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ORIGINAL PAPER

Kinetics of the Release of Sugars from the Enzymatic and Physico-Chemical Pre-treated Sugarcane Bagasse and Residual Forest Biomass

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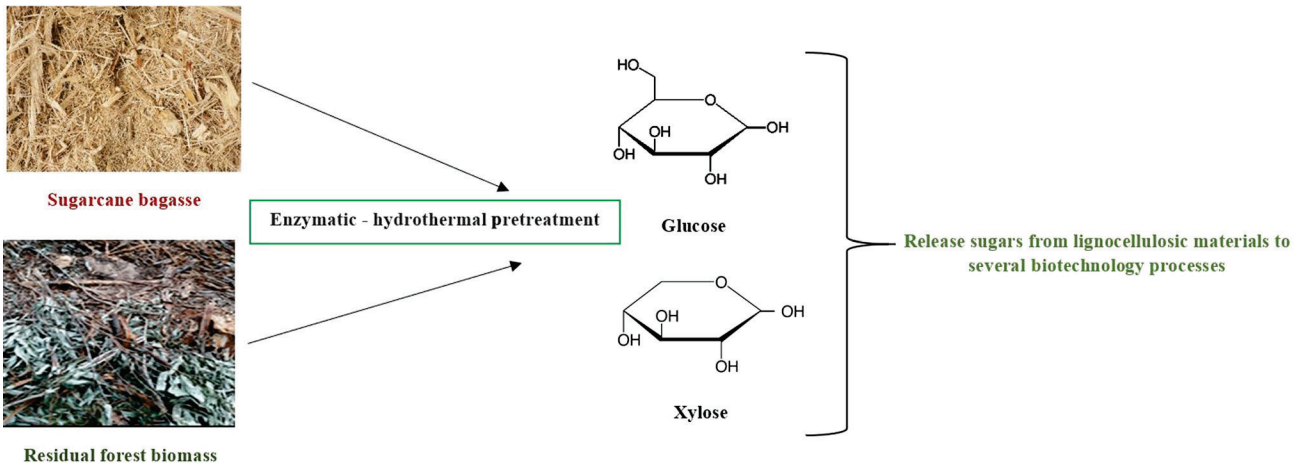
Abstract

Several pre-treatments are used to release sugars from lignocellulosic materials that are used to produce second-generation ethanol (2G). This study aimed to evaluate the kinetic release of glucose and xylose through the enzymatic and physical treatments of sugarcane bagasse and residual forest biomass, focusing on the ratio between hexose and pentose. Enzymatic hydrolysis after hydrothermal pre-treatment under different conditions, at 170, 170 and 190 °C, 170 and 190 °C with sulfuric acid, and 170 and 190 °C with the Organosolv solvent, all of them for 10 min, were performed with sugarcane bagasse and residual forest biomass, and the kinetic parameters of sugar release were evaluated. The results indicated that compared to hydrothermal and combined hydrothermal and dilute acid hydrolysis, organosolvation process led to higher release of glucose in hydrolysates from both biomasses, with a maximum yield of 14.12 and 33.33 g L^{-1}, respectively. On the other hand, the highest glucose/xylose ratio (about 19), which will facilitate its subsequent use for fermentation, was obtained from sugarcane bagasse after hydrothermal treatment at 170 and 190 °C. This ratio was higher for all treatments when compared to untreated biomass, which indicated that temperature and acid affected xylose instead of glucose.

Graphical Abstract



Keywords Sugarcane bagasse · Residual forest biomass · organosolvation process · Enzymatic pretreatment · Glucose and xylose released and hydrothermal process

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Statement of Novelty

The research presents important parameters in the microbial utilization of two lignocellulolytic residues, with emphasis not only on their main available fermentable sugars, but also on the ratio between hexoses and pentoses.

Introduction

First-generation ethanol (1G) is almost entirely produced using only simple sugars or starch, with readily available sugars. Second-generation ethanol (2G) is produced from lignocellulosic materials such as forest and agricultural wastes (Buckeridge et al., 2008) [1, 2]. Lignocellulosic materials mainly contain cellulose, hemicellulose, lignin, and other minor components such as ash and extractives [3, 4]. These substrates can potentially be used as raw materials in industrial processes to produce food, fuel, chemical inputs, enzymes, and various consumer goods [5, 6].

During sugarcane processing, for example, large amounts of lignocellulosic residues are generated during the production of first-generation ethanol and sugar. Sugarcane bagasse contain about 50% cellulose, 25% hemicellulose, and 25% lignin [7, 8].

Residual forest biomass (BFR) consists of branches, leaves, and bark of Eucalyptus (*Eucalyptus globulus*), Cork oak (*Quercus suber*), and Wild Pine (*Pinus pinaster*) (MAMAOT/ICNF). They can be used to produce wooden pallets (used as an alternative for heating in homes), heat production systems, power and cogeneration in industries, with an estimated production of approximately three million tons per year [9, 10].

For the production of second-generation ethanol, lignocellulosic biomass needs to be transformed into monomers (glucose and xylose) to be eventually converted to ethanol, consisting one of the most important stages of the process, regarding the cost and influence in the subsequent stages of hydrolysis [11–13]. Through hydrolysis, i.e., the addition of water molecules, hemicellulose and cellulose are converted to their monomers, consisting of pentoses and hexoses. This reaction requires a catalyst, which can be an acid, in case of acid hydrolysis, or an enzyme for enzymatic hydrolysis [12, 14].

Pretreatments can be chemical, physical, biological, or a combination of all. The main objective of pretreatment is to degrade the structural matrix of lignocellulosic biomass by exposing the cellulose and hemicellulose chains, making them more accessible to hydrolytic agents for acidic and enzymatic hydrolysis; thus, increasing the efficiency of the process [12, 15, 16].

Physical treatments include mechanical grinding, crushing, or crushing and steam blast [17]. The latter consists of the most widely used physical–chemical pretreatment for lignocellulosic biomass [18, 19]. It is a hydrothermal pretreatment process or also known as liquid hot water. These processes use compressed hot water (pressure above saturation point), and the operation temperatures between 150 and 230 °C. The reaction time may vary from seconds up to hours and in the sequence depressurized [20]. The mechanical effects occur due to the rapid reduction in pressure, and the fibers get separated by decompression [21]. In this method, lignin is partially fragmented, which facilitates the action of water, acids, or enzymes, and increases the hydrolytic potential of cellulose [22].

Chemical methods constitute the main method of treatment and include acidic, alkaline, or oxidative treatments. All types of treatment modify the chemical and structural properties of the cell wall, which increases the accessibility to the layers of hemicellulose and cellulose [13]. Solvents are also used for chemical treatment, and a process called "organosolvation" uses a mixture of acid and organic solvent, usually ethanol, to break the internal bonds of lignin and hemicellulose [3, 21]. Acid hydrolysis may lead to the formation of fermentation inhibiting components, such as 5-hydroxymethylfurfural [23].

Enzymatic hydrolysis is a process that has been extensively studied due to the absence of the formation of secondary products, no use of chemical compounds, or high pressure and temperature [24]. The catalyst, besides its biological origin, performs specific reactions, and thus, has a lesser impact on the environment [25]. Cellulases are enzymes capable of hydrolyzing lignocellulosic materials and have an enzyme complex composed of endoglucanases, exoglucanases, and β-glycosidases that act synergistically in cellulolytic fibers. Endoglucanases internally hydrolyze cellulose chains to produce shorter-length polysaccharides. The exoglucanases hydrolyze the non-reducing terminals at the ends of cellulose, releasing cellobiose, and β-glycosidases hydrolyze cellobiose to glucose [12, 26]. The fungi *Trichoderma reesei* and *Aspergillus niger* are considered to be producers of cellulases, with *T. reesei* known to produce large amounts of endoglucanases, while *A. niger* is an efficient producer of β-glycosides [27]. Enzymatic hydrolysis has several benefits, such as the absence of by-products that originate due to the degradation of sugars, performs specific reactions causing lesser environmental impacts compared to chemical hydrolysis, low energy consumption due to lighter reaction conditions, and lower investment and maintenance costs of equipment (Ballesteros, 2010) [28].

The biological pretreatments usually use fungi and some bacteria, which secrete extracellular enzymes, such as lignin peroxidases and laccases, responsible for removing lignin [16]. The effectiveness of fungi has been studied for pretreatment to increase the enzymatic saccharification of lignocellulosic biomass in ethanol production [15]. More recently, Codato et al., [29] evaluated the effectiveness of ethanol fermentation from bagasse hydrolyzed after the solid-state cultivation (SSC) of *A. niger* and *T. reesei* and compared it to the acid hydrolysis method. The results indicated that fermentation yield was 0.40 g of ethanol per gram of glucose, about 78% of the maximum stoichiometry, due to the lower initial concentration of inhibitors in the non-acid hydrolysates generated by SSC.

The quantity of hexose and pentose and the ratio between them are used to determine the type of alcoholic yeast to be used for fermenting the hydrolysates obtained from the different lignocellulosic materials. The production of ethanol from plant materials could be feasible if both pentoses and hexoses are converted to ethanol. *Saccharomyces cerevisiae*, the most important yeast used in bioethanol production, is unable to convert pentoses to ethanol, and it can only use the six-carbon fraction of the biomass hydrolysates. Other yeast species such as *Scheffersomyces (Pichia)*, *Candida*, and *Pachysolen* can convert pentoses to ethanol and other alcohols [30, 31].

This study aimed to evaluate the kinetic release of glucose and xylose through the enzymatic and physical treatment of sugarcane bagasse and residual forest biomass, focusing on the relationship between hexoses and pentoses for the subsequent ethanol fermentation.

Materials and Methods

Lignocellulosic Biomass

Sugarcane bagasse (SB) used in the experiments were collected from a sugarcane processing industry located in the city of Araras, São Paulo, Brazil. Residual eucalyptus forest biomass (RFB), consisting of branches, leaves, and bark, was Kindly provided by The Navigator Company (Cacia, Portugal) and was previously crushed. Upon reception the samples were dried in an oven at 50 °C for approximately 48 h to reduce the moisture content to less than 10% (w/w) and milled in a knife mill (Fritsch, Germany) to particles smaller than 6.0 mm.

Chemical Characterization of the Solid Fraction

Lignocellulosic raw materials (bagasse and residual forest biomass) were ground to particle size smaller than 0.5 mm and characterized by quantitative acid hydrolysis according to NREL (SLUITER et al., 2011). The samples were mixed with 72% (w/w) sulfuric acid for 60 min at 30 °C, diluted with water to reach 4% (w/w) sulfuric acid concentration, and hydrolyzed for 60 min in an autoclave at 121 °C. The solids obtained were used to determine the acid-insoluble lignin (Klason lignin) content after correcting for the ash content. Ash was determined by igniting the contents at 550 °C for 5 h. In the liquid fraction, monosaccharides (glucose, xylose andarabinose) and acetic acid were determined by HPLC (Agilent 1100 Series, Germany), using an Aminex HPX-87H column (Bio-Rad, Hercules, CA), operating at 50 °C. The samples were eluted after injecting 20 µL, with sulfuric acid (5 mM) as the mobile phase, at a flow rate of 0.4 mL min^{-1} [32].

Hydrothermal Treatment

The hydrothermal treatments (autohydrolysis) were conducted using a stainless-steel reactor (Parr Instruments Company, Moline, Illinois, USA) with a total volume of 2 L. The reactor was equipped with two four-blade turbine impellers, heated by an outer fabric blanket, cooled by cold water circulating through an internal stainless-steel circuit, and temperature-controlled via a Parr PID controller. The biomass was mixed with water in the reactor to obtain a liquid–solid ratio (LSR) of 8 mg of water per mg dry raw material). The agitation speed was set at 150 rpm, and the reactor was heated to reach 170 or 190 °C (non-isothermal conditions), hydrothermal at 170 °C, hydrothermal at 170 °C and 190 °C, hydrothermal at 170 and 190 °C with 0.5% v/v of H_2SO_4, and hydrothermal at 170 °C and 190 °C with the organosolv solvent (1:1). After reaching the desired temperature, the reactor was maintained in that state for 10 min and then quickly cooled. The liquid and solid fractions were separated using a hydraulic press (Sotel, Portugal), up to 200 kg cm^{-2}, and the recovered liquid fraction was filtered (Whatman #1 Filter Paper) to remove the remaining solids. The solid fraction was washed at room temperature with two volumes of water (based on the water volume used in the pre-treatment), filtered, and dried at 50 °C before analyzis for chemical composition.

Enzymatic Pretreatment

The enzymatic hydrolysis of the pretreated solids was performed with Cellic CTec2 enzymes kindly provided by Novozyme® (Denmark). The conditions of the assays were fixed for an enzyme load of 5% (w/v) 8 FPU/g substrate at 180 rpm and 50 °C for 72 h. The reaction mixture contained 0.05 M citrate buffer (pH 5.0) and 0.02% w/v sodium azide. Immediately after sampling, the enzymes were inactivated

Table 1 Chemical composition of raw materials

	Residual Forest Biomass (g 100 g^{-1})	Sugarcane bagasse (g 100 g^{-1})
Glucose	34.55	45.20
Hemicellulose	22.61	29.77
Xylan	15.68	20.96
Arabinan	5.48	5.42
Acetyl groups	1.45	3.39
Klason lignin	30.08	20.69
Ash	5.72	1.93
Others (by difference)	7.03	2.40

(100ºC, 5 min), and after filtration (0.22 µm nylon filters), the hydrolysates were analyzed by HPLC as described above; the final sugar concentrations were corrected with substrate and enzyme blanks.

Sugar Release Kinetics

Glucose and xylose were quantified in the samples by HPLC. The sugar profile was fit to a saturation model by double reciprocal plot according to Eq. 1, where "C" is the concentration of glucose and xylose released during time "t", "C_{max}" is the maximum concentration of sugars and "k" is the constant that indicates the treatment time for ½C_{max}.

$$C = \frac{C_{max}t}{k+t} \quad (1)$$

Results and Discussion

Characterization of Sugarcane Bagasse and Residual Forest Biomass

Table 1 shows the chemical characterization of lignocellulosic material. Both sugarcane bagasse and residual forest biomass had a glucose/xylose ratio of around 2.2, which supported the fermentation of hexoses. Extractives are structural components of the cell wall that include salts, sugars and water-soluble polysaccharides, fatty acids or esters, long-chain alcohols, waxes, resins, steroids, phenolic compounds and glycosides, with higher content in the RFB.

Glucose and Xylose Profiles from Hydrolysis of Residual Forest Biomass

Tables 2 and 3 show the release of glucose and xylose from the residual forest biomass after treatment. For both sugars, the maximum possible concentrations were obtained by using the Organosolv process solvent.

The amount of xylose released was considerably lower than the amount of glucose released and, in the case of the 170 + 190 °C treatment with H_2SO_4 in the RFB, the amount of xylose released was practically negligible, which led to a high value of "k" (318.73 h), implying that xylose was not released. However, it should be noted that most of the xylose was initially released in the hydrothermal pre-treatment step, in a xylooligosaccharides form, remaining a smaller fraction of hemicellulose to be converted in enzymatic hydrolysis. The use of acids in

Table 2 The kinetic parameters of glucose release (C) after treatment time (t) from the residual forest biomass

Treatment	Fit	C_{max} (g L^{-1})	K (h)
170 °C	$C^{-1} = 0.9963t^{-1} + 0.2651$ ($R^2 = 0.9798$)	3.77	3.76
170 + 190 °C	$C^{-1} = 1.2163t^{-1} + 0.1494$ ($R^2 = 0.9417$)	6.69	8.14
170 + 190 °C with H_2SO_4	$C^{-1} = 1.5728t^{-1} + 0.1196$ ($R^2 = 0.9964$)	8.32	13.15
Organosolv	$C^{-1} = 0.4866t^{-1} + 0.0708$ ($R^2 = 0.9894$)	14.12	6.87

C_{max}: maximum sugar content released; k: treatment time for ½C_{max}

Table 3 The kinetic parameters of xylose release (C) after treatment time (t) from the residual forest biomass

Treatment	Fit	C_{max} (g L^{-1})	K (hours)
170 °C	$C^{-1} = 5.7845t^{-1} + 0.9386$ ($R^2 = 0.9916$)	1.07	6.20
170 + 190 °C	$C^{-1} = 11.527t^{-1} + 1.3139$ ($R^2 = 0.9704$)	0.76	8.77
170 + 190 °C with H_2SO_4	$C^{-1} = 872.49t^{-1} + 2.7359$ ($R^2 = 0.9974$)	0.37	318.73
Organosolv	$C^{-1} = 26.983t^{-1} + 0.8175$ ($R^2 = 0.9975$)	1.22	33.01

C_{max}: maximum sugar content released; k: treatment time for ½C_{max}

treatments is generally recommended to break hemicellulose bonds, leading to an increase in the yield of xylose [15]. However, H_2SO_4 might have influenced the lower concentration of xylose, thus affecting the release time of RFB and SB. Saha et al. [33], while studying wheat straw, obtained the highest glucose yields in the temperature range of 160 to 180 °C for the same sulfuric acid concentration (0.5% v/v). However, in the case of glucose, for the same amount of acid, the range was smaller, with an optimum temperature below 160 °C. Therefore, our results also suggested that temperature might have a combined effect with a greater effect of acids on xylose, which led to the release and degradation of the sugar. However, due to the interweaving of hemicellulose and cellulose fibers, cellulose can also undergo degradation, depending on the conditions used in the process (Moiser et al., 2005,[13]. In this sense, chemical and physical methods are essential for the removal of lignin and hemicellulose without damaging the cellulosic chain, preserving the characteristics for the next steps.

The release profiles of these sugars are presented in Figs. 1 and 2. The relationship between glucose and xylose is almost tenfold elevated with the Organosolv solvent and remains almost constant during the treatment period (Fig. 3). Glucose/xylose profiles for the 170 + 190 °C treatment with H_2SO_4 is not shown in this figure because the amount of xylose released was much lower than that of glucose in an equivalent period, which greatly increased the ratio between the sugars. The Organosolvation method is a promising pretreatment strategy since it demonstrated its potential to obtain sugars from lignocellulosic materials [34]. High lignin removal (around 70%) is one of its many advantages, with a minimum loss of cellulose (less than 2%). Therefore, Organosolvation seems to be the most suitable pretreatment method for plant biomass with high lignin contents, such as for residual forest biomass (Table 1).

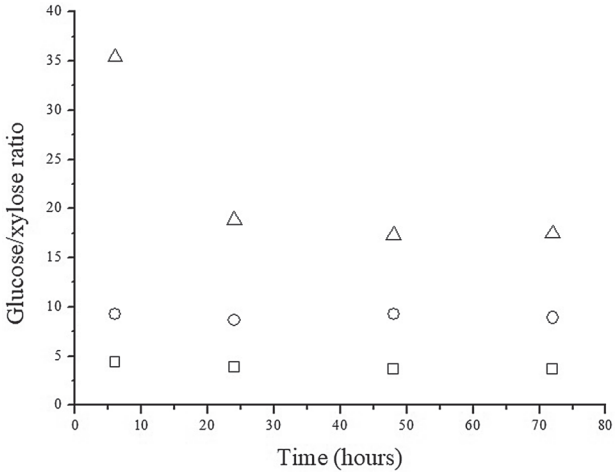

Fig. 2 Glucose/xylose ratio obtained by hydrothermal treatment 170 °C (□), 170 + 190 °C (○), and 170 + 190 °C with Organosolv (Δ) of residual forest biomass

Glucose and Xylose Profiles from Hydrolysis of Sugarcane Bagasse

Tables 4 and 5 show the release of glucose and xylose from sugarcane bagasse after treatment. For both the sugars, the maximum possible concentrations were obtained using the Organosolv solvent.

The release profiles of these sugars are presented in Fig. 3. The maximum glucose released was around 33 g L^{-1} using the Organosolv solvent after enzymatic

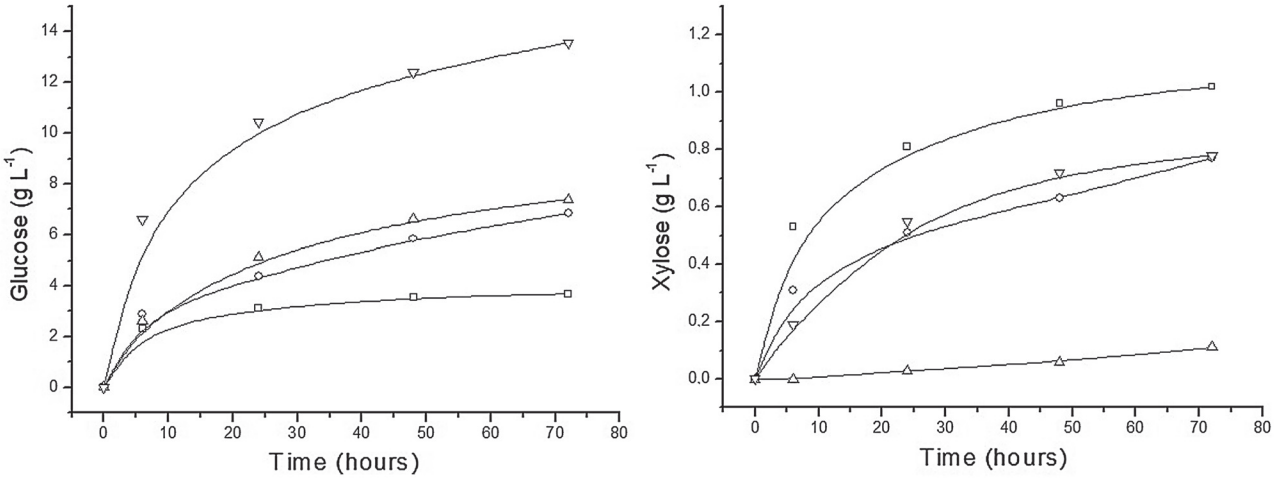

Fig. 1 Glucose and xylose release profiles obtained by hydrothermal treatment 170 °C (□), 170 + 190 °C (○), 170 + 190 °C with H_2SO_4 (Δ), and 170 + 190 °C with Organosolv (∇) of residual forest biomass

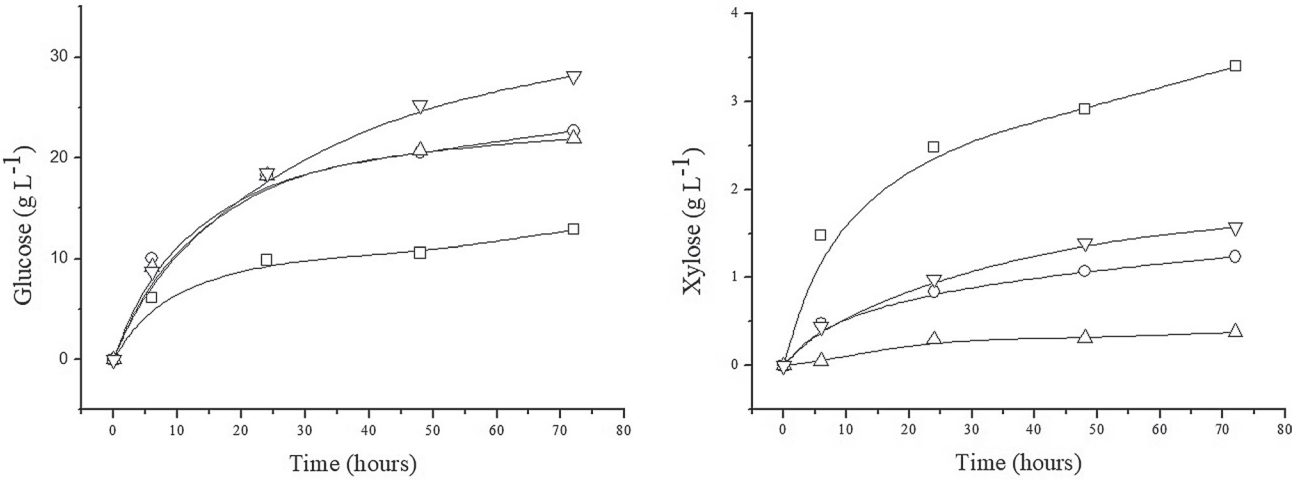

Fig. 3 Glucose and xylose release profiles obtained by hydrothermal treatment 170 °C (□), 170 + 190 °C (○), 170 + 190 °C with H_2SO_4 (Δ), and 170 + 190 °C with Organosolv (∇) of sugarcane bagasse

Table 4 The kinetic parameters of glucose release (C) after treatment time (t) from sugarcane bagasse

Treatment	Fit	C_{max} (g L^{-1})	K (hours)
170 °C	$C^{-1} = 0.5173t^{-1} + 0.078$ ($R^2 = 0.9768$)	12.82	6.63
170 + 190 °C	$C^{-1} = 0.3552t^{-1} + 0.0402$ ($R^2 = 0.9988$)	24.88	8.84
170 + 190 °C H_2SO_4	$C^{-1} = 0.4148t^{-1} + 0.0391$ ($R^2 = 0.9989$)	25.58	10.61
Organosolv	$C^{-1} = 0.5105t^{-1} + 0.03$ ($R^2 = 0.9971$)	33.33	17.02

C_{max}: maximum sugar content released; k: treatment time for ½C_{max}

Table 5 The kinetic parameters of xylose release (C) after treatment time (t) from sugarcane bagasse

Treatment	Fit	C_{max} (g L^{-1})	K (hours)
170 °C	$C^{-1} = 2.3606t^{-1} + 0.2854$ ($R^2 = 0.9880$)	3.50	8.27
170 + 190 °C	$C^{-1} = 7.9483t^{-1} + 0.7681$ ($R^2 = 0.9861$)	1.30	10.35
170 + 190 °C H_2SO_4	$C^{-1} = 119.42t^{-1} + 0.1361$ ($R^2 = 0.9813$)	7.35	877.44
Organosolv	$C^{-1} = 10.552t^{-1} + 0.5187$ ($R^2 = 0.9971$)	1.93	20.34

C_{max}: maximum sugar content released; k: treatment time for ½C_{max}

pretreatment. Furthermore, the ratio between glucose and xylose remained almost constant during treatment with these conditions (Fig. 4). Cortez et al. [35] evaluated the enzymatic hydrolysis of seven sugarcane hybrids with different levels of cell wall components as substrates for ethanol production from cellulosic and hemicellulosic fractions using the yeast *Pichia stipitis* NRRL Y-7124. The results showed that the concentration of hemicellulose had a greater negative effect on the enzymatic conversion of cellulose compared to the concentration of lignin. Mussatto et al. [36] showed that hemicellulose negatively influenced the effect of enzymes on cellulose fibers, and a greater quantity of cellulose was converted after pretreatment with a diluted acid due to the removal of hemicellulose. Studies have reported that the presence of lignin can indirectly affect the action of cellulase due to its association with hemicellulose, indicating that lignin does not act directly on cellulose accessibility but can limit the access to hemicellulose [37, 38]. According to Larsen et al. [39] and Kang et al. [40], hydrolytic enzymes might convert the remaining solid fraction of the pretreated material, composed mostly of cellulose, to produce glucose. Therefore, to completely recover monosaccharides from biomass, the cellulose fraction obtained in the pretreated solid must be hydrolyzed to be eventually converted to glucose (Ballesteros, 2010) [22].

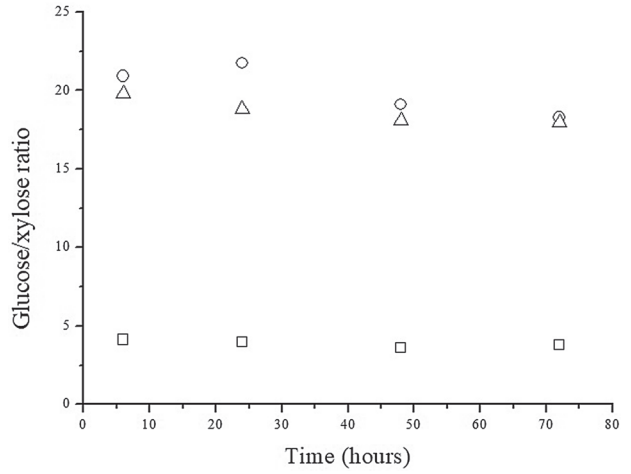

Fig. 4 Glucose/xylose ratio obtained by hydrothermal treatment 170 °C (□), 170 + 190 °C (○), and 170 + 190 °C with Organosolv (Δ) of sugarcane bagasse

Table 6 shows the glucose/xylose ratio obtained from residual forest biomass and sugarcane bagasse for the maximum sugar released with different treatments. The results indicated that the highest ratio for sugarcane bagasse was obtained through the 170 + 190 °C treatment (19.14). However, the maximum glucose was released after treatment with the Organosolv solvent (33.33 g L^{-1}). For the residual forest biomass, the highest proportion was obtained through the 170 + 190 °C treatment with H_2SO_4 due to the lower content of xylose than that of glucose. These results suggested that Organosolvation was a more efficient treatment to release sugars, considering that both the maximum glucose concentrations showed a very similar ratio in the 170 + 190 °C treatment.

The ratio between glucose and xylose is important for the application of the hydrolysates in ethanol fermentation since yeasts tend to prioritize hexoses for conversion to alcohols. *Saccharomyces cerevisiae*, a typical yeast used industrially, can rapidly ferment glucose and fructose into ethanol; additionally, they have a high tolerance to the product obtained and temperature variations [41]. However, they cannot ferment sugars such as xylose. These sugars are interesting in lignocellulosic biomass, a fact that has motivated research on yeasts capable of fermenting such sugars to produce "second-generation ethanol" [31, 42, 43].

A high ratio of glucose/xylose in the hydrolyzed biomass would enable the conversion of sugars into ethanol by *S. cerevisiae* strains. Alternatively, a yeast species that efficiently utilizes five-carbon and six-carbon sugars in this process would be advantageous. A promising species of yeast is *Meyerozyma guilliermondii* (formerly called *Candida guilliermondii*), which can produce ethanol and xylitol from sugarcane bagasse acid hydrolysates and hydrolyzed soybean hull [30, 44].

Conclusions

Sugarcane bagasse and residual forest biomass have a sugar composition that maintains a glucose/xylose ratio of around 2.2, which facilitates biotechnological use. The sugars profiles indicated that the Organosolvation process led to a greater release of glucose and xylose considering the maximum potential concentration (C_{max}) estimated for both forest residues biomass and sugarcane bagasse hydrolysates. In addition, the application of the Organosolv treatment maintained a high ratio between hexoses and pentoses in the hydrolysates, which is recommended for subsequent ethanol fermentation by yeast.

Table 6 Glucose/xylose ratio for residual forest biomass and sugarcane bagasse with the maximum sugar released after different treatments

Vegetal biomass	Residual forest	Sugarcane bagasse
Without treatment	2.20	2.16
After treatment 170 °C	3.52	3.66
After treatment 170 + 190 °C	8.80	19.14
After treatment 170 + 190 °C with H_2SO_4	22.49	3.48
After treatment with Organosolv	11.57	17.27

Author Contributions All authors contributed to the study conception and design.

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Data Availability The datasets generated during the current study are not publicly available because they are being held for publication, being made available by the corresponding author upon reasonable request.

Declarations

Competing interest The authors have no competing interest.

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