In the first set of experiments, makeup water (fresh water) was added to the liquor to reach the initial amount of 3.5 L. To this liquor–water mixture was then added 350 g of raw sugarcane bagasse, and a new autohydrolysis batch was carried out. This process of reusing the liquor and adding makeup water was repeated sequentially to complete the set of 5 sequential batches of autohydrolysis. The second set of experiments presented only one difference: before the makeup water was mixed with the liquor, it was used to wash the solid three times before going to the new batch. It is important to mention that the first batch autohydrolysis, in both experimental set ups, corresponded to a traditional autohydrolysis, so it was used as a reference to evaluate the benefits generated by the water-reuse approach adopted in this work, as depicted in Fig. 1. The standard autohydrolysis, as in the experiments 1–1 and 2–1, used 3.5 L of fresh water per batch, so 17.5 L of water should be used for five batches.

Table 1
Volume of liquor reused, and makeup water added in each batch of autohydrolysis.[a] The code of the samples means "set-batch".

Sample Code	Volume of Liquor (L)	Volume of Makeup Water (L)
Set 1		
1–1	0.00	3.50
1–2	2.80	0.70
1–3	2.80	0.70
1–4	2.85	0.65
1–5	2.85	0.65
Total fresh water (L)		6.20
Set 2		
2–1	0.00	3.50
2–2	2.80	0.70
2–3	2.80	0.70
2–4	2.80	0.70
2–5	2.85	0.65
Total fresh water (L)		6.25

[a] The difference between the experimental data from set 1 and set 2 relies on the use of water: directly added into the next batch (Set 1) or washing the solids prior to being added to the next batch (Set 2).

However, in our proposed experimental set up, we used 6.2 L and 6.25 L, respectively, in the first and second set ups (Table 1).

The solid fractions, in both sets, were frozen for later use on the enzymatic hydrolysis (Section 2.3). Samples had to be frozen so that all of them could be submitted to the enzymatic hydrolysis at the same time, reducing experimental errors associated with that step. Figure S1 of the Supplementary Material graphically summarizes the assays. In both experimental sets, after each batch, samples of solid and liquor were taken for further analysis (Section 2.4).

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis was carried out following a previously reported study with some modifications [7]. The reserved solids were submitted to enzymatic hydrolysis in 250 mL Erlenmeyer flasks under 50 °C and 150 rpm orbital agitation. The commercial Enzyme Complex Cellic® CTec3 (221 FPU/mL) measured by filter paper assay was used, under a dosage of 10 FPU/g of pretreated biomass. The liquid-to-solid ratio (LSR) was 10 mL/g substrate and the assays lasted 48 h. A citric acid/sodium citrate buffer 0.1 mol·L^{-1}, pH 5.0 was added to the assays to reach the desired LSR. All the hydrolyzes were performed in triplicate. Liquid phases were analyzed by HPLC, and solids were discarded.

2.4. Analysis of the raw sugarcane bagasse, liquors, and solids after autohydrolysis and liquid phase of enzymatic hydrolysis

The raw bagasse was analyzed following the NREL protocol for structural carbohydrates and lignin [26]. Solids obtained from the autohydrolysis assays were washed with water until the post-washing liquid presented neutral pH and analyzed following the same protocol as the raw sugarcane bagasse. The liquor (liquid phase) from autohydrolysis assays was analyzed by high-performance liquid chromatography, HPLC (Shimadzu, PROMINENCE LC-20AD, Shimadzu do Brasil Comércio Ltda., Barueri, SP, Brazil) for sugars, acetic acid, HMF (5-Hydroxymethylfurfural), and furfural, using a refractive index detector and a BioRad Aminex HPX-87 H column, eluted with 0.005 M sulfuric acid, at a constant flow rate of 0.6 mL/min at 45 °C. Liquid phases from the enzymatic hydrolysis were also analyzed by HPLC for sugars and eluted with deionized water at a flow rate of 0.6 mL/min. All liquid samples were filtered on 0.22 μm syringe filters prior to injection.

2.5. Calculation

2.5.1. Severity of the autohydrolysis

The pretreatment severity (S_0) was calculated by Eq. (1):

$$S_0 = \log R_0 = \log \left\{ \int_0^t \exp[(T-100)/(14.75)]dt \right\} \quad (1)$$

where R_0 is the severity factor, t is the reaction time (min), T is the temperature of the treatment (expressed in °C), 100 is the temperature of reference (expressed in °C), and 14.75 is an empirical parameter reported in the literature [26,27].

2.5.2. Cellulose to glucose yield

The conversion yield (x) was calculated using Eq. (2):

$$x \quad (\%) = 100 \frac{0.2 \bullet A}{20 \bullet B \bullet C} \quad (2)$$

where A is the correspondent glucose concentration after enzymatic hydrolysis (g·L^{-1}), B is the cellulose fraction of the solids submitted to the enzymatic step (dimensionless) and C is the glucose hydration factor (180/162), 0.2 is the volume (L) of each enzymatic hydrolysis assay, and 20 is the mass of dry solids for the assays (g) [26].

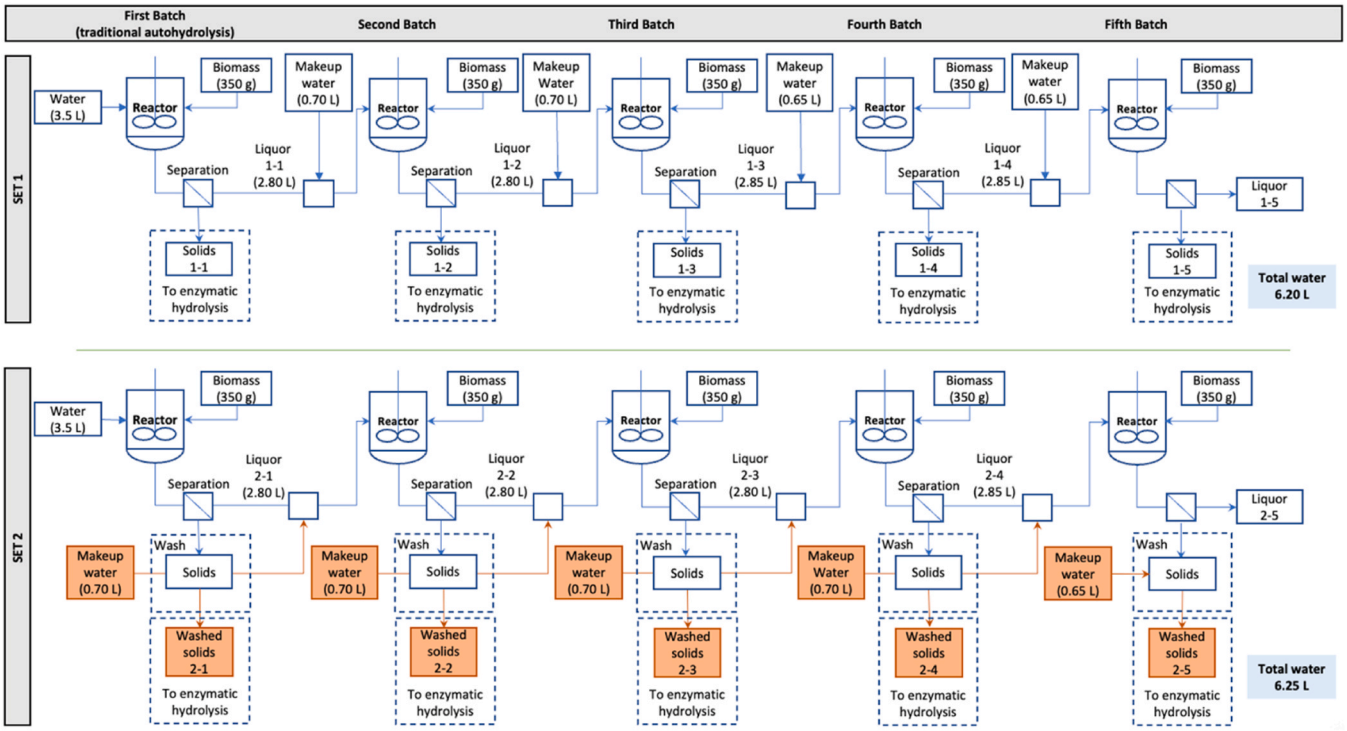

Fig. 1. Flowchart of the traditional and sequential autohydrolysis' assays by using reused water. The blue boxes represent the first experimental set up (1-X), while the orange boxes represent the second experimental set up (2-X), where the makeup water was used to wash the solid from the prior run.

2.5.3. *Water savings*

The total water savings were obtained by the sum of volumes of the reused liquors. The reduction, when compared to the traditional autohydrolysis, was calculated by the division between the water saved and water used if no liquor was reused.

2.5.4. *Statistical analysis*

A statistical analysis was conducted using OriginPro 2021 version 9.8.0.200 software with a significance level (p) set at 0.05. A data evaluation was performed through an analysis of variance (ANOVA) with pairwise mean comparisons using Tukey's statistical tests.

3. Results

3.1. *Effects of water reuse on the products of autohydrolysis*

Fig. 2 and Table S1 of the Supplementary Material show the main components (lignin and structural carbohydrates) of the raw sugarcane bagasse and solid fractions obtained after each batch of pretreatment. Overall, both sets of experiments presented similar behavior. For the raw sugarcane bagasse, the content of lignin, hemicellulose, and cellulose was found to be equal to 21.38 ± 0.25 %, 29.94 ± 0.50 %, and 36.01 ± 0.30 %, respectively. In particular for hemicellulose (expressed as the summation of xylan, arabinan, and acetyl), the total content was around 24 %. The overall composition of sugarcane bagasse is in agreement with typical values reported in the open literature. For instance, Sluiter et al. [28] have described that a typical composition observed for different hydrolysis methods reveals as main constituents the following components: glucan (45.2 ± 1.9 %), xylan (23.4 ± 1.2 %), arabinan (2.5 ± 0.3 %), galactan (1.0 ± 0.2 %), acetyl (4.3 ± 1.0 %), and total lignin (23.1 ± 1.6 %).

As expected, given the characteristics of autohydrolysis as a pretreatment, there was little or no degradation of lignin at all. In fact, the lignin content measured increased when compared to its initial content, due to degradation of the carbohydrates and formation of pseudo-lignin during the pretreatment [29]. As soon as the carbohydrate percentages stopped to decrease, the lignin content reached a plateau, clearly visible from the third batches of both sets, and so on.

Hemicellulose had a rapid decrease at the first batch, when compared to the raw bagasse, as the concentration of hydronium ions started to increase with high pressure and temperature and catalyze the polymer degradation, which is the main effect autohydrolysis causes on the biomass. However, although the severity of the treatment was the same for each batch, calculated as 3.96 by Eq. (1), hemicellulose and cellulose values continued to decrease throughout the batches. That can be easily explained by the fact that, as water was being reused from the previous batch, some components were being accumulated, especially acetic acid (see Fig. 3 and Table S2 of the Supplementary Material), and the medium in which the new autohydrolysis was being carried out was more effective towards the removal of hemicellulose from biomass than the clean water used in the first batch. According to Rocha et al. [30], severity values below 5.1 are generally associated with the hydrolytic conversion of hemicellulose below 60 %, not improving the enzymatic conversion of the cellulosic material.

Following the pattern observed in the lignin, hemicellulose and cellulose percentages also reached a plateau after the third batch, although acetic acid concentration continued to increase on the reused medium. That plateau shows that the treatment reached its maximum capacity of biomass modification, especially hemicellulose degradation. The interaction between the macromolecules in the structure of the remaining solid was intricate in such way that the increasing harshness of the medium composition was not enough to disassemble. Increasing the severity of the treatment by changing parameters like temperature and duration of the treatment could lead to a new plateau.

Expectations were that the liquor throughout the batches would be increasingly richer in furfural, since the xylose extracted from the hemicellulose of the raw material is inevitably converted under a high temperature. However, as seen in Table S2 (Supplementary Material), the furfural concentration increased but soon reached a plateau, between batches 2 and 3. The harshness of the reused medium was also

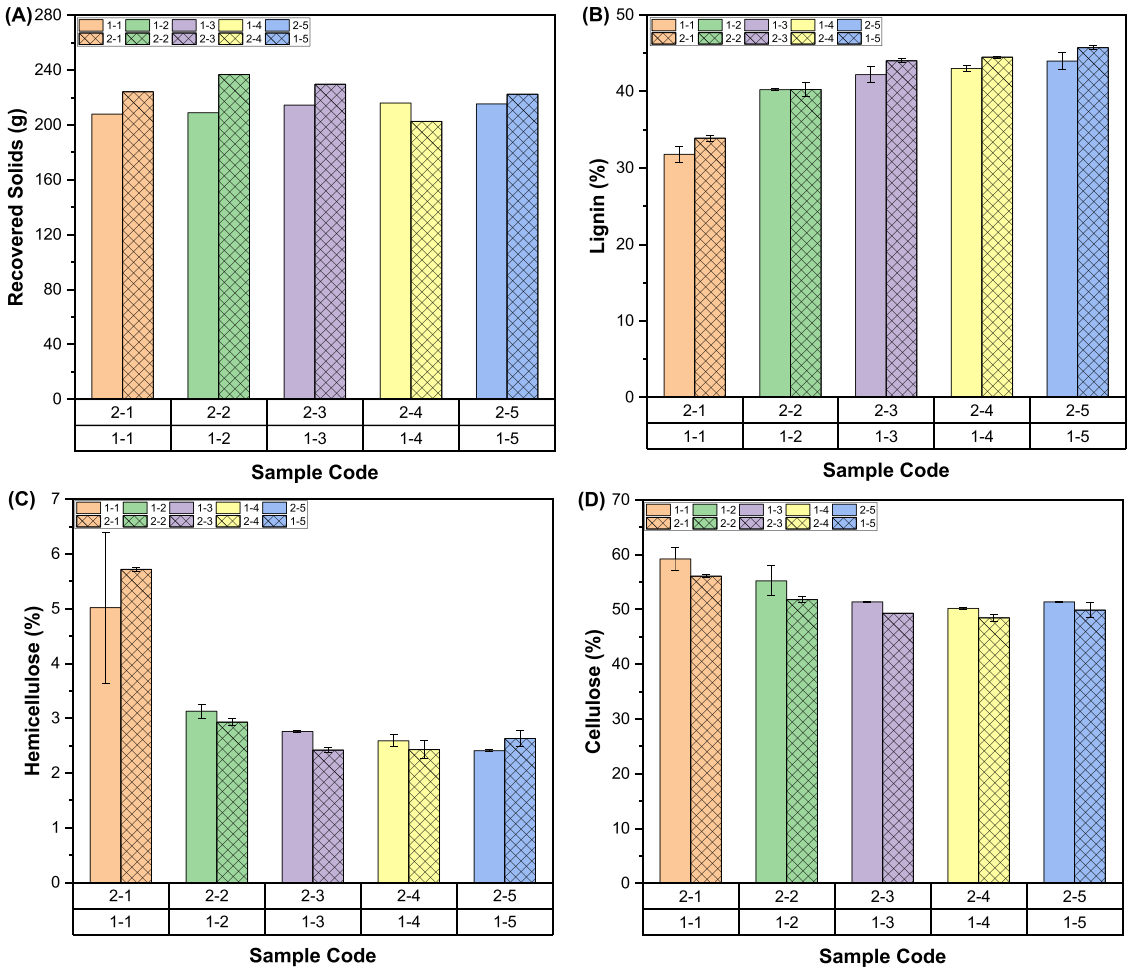

Fig. 2. Experimental data for (A) recovered solids, (B) lignin, (C) hemicellulose, and (D) cellulose from sets 1 and 2 of autohydrolysis' experiments.

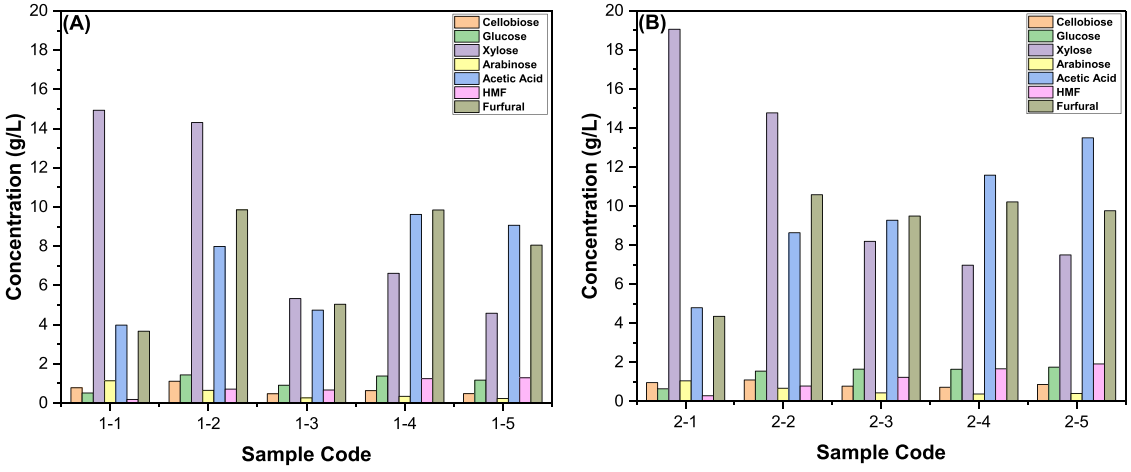

Fig. 3. Composition of the liquors obtained after each batch of autohydrolysis for (A) first set (Set 1) and (B) second set (Set 2) of experiments.

enough to convert furfural into some non-quantified derivative. On the other hand, acetic acid and HMF did accumulate through the batches and did not seem to be reaching a plateau. This is an interesting fact, since both molecules are building blocks and HMF, specifically, is a promising molecule for biomaterial, chemical, and transportation markets [31,32].

Based on Fig. 3 and Table S2 (Supplementary Material) results, cellobiose, glucose, xylose, and arabinose continue to be released in the same way or even in larger quantities throughout the cycles of autohydrolysis (N.B. in both cases, their content should increase over the autohydrolysis cycles). However, since the pH of the medium is likely to become more acidic (concentration of acetic acid and other acids increases), the tendency is for more of these molecules (glucose, arabinose, and xylose) to be degraded into furfural and HMF [33].

3.2. Effects of water reuse on enzymatic hydrolysis

The solid samples obtained after each autohydrolysis were enzymatically hydrolyzed in triplicate to convert cellulose into glucose and to observe any adverse effects of water reuse on glucose yield. Table 2 displays the concentration and yield of glucose obtained after hydrolysis.

Each sequential batch of the first set presented a significant drop in the glucose concentration when compared to the previous one. One of the main reasons for the reduction in the glucose yield is the accumulation of a wide variety of enzyme-inhibiting molecules in the liquor such as acetic acid, oligosaccharides, polyphenols, and monosaccharide derivatives (e.g., HMF and furfural, which can be degraded into dozens of other compounds). In this study, only three of these molecules were quantified (acetic acid, HMF, and furfural), as shown in Table S2 (Supplementary Material), clearly indicating a trend towards an increase in their concentrations as a function of the number of cycles, suggesting that the same behavior may be observed for the other inhibitors present in the liquor. Since solids of the first set were only separated from the liquor by a single filtration, there was still a certain volume of liquor impregnated in the solid (equal to the makeup water volume shown in Table 1), filled with inhibitors, and carried to the enzymatic step.

Maintaining the overall water savings but using makeup water to wash the solids before adding to the recovered liquor was the alternative to minimize the inhibitors' effects on the hydrolysis, so this was executed on the second set of experiments and the results showed a huge improvement.

A statistical evaluation of the experimental data in Table 2 was performed to give insight into the process behavior based on the ANOVA statistical test (95 % confidence level, $p = 0.05$). For the first set of experiments (Set 1), according to Fig. 4A, the mean values of glucose concentration determined from each experimental condition carried out in triplicate were significantly different. An exponential decrease in the glucose concentration throughout the sequential batches for Set 1 was a result of the presence of inhibitors in the liquid phase in the batches as discussed earlier. Considering the Tukey test results for Set 1, only a comparison between the batches (1–5; 1–4), (1–4; 1–3), and (1–3; 1–2) indicates that they cannot be considered significantly different.

On the other hand, when comparing the mean values of the glucose concentration in Fig. 4B (Set 2), it is reasonable to consider that the slight U-shape of the mean value indicated a significant statistical difference. However, the exponential decay of glucose concentration was no longer observed, suggesting the benefits of employing the washing step in the sequential batches. According to the Tukey test, only the results from the batches (2–5; 2–1) and (2–3; 2–1) are considered significantly different.

When looking at the hydrolysis yield (see Fig. 5), the values remained the same throughout the batches, showing that the makeup water detour to wash the solids had a significant effect on the subsequent hydrolysis, canceling the negative impact the inhibitors had on the first set. Fig. 5 also shows that there was a clear difference between enzymatic hydrolysis efficiency in the first and second sets.

The fact that no additional clean water was used to wash the solids, which is very common among studies presented in the literature [34, 35], and the enzymatic hydrolysis yield was not affected, shows a huge potential for water reuse during autohydrolysis without compromising the overall glucose yield.

Considering that the cellulose content in the samples varied from approximately 48–59 %, it is possible to estimate that the enzymatic load in the hydrolysis assays varied from 17 FPU/g of cellulose to 21 FPU/g of cellulose. According to the manufacturer Novozyme, 50 kg of the Cellic Ctec 3 enzyme complex produces 1 ton of ethanol from biomass. Bearing in mind that the enzymatic activity of this complex was approximately 220 FPU/mL (density of the enzyme solution equal to 1.1 g mL^{-1}), and that 1 g of glucose (~0.9 g of cellulose) generated 0.51 g of ethanol, it is reasonable to assume that the enzyme load recommended by the manufacturer for hydrolysis of biomass would be 5 FPU/g of cellulose.

In the enzymatic hydrolysis assays performed in this study, the enzyme was used in excess to ensure that all cellulose available after pretreatment was converted to glucose. The hydrolysis conditions were also established in such a way that the maximum possible cellulose conversion was achieved, for instance, long reaction time (48 h), low solid loading (10 % wt/vol, LSR 10 mL/g of substrate), and buffered reaction medium (pH equal to 5) in order to keep the enzymes stable and active. Therefore, the conditions settled for the enzymatic hydrolysis were not a limiting factor for the cellulose conversion, and any oscillation in this response was probably due to the efficiency of the pretreatment process.

It is worth mentioning that in the absence of pretreatment, the cellulose conversion would be less than 20 %, even using enzymes in excess, and the pretreated biomasses in this study reached a maximum conversion of around 59 %, which is in very close agreement with the cellulose conversion values presented in the open literature. For example, Bordignon et al. [36] have employed a combined ozonolysis and liquid hot water process along with a 4:1 mixture of Cellic CTec2 and HTec2 (180 FPU mL^{-1} FPase activity; 13,213 UI mL^{-1} xylanase activity; 7240 UI mL^{-1} β-glucosidase activity) to pretreat sugarcane bagasse, reaching a 59 % glucan conversion.

Table 2
Concentration and yield of glucose after enzymatic hydrolysis of the recovered solids.

Sample Code	Glucose Concentration (g/L)	Yield (g Glucose/100 g Cellulose) (%)
Raw Sugarcane Bagasse	10.57 ± 1.32	26.41 ± 3.31
1–1	34.05 ± 1.22	51.94 ± 1.86
1–2	28.95 ± 0.65	47.38 ± 1,06
1–3	27.65 ± 0.95	48.79 ± 1.67
1–4	25.69 ± 1.03	46.24 ± 1.85
1–5	24.43 ± 0.31	44.87 ± 0.56
2–1	33.24 ± 0.54	53.42 ± 0.86
2–2	30.79 ± 0.45	53.49 ± 0.77
2–3	27.89 ± 2.22	50.92 ± 4.05
2–4	29.04 ± 0.89	53.89 ± 1.66
2–5	31.20 ± 1.25	56.27 ± 2.25

3.3. Water consumption

Reducing water consumption in industrial facilities is not only an economic issue, but an environmental one as well. The experimental strategy adopted in this study helped by saving more than 11 L of clean water when compared to the traditional autohydrolysis, that used up to 17.5 L in five batches (see Section 2.5.3 for calculations). This represents a 64 % reduction, without reducing the glucose yield at the final step. As a matter of fact, reducing water consumption means also reducing effluent generation and equipment maintenance costs, and, as a consequence, total costs and environmental impact, which, at industrial scale, can lead to technical and economic feasibility.

4. Conclusions

In this study, water consumption was reduced during a sequential set of autohydrolysis batches, reusing the liquor obtained from the previous batch to start the next one. By using makeup water to wash the solids from the next batch, approximately 11.25 L of water was saved at the end of the fifth batch, from the 17.5 L that would be used in five standard autohydrolysis batches. This represents a 64 % reduction, without compromising the glucose yield at the enzymatic hydrolysis, which remained around 53 %, demonstrating significant potential for water reuse during autohydrolysis. Approximately 94 % of the hemicellulose

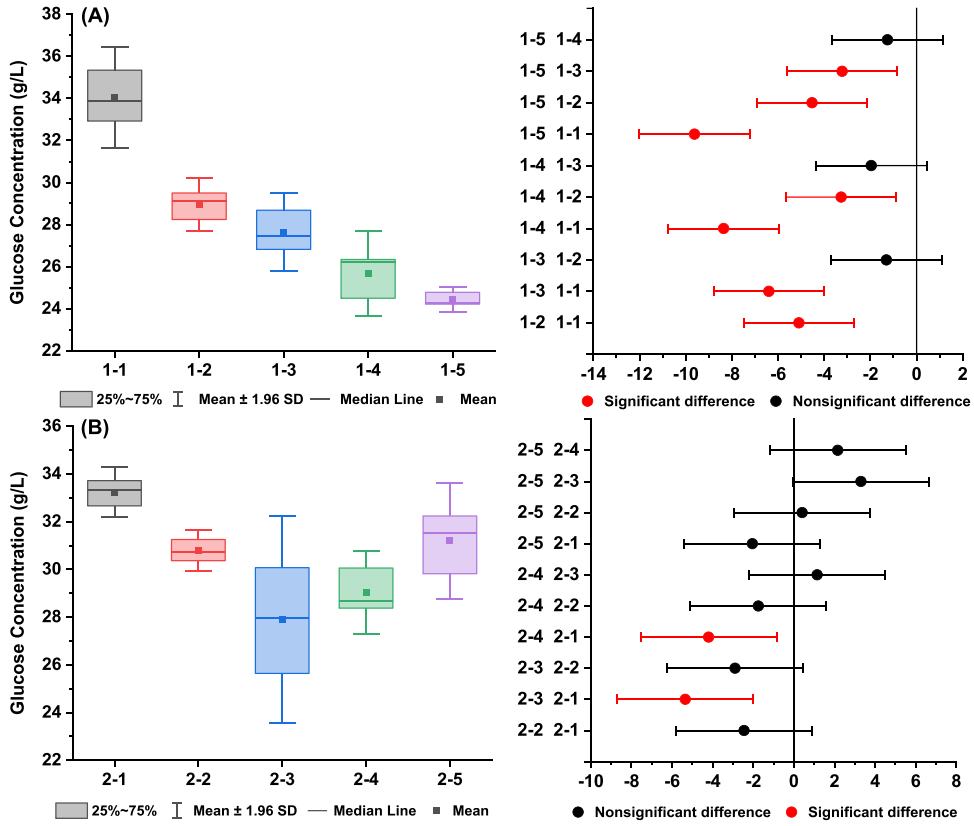

Fig. 4. Box charts and means comparison using Tukey test for (A) first set (Set 1) and (B) second set (Set 2). The colors gray, red, blue, green, and purple refer to the individual batches from sets 1 and 2.

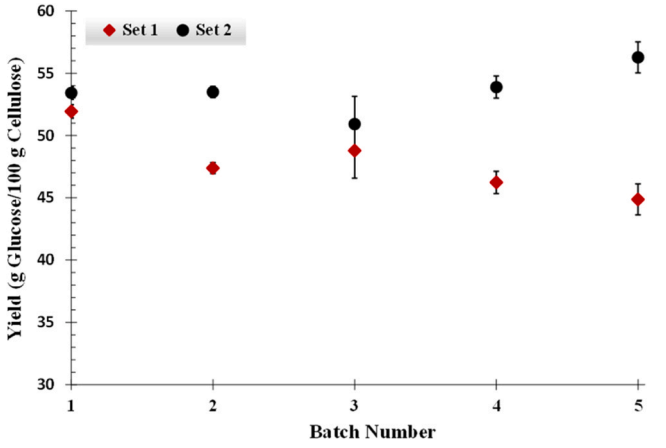

Fig. 5. Comparison between first and second set of experiments in terms of glucose yield.

was removed during autohydrolysis pretreatment, which was associated with liquor recycling that improved its removal up to the third batch and stabilized thereafter. The liquors obtained after the batches presented an increasing concentration of acetic acid and HMF, which are important building blocks for different industries.

Statistical evaluation of the experimental data revealed that the decay of glucose concentration was no longer observed, indicating the benefits of including the washing step in sequential batches. As for hydrolysis yield, the values remained consistent across all batches, highlighting the significant effect of diverting makeup water to wash the solids on subsequent hydrolysis. This action effectively counteracted the negative impact of inhibitors observed in the first set of experiments.

Autohydrolysis was already an interesting biomass pretreatment, since it is a simple process that uses no catalyst chemicals. Now, with the obtained positive results for water reuse, not only by reducing the overall water usage, but by improving the process in the hemicellulose removal as well, the liquor shows a concentration in some commercial products of everything going in the direction of improving the process in terms of economic and environmental sustainability.

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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.

Data availability

Data will be made available on request.

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Author contributions

F.B.P.C., D.S.R., F.M., and R.G. conceived of and designed the experiments, analyzed the data, and wrote the paper; F.B.P.C. performed the experiments. All authors have read and agreed to the published version of the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.scenv.2024.100097.

References

[1] C.-T. Buruiana, C. Vizireanu, G. Garrote, J.C. Parajó, Optimization of corn stover biorefinery for coproduction of oligomers and second generation bioethanol using non-isothermal autohydrolysis, Ind. Crop. Prod. 54 (2014) 32–39, https://doi.org/10.1016/j.indcrop.2014.01.003.

[2] M.E. Vallejos, M.D. Zambon, M.C. Area, A.A. da Silva Curvelo, Low liquid–solid ratio (LSR) hot water pretreatment of sugarcane bagasse, Green Chem. 14 (7) (2012) 1982–1989, https://doi.org/10.1039/C2GC35397K.

[3] CONAB, Acompanhamento da Safra Brasileira de Cana de Açúcar, 02/2015. Ministério da Agricultura, Pecuária e Abastecimento, ⟨http://www.conab.gov.br⟩, 2015.

[4] D. Maga, N. Thonemann, M. Hiebel, D. Sebastião, T.F. Lopes, C. Fonseca, F. Gírio, Comparative life cycle assessment of first- and second-generation ethanol from sugarcane in Brazil, Int. J. Life Cycle Assess. 24 (2) (2019) 266–280, https://doi.org/10.1007/s11367-018-1505-1.

[5] D.H. Fockink, A.R.C. Morais, L.P. Ramos, R.M. Łukasik, Insight into the high-pressure CO2 pre-treatment of sugarcane bagasse for a delivery of upgradable sugars, Energy 151 (2018) 536–544, https://doi.org/10.1016/j.energy.2018.03.085.

[6] L.L. Gai, E.F. Ren, W. Tian, D.B. Niu, W.D. Sun, F.X. Hang, K. Li, Ultrasonic-assisted dual-alkali pretreatment and enzymatic hydrolysis of sugarcane bagasse followed by Candida tropicalis fermentation to produce xylitol, Front. Nutr. 9 (2022), https://doi.org/10.3389/fnut.2022.913106.

[7] F.R. Paz-Cedeno, J.M. Carceller, S. Iborra, R.K. Donato, A.F.R. Rodriguez, M. A. Morales, E.G. Solorzano-Chavez, I.U.M. Roldan, A.V. de Paula, F. Masarin, Stability of the Cellic CTec2 enzymatic preparation immobilized onto magnetic graphene oxide: assessment of hydrolysis of pretreated sugarcane bagasse, Ind. Crop. Prod. 183 (2022), https://doi.org/10.1016/j.indcrop.2022.114972.

[8] M.C.D. Santo, F.T. Thema, V.D.A. Pellegrini, A.O. Kane, F.E.G. Guimaraes, J. G. Filgueiras, E.H. Novotny, E.R. DeAzevedo, I. Polikarpov, When the order matters: impacts of lignin removal and xylan conformation on the physical structure and enzymatic hydrolysis of sugarcane bagasse, Ind. Crop. Prod. 180 (2022), https://doi.org/10.1016/j.indcrop.2022.114708.

[9] W.J. Ying, J. Yang, J.H. Zhang, In-situ modification of lignin in alkaline-pretreated sugarcane bagasse by sulfomethylation and carboxymethylation to improve the enzymatic hydrolysis efficiency, Ind. Crop. Prod. 182 (2022), https://doi.org/10.1016/j.indcrop.2022.114863.

[10] P. Manzanares, The role of biorefinering research in the development of a modern bioeconomy, Acta Innov. (37) (2020) 47–56, https://doi.org/10.32933/ActaInnovations.37.4.

[11] A.K. Chandel, V.K. Garlapati, S.P. Jeevan Kumar, M. Hans, A.K. Singh, S. Kumar, The role of renewable chemicals and biofuels in building a bioeconomy, Biofuels Bioprod. Biorefin. 14 (4) (2020) 830–844, https://doi.org/10.1002/bbb.2104.

[12] A. Duque, C. Álvarez, P. Doménech, P. Manzanares, A.D. Moreno, Advanced bioethanol production: from novel raw materials to integrated biorefineries, Processes 9 (2) (2021) 206, https://doi.org/10.3390/pr9020206.

[13] Sushma, S. Chamoli, S. Upadhyayula, F. Parveen, Biofuels and fine chemicals from lignocellulosic biomass: a sustainable and circular economy, Handb. Biomass Valoriz. Ind. Appl. (2022) 41–53, https://doi.org/10.1002/9781119818816.ch3.

[14] E. van der Pol, R. Bakker, A. van Zeeland, D. Sanchez Garcia, A. Punt, G. Eggink, Analysis of by-product formation and sugar monomerization in sugarcane bagasse pretreated at pilot plant scale: differences between autohydrolysis, alkaline and acid pretreatment, Bioresour. Technol. 181 (2015) 114–123, https://doi.org/10.1016/j.biortech.2015.01.033.

[15] H.A. Ruiz, R.M. Rodríguez-Jasso, B.D. Fernandes, A.A. Vicente, J.A. Teixeira, Hydrothermal processing, as an alternative for upgrading agriculture residues and marine biomass according to the biorefinery concept: a review, Renew. Sustain. Energy Rev. 21 (2013) 35–51, https://doi.org/10.1016/j.rser.2012.11.069.

[16] M.-Q. Zhu, J.-L. Wen, Y.-Q. Su, Q. Wei, R.-C. Sun, Effect of structural changes of lignin during the autohydrolysis and organosolv pretreatment on Eucommia ulmoides Oliver for an effective enzymatic hydrolysis, Bioresour. Technol. 185 (2015) 378–385, https://doi.org/10.1016/j.biortech.2015.02.061.

[17] M.E. Vallejos, M.D. Zambon, M.C. Area, A.Ad.S. Curvelo, Low liquid-solid ratio fractionation of sugarcane bagasse by hot water autohydrolysis and organosolv delignification, Ind. Crops Prod. 65 (2015) 349–353, https://doi.org/10.1016/j.indcrop.2014.11.018.

[18] J.S. Gütsch, T. Nousiainen, H. Sixta, Comparative evaluation of autohydrolysis and acid-catalyzed hydrolysis of Eucalyptus globulus wood, Bioresour. Technol. 109 (2012) 77–85, https://doi.org/10.1016/j.biortech.2012.01.018.

[19] A.A. Modenbach, S.E. Nokes, The use of high-solids loadings in biomass pretreatment—a review, Biotechnol. Bioeng. 109 (6) (2012) 1430–1442, https://doi.org/10.1002/bit.24464.

[20] F. Vargas, E. Domínguez, C. Vila, A. Rodríguez, G. Garrote, Agricultural residue valorization using a hydrothermal process for second generation bioethanol and oligosaccharides production, Bioresour. Technol. 191 (2015) 263–270, https://doi.org/10.1016/j.biortech.2015.05.035.

[21] M.J. Selig, S. Viamajala, S.R. Decker, M.P. Tucker, M.E. Himmel, T.B. Vinzant, Deposition of lignin droplets produced during dilute acid pretreatment of maize stems retards enzymatic hydrolysis of cellulose, Biotechnol. Prog. 23 (6) (2007) 1333–1339, https://doi.org/10.1021/bp0702018.

[22] A.A. Modenbach, S.E. Nokes, Enzymatic hydrolysis of biomass at high-solids loadings – a review, Biomass Bioenergy 56 (2013) 526–544, https://doi.org/10.1016/j.biombioe.2013.05.031.

[23] L.H.S. Martins, S.C. Rabelo, A.Cd Costa, Effects of the pretreatment method on high solids enzymatic hydrolysis and ethanol fermentation of the cellulosic fraction of sugarcane bagasse, Bioresour. Technol. 191 (2015) 312–321, https://doi.org/10.1016/j.biortech.2015.05.024.

[24] S.M. Kim, B.S. Dien, M.E. Tumbleson, K.D. Rausch, V. Singh, Improvement of sugar yields from corn stover using sequential hot water pretreatment and disk milling, Bioresour. Technol. 216 (2016) 706–713, https://doi.org/10.1016/j.biortech.2016.06.003.

[25] F.B.P. Carvalho, Pré-tratamento de bagaço de cana-de-açúcar por autohidrólise, com ênfase na reutilização de água, Intitute of Chemistry, University of Brasilia, Brasília, 2018, p. 88. https://doi.org/10.26512/2018.02.D.32456.

[26] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, D. Crocker, Determination of Structural Carbohydrates and Lignin in Biomass, NREL/TP-510-42618 - NREL - National Renewable Energy Laboratory. Technical Report. Laboratory Analytical Procedures (LAP), 2012.

[27] R.P. Overend, E. Chornet, J.A. Gascoigne, B.S. Hartley, P.M.A. Broda, P.J. Senior, Fractionation of lignocellulosics by steam-aqueous pretreatments, Philos. Trans. R. Soc. Lond. Ser. A Math. Phys. Sci. 321 (1561) (1987) 523–536, https://doi.org/10.1098/rsta.1987.0029.

[28] J.B. Sluiter, H. Chum, A.C. Gomes, R.P.A. Tavares, V. Azevedo, M.T.B. Pimenta, S. C. Rabelo, K. Marabezi, A.A.S. Curvelo, A.R. Alves, W.T. Garcia, W. Carvalho, P. J. Esteves, S. Mendonça, P.A. Oliveira, J.A.A. Ribeiro, T.D. Mendes, M.P. Vicentin, C.L. Duarte, M.N. Mori, Evaluation of Brazilian sugarcane bagasse characterization: an interlaboratory comparison study, J. AOAC Int. 99 (3) (2019) 579–585, https://doi.org/10.5740/jaoacint.15-0063.

[29] Y. Pu, F. Hu, F. Huang, B.H. Davison, A.J. Ragauskas, Assessing the molecular structure basis for biomass recalcitrance during dilute acid and hydrothermal pretreatments, Biotechnol. Biofuels 6 (1) (2013) 15, https://doi.org/10.1186/1754-6834-6-15.

[30] G.J.M. Rocha, C. Martin, I.B. Soares, A.M. Souto Maior, H.M. Baudel, C.A.M. de Abreu, Dilute mixed-acid pretreatment of sugarcane bagasse for ethanol production, Biomass Bioenergy 35 (1) (2011) 663–670, https://doi.org/10.1016/j.biombioe.2010.10.018.

[31] C.-M. Liu, S.-Y. Wu, From biomass waste to biofuels and biomaterial building blocks, Renew. Energy 96 (2016) 1056–1062, https://doi.org/10.1016/j.renene.2015.12.059.

[32] X. Tang, J. Wei, N. Ding, Y. Sun, X. Zeng, L. Hu, S. Liu, T. Lei, L. Lin, Chemoselective hydrogenation of biomass derived 5-hydroxymethylfurfural to diols: key intermediates for sustainable chemicals, materials and fuels, Renew. Sustain. Energy Rev. 77 (2017) 287–296, https://doi.org/10.1016/j.rser.2017.04.013.

[33] K. Dussan, B. Girisuta, M. Lopes, J.J. Leahy, M.H.B. Hayes, Conversion of hemicellulose sugars catalyzed by formic acid: kinetics of the dehydration of D-xylose, L-arabinose, and D-glucose, ChemSusChem 8 (8) (2015) 1411–1428, https://doi.org/10.1002/cssc.201403328.

[34] M.T. Ashraf, M.H. Thomsen, J.E. Schmidt, Hydrothermal pretreatment and enzymatic hydrolysis of mixed green and woody lignocellulosics from arid regions, Bioresour. Technol. 238 (2017) 369–378, https://doi.org/10.1016/j.biortech.2017.04.065.

[35] B.-Y. Chen, B.-C. Zhao, M.-F. Li, Q.-Y. Liu, R.-C. Sun, Fractionation of rapeseed straw by hydrothermal/dilute acid pretreatment combined with alkali post-treatment for improving its enzymatic hydrolysis, Bioresour. Technol. 225 (2017) 127–133, https://doi.org/10.1016/j.biortech.2016.11.062.

[36] S.E. Bordignon, E. Ximenes, O.M. Perrone, Cd.C. Carreira Nunes, D. Kim, M. Boscolo, E. Gomes, E.X.F. Filho, R. da Silva, M.R. Ladisch, Combined sugarcane pretreatment for the generation of ethanol and value-added products, Front. Energy Res. 10 (2022), https://doi.org/10.3389/fenrg.2022.834966.