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Xylan Solubilization from Partially Delignified Biomass, and Residual Lignin Removal from Solubilized Xylan

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Abstract: Xylan is a macromolecule of industrial interest that can be solubilized from lignocellulosic materials, such as sugarcane bagasse, which is a renewable source. However, the solubilization methods of xylan need to be better developed for use in industrial applications. The main objective of this study was to evaluate xylan solubilization methods with higher yields and purity by using biomasses/fractions of sugarcane: leaf and stem, internode, node, and external fraction. Two strategies were evaluated by applying diluted sodium chlorite, sodium sulfite, and hydrogen peroxide: a delignification of the biomass before xylan solubilization; and the delignification of the solubilized xylan for residual lignin removal. The delignification of the biomass before the xylan solubilization enabled to identify material and specific conditions for yields higher than 90%. Residual lignin varied from 3.14 to 18.06%, with hydrogen peroxide in alkaline medium partial delignification shown to be effective. The delignification of xylan presented better results using diluted hydrogen peroxide, with a reduction of 58.44% of the initial lignin content. The solubilized xylans were used as a substrate for xylanase activities, resulting in higher activity than commercial xylan. In the delignification of the biomasses, hydrogen peroxide was the reagent with better results concerning the yield, purity, and solubility of the xylan. This reagent (diluted) was also better in the delignification of the solubilized xylan, resulting in lower residual lignin content. The solubility and purity tests (low salt content) indicated that the solubilized xylan presented characteristics that were similar to or even better than commercial xylan.

Keywords: hemicellulose; delignification; xylanase activity; enzymatic substrate; sugarcane bagasse; biomass heterogeneity

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1. Introduction

Xylan is the main hemicellulose present in sugarcane bagasse. Sugarcane bagasse consists of a lignocellulosic biomass that offers the advantage of being abundant in the context of ethanol and sugar production [1]. Sugarcane bagasse comprises xylan, lignin, and cellulose; the latter appearing in a high amount [2]. These components represent around 90% of the dry mass of the bagasse; the remaining 10% consists of extractives and ashes [3]. These macromolecules are highly organized in the plant cell wall, which provides high recalcitrance for this biomass to be converted into bioethanol [4], and for its macromolecules to be solubilized [1,5]. Xylan is an interesting polysaccharide to work with as feedstock for several industrial products. However, some difficulties regarding its solubilization from biomass need to be solved in the exploration of its potential.

The challenge of using biomass macromolecules to generate products with aggregate value is due to the characteristics of carbohydrates, such as xylan, which are highly protected against degradation by microorganisms or enzymes and chemical treatment. This resistance of the lignocellulosic material to the microorganism attack or the pretreatment is

called recalcitrance [4,6]. Another factor that makes the conversion of the lignocellulosic by biotechnological routes more difficult is its heterogeneity. By laying out the sugarcane stem, it can be divided into several fractions, based on its morphology: external fraction (containing the epidermis), node, and internode [7–9]. Among these fractions, the external fraction has been shown to be the one with the most resistance to acid pretreatment. This characteristic is related to the composition of this fraction, a fraction of tissue that is rigid and dense in its extremity [10] with a high number of vascular bundles close to the epidermis [7]. The internode and the node—the less recalcitrant fractions—are mostly formed by parenchyma storage cells [7] and, at a smaller proportion, vascular bundles, and the node presents vascular bundles in a higher number and diameter than the internode [7].

Xylan solubilization (macromolecule) can be performed with a high yield from sugarcane bagasse [11,12], giant bamboo [13], and Eucalyptus [14]. However, the polysaccharide presents some content of residual lignin, and there is possible salt presence, which would come from the solubilization process (the neutralization step) [11,15]. Some studies suggested that a delignification process of the biomass could result in xylan solubilization with low lignin content [16–18]. Moreover, the delignification of solubilized xylan could be a promising strategy that has not yet received its deserved attention. It is important to mention that hemicellulose/xylan can be solubilized, producing oligomers such as xylooligosaccharides (XOS) and xylose. For the purpose of xylan fragmentation/hydrolysis into XOS and xylose, hydrothermal treatments (and acids) can be successfully applied [1,8,10,14].

The quality of hemicellulose could define its application and product generation. A hemicellulose with high purity could be required for certain applications; however, impure compounds such as lignin could be present with no effect on other products. The lignin–carbohydrate complex makes it difficult to isolate hemicellulose, which shows residual lignin [19]. Obtaining high-purity hemicellulose certainly makes the economic aspect unfeasible on an industrial scale for certain products. Moreover, the products from hemicellulose could be evaluated according to the need for hemicellulose purity. Hemicelluloses have been shown to produce gels, films, coatings, adhesives, gelling, stabilizing, and viscosity-enhancing additives in food and pharmaceuticals [20]. In the energy context, hemicellulose can be hydrolyzed into fermentable sugars for fermentation (ethanol) [21]. With a catalytic approach, hemicellulose can be converted into intermediate compounds such as xylitol, furfural, and levulinic acid used for the production of chemicals and polymers [19]. Therefore, understanding the chemical compositions of the hemicelluloses and their impurities could assist in improving its industrial applications.

Considering the problems discussed, this study aimed to evaluate two strategies of xylan solubilization reducing residual lignin: delignifying the biomass before the xylan solubilization and delignifying the solubilized xylan. In order to verify which strategy would bring the greatest benefit, xylan yield, lignin content, and salt presence were monitored. Complementarily, the xylan was evaluated as a substrate for enzymatic activity in opposition to the commercial one. Enzymatic activity determination requires a great-quality substrate. In the present context, xylan was evaluated as a substrate for endoxylanase activity. Currently, a producer of xylan for use as a substrate for endoxylanase activity has discontinued the commercial product. For this reason, a protocol for xylan production focusing on the quality (i.e., the possibility of controlling the amount of contaminants) is required.

2. Material and Methods

2.1. Sugarcane Bagasse and Sugarcane for Separating the Fractions

The sugarcane (leaf, stem, and bagasse) was supplied by the Sugarcane Technology Center (CTC) in the city of Piracicaba-SP, Brazil. The stem fractions were separated into external (containing the epidermis), internode, and node, as reported elsewhere [2,8]. The separation was conducted manually, with a cut at 2–3 mm of the extremity to remove the external fraction. The stem, free of the external fraction, was fractioned into node and internode. The fractions were pressed to remove the saccharose, washed with distilled

water, and dried in stoves at 50 °C. The samples were milled and selected, passing through a 20-mesh sieve.

2.2. Delignification Strategies

Two strategies were evaluated: (i) a delignification of the sugarcane biomasses before solubilizing the xylan with a hydrogen peroxide standard method [12]; (ii) a treatment of the isolated xylan for delignification using sodium chlorite, hydrogen peroxide, and sodium sulfite (Figure 1).

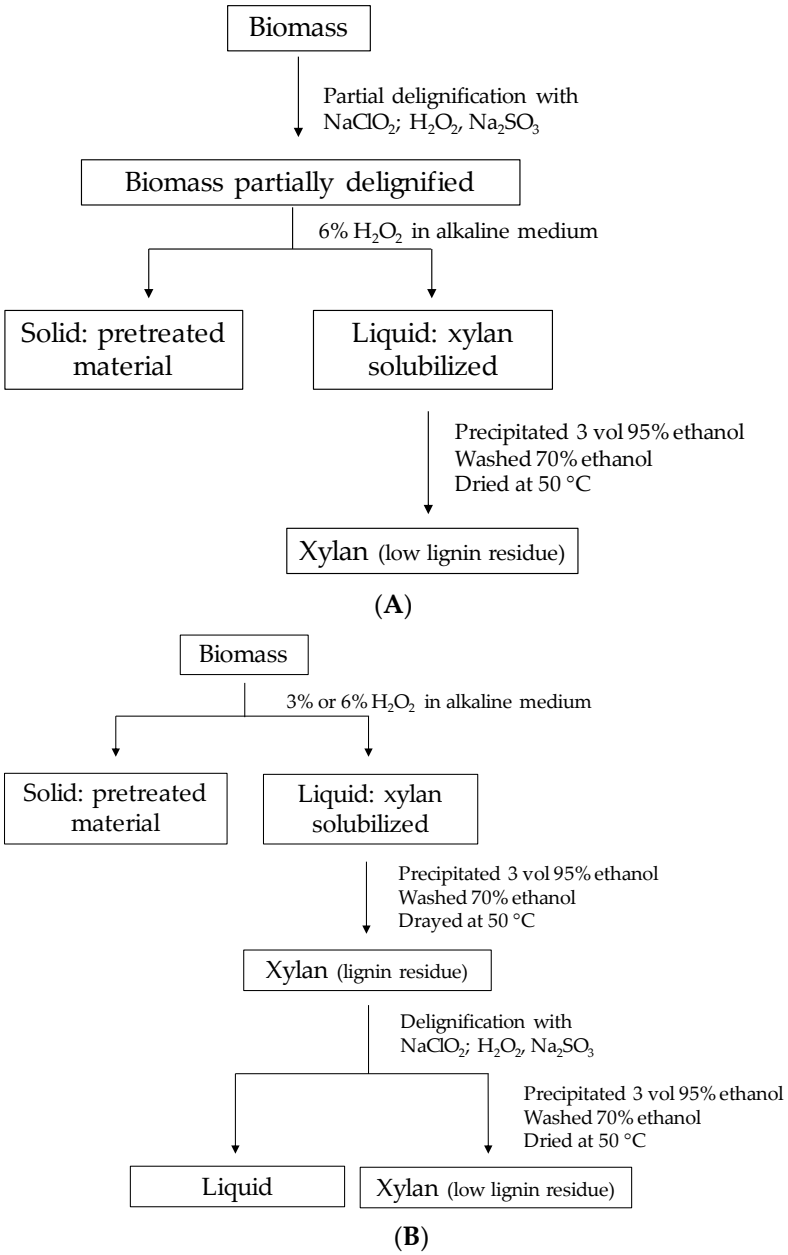

Figure 1. Strategies for high-quality xylan production with partial biomass delignification (**A**) and lignin removal from the solubilized xylan (**B**).

The delignification was conducted with specific reagents for lignin, such as sodium chlorite, hydrogen peroxide, and sodium sulfite in an alkaline medium. The sodium chlorite was compared to delignification processes [9], the peroxide to whitening the cellulosic pulp [22], and the sulfite to biomass pretreatment [23]. The reactions occurred under specific conditions for each reagent: sodium chlorite: 5, 10, and 20% (*m*/*m*) at 80 °C for 3 h,

using 10 g of material, in a reactional volume of 200 mL in a liquid medium with deionized water; hydrogen peroxide 5, 10, and 20% (*m*/*m*) at 25 °C for 4 h, also in the volume of 200 mL in liquid medium with deionized water; sodium sulfite 5, 10, and 20% (*m*/*m*) at a temperature of 121 °C/30 min, in a volume of 200 mL in liquid medium with deionized water. After the delignification processes, the samples were washed in running water until the pH was neutral, and the solid part was dried in an oven at 50 °C.

The standard xylan solubilization (Item 2.3) was conducted with the partially delignified biomasses. In the case of the delignification of the solubilized xylan, after the application of the treatments, the samples were washed with ethanol following the same methodology of xylan solubilization. Later, they were dried and washed for chemical characterization. Regarding these strategies, the solubilization yield, mass recovery, and residual lignin content were determined.

2.3. Xylan Solubilization

The xylan solubilization was conducted in triplicate with sugarcane fractions (leaf and stem: internode, node, and external fraction), sieved fractions of bagasse (provided by the industry), and with whole bagasse. The solubilization was also performed with the biomass partially delignified. The solid–liquid proportion was 1:20, using 10 g (dry mass) biomass in 200 mL of solution. The reaction was conducted with hydrogen peroxide in concentrations of 6% and 3% (*m*/*v*), adding 5 mol/L sodium hydroxide, up to a pH 11.6. The reaction volume was increased up to 200 mL with deionized water and shaken at 75 rpm, at 25 °C, for 4 h. After this period, the samples were neutralized with hydrochloric acid 5 mol/L up to a pH between 5–6. After neutralization, the samples were oven heated at 50 °C for concentration and volume reduction to around 1/3 to decrease the ethanol needed in the precipitation step. The xylan was precipitated by adding 3 volumes of ethanol 95%. After approximately 24 h, the liquid fraction was separated from the precipitated material (xylan), the supernatant was discarded, and a new wash was conducted with 70% ethanol. This procedure was repeated three times in order to avoid the formation of salt in the precipitated material. The precipitated material (xylan) was dried in an oven at 50 °C for around 72 h [12]. The xylan solubilization yield was calculated based on the amount of material solubilized in relation to the content of xylan in the raw biomass according to the chemical composition.

2.4. Chemical Characterization of Xylan

The solubilized xylan was characterized by its chemical composition, hydrolyzing about 300 mg of xylan and adding 3 mL of H_2SO_4 72% (*m*/*m*). The reaction occurred at 45 °C for 7 min and was interrupted by the addition of 84 mL of distilled water. This mixture was autoclaved at 121 °C for 30 min [12]. The liquid content was filtered in a porous plate filter n° 4, previously tared. The solid residue was washed with distilled water and dried in an oven at 105 °C up to constant mass for determining the residual lignin. The soluble fraction was used to determine the sugar and acetic acid contents by liquid chromatography. The acid-soluble lignin content was determined by absorbance measure in 205 nm. For the calculation of the soluble lignin concentration, an absorptivity of 105 L/g.cm was used in this wavelength.

The concentrations of glucose, xylose, arabinose, and acetic acid were determined by HPLC using a Bio-Rad Aminex HPX-87H (300 × 7.8 mm) column kept at 45 °C, a detector of refraction beginning WATERS 2414, a mobile phase of H_2SO_4 0.05 mol/L, a flow of 0.6 mL/min, and an injected sample volume of 20 µL. The samples were previously filtered in a syringe filter of 0.22 µm.

2.5. Xylan Solubility and Solution Conductivity

The xylan solubility was determined by preparing a 1% xylan solution in sodium acetate buffer 50 mmol/L, pH 4.8 [5]. The solution was heated up to the boiling point in a microwave oven; then, the solution was cooled and remained under 80 rpm agitation

for one night at 25 °C. The solution was centrifuged at 10,000× *g* for 15 min to separate the insoluble material (not solubilized xylan). The solid material was dried in an oven at 105 °C, up to constant mass.

The conductivity was determined for the 1% xylan solution and sodium acetate buffer 50 mmol/L, pH 4.8. The solution conductivity was measured in a conductometer to verify the presence of salt (from the neutralization step) in the solubilized xylan.

2.6. Xylan as a Substrate for Xylanase Enzymatic Activity

The enzymatic activity of xylanase (in the Celluclast cocktail—Novozymes) was determined according to the method described by Bailey, Biely, and Poutanen [24]. The quantification of reducing sugars from xylan was conducted by the 3,5-dinitrosalicilic acid method (DNS) [25]. An aliquot of 0.9 mL of 1% xylan solution (solubilized xylans and commercial birchwood xylan—Sigma Aldrich Chem. Co., St. Louis, MO, USA) was added to 0.1 mL of enzymatic extract and incubated at 50 °C for 5 min. The reaction was interrupted by adding 1.5 mL of DNS and the tubes were boiled in water for 5 min. After cooling, the absorbance was read at 540 nm. A control for each assay was also prepared by adding the DNS before the enzymatic extract. The absorbance of these controls was discounted from the respective samples.

The standard curve was built with xylose solutions (Merck) 0.25, 0.5, 0.75, 1, 1.5, and 2 mg/mL. A unit of enzymatic activity was defined as the amount of enzyme able to catalyze the liberation of 1 µmol of sugar per minute, expressed in xylose.

2.7. Nuclear Magnetic Resonance (NMR) Spectroscopy

^{1}H NMR spectra of bagasse xylan and external fraction xylan were performed using a Bruker Avance III HD 600 NMR spectrometer. Samples were measured in D_2O. Bagasse xylan: ^{1}H NMR (D_2O, 600 MHz), δ (ppm): 5.20 (m), 4.35 (H-1, d, 7.0 Hz), 3.99 (H-5_{eq}, m), 3.68 (H-4, m), 3.42 (H-3, t, 8.6 Hz), 3.29 (H-5_{ax}, t, 8.1 Hz), 3.20 (H-2, t, 8.1 Hz). External fraction xylan: ^{1}H NMR (D_2O, 600 MHz), δ (ppm): 8.46 (s), 5.39 (m), 4.49 (H-1, m), 4.34 (s), 4.33 (s), 4.12 (H-5_{eq}, m), 3.94 (s), 3.80 (H-4, m), 3.57 (H-3, m), 3.39 (H-5_{ax}, m), 3.30 (H-2, m), 1.92 (s).

2.8. Statistical Analysis

The design expert 6.0 was used for the statistical study, which consisted of the Tukey test to verify significant differences among the triplicate results. When the *p*-value was inferior to 0.05 ($p < 0.05$), differences were considered statistically significant.

3. Results and Discussion

3.1. Partial Delignification of the Sugarcane Biomass Previously to the Xylan Solubilization

The following topics are related to the results of the xylan solubilization conducted with previous biomass delignification and using different concentrations of sodium sulfite, sodium chlorite, and hydrogen peroxide. The sugarcane biomasses submitted to delignification and subsequent xylan solubilization were bagasse, internode, node, external fraction, and leaf. The xylan solubilization yields, the residual lignin content, the solution conductivity, and the solubility were determined.

The content of total hemicellulose/xylan (i.e., the sum of anhydroxylose, anhydroarabinose, acetil groups) was 28.55% for bagasse, 30.51% for internode, 29.47% for node, 28.55% for external fraction, and 23.40% for leaf. The complete chemical composition of the biomass can be accessed in a previous publication [2]. The xylan content in the material was used to calculate the solubilization yield.

The presence of xylan in extracts obtained from bagasse and the external fraction was confirmed by ^{1}H nuclear magnetic resonance (Figures 2 and 3, respectively). In both spectra, the signals were assigned to D-xylopyranosyl units (H-1-H$5_{ax/eq}$) and arabinosyl residue, linked to xylose residue (5.20 ppm and 5.39 ppm for extracts obtained from bagasse and external fraction, respectively) [26–28].

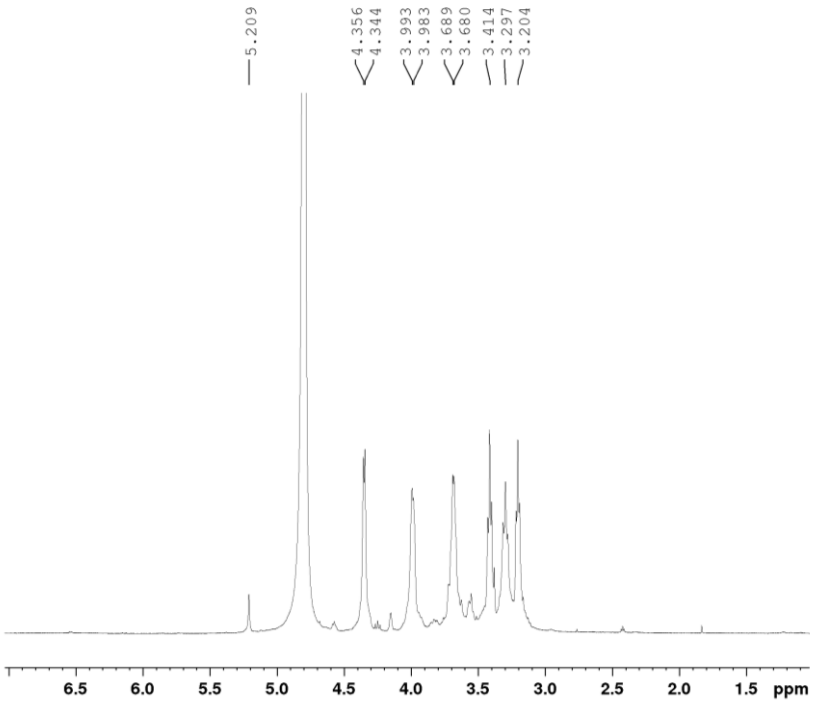

Figure 2. Xylan solubilized from sugarcane bagasse ^{1}H NMR spectrum.

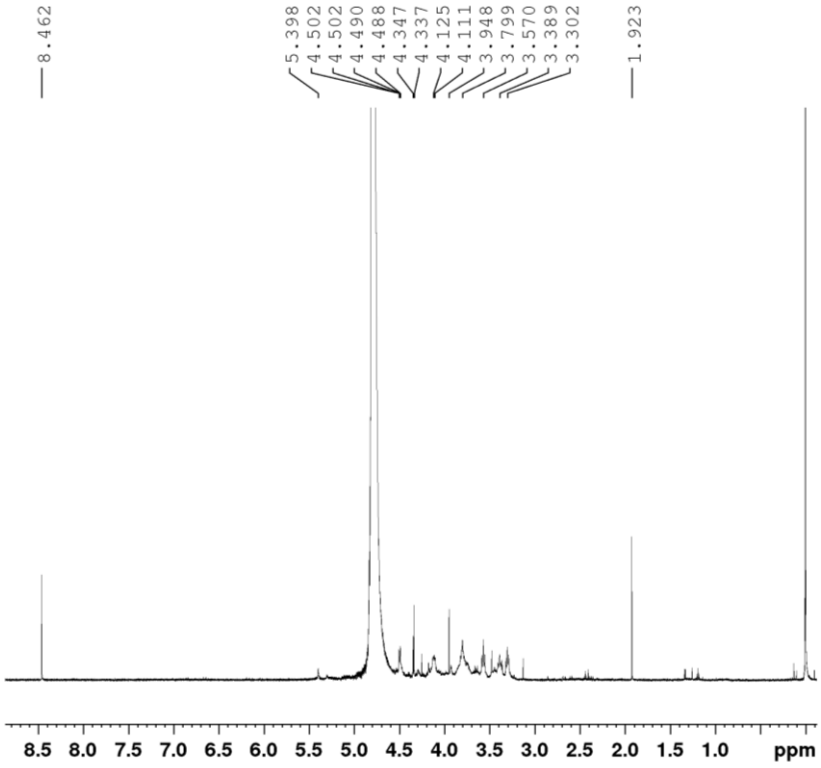

Figure 3. Xylan solubilized from external fraction of sugarcane bagasse ^{1}H NMR spectrum.

3.2. Partial Delignification with Sodium Sulfite

Xylan solubilization from a previously delignified material with sodium sulfite resulted in the best yields of 77.29, 61.58, 98.43, and 90.76% corresponding, respectively, to bagasse, internode, node, external fraction, and leaf (Table 1). Most of the best results occurred applying 10% (*m*/*m*). The exception was observed for xylan solubilization from

the internode delignified with 5% of sodium sulfite. Probably, the internode, as a less recalcitrant material compared to the external fraction, as reported in the literature [8], required a lower sodium sulfite charge. On the other hand, a higher charge of the delignification chemical could not contribute to the xylan solubilization affecting the polysaccharides. A yield of 53% of xylan solubilization was reported for sugarcane bagasse submitted to alkaline sulfite pretreatment [29]. The subsequent xylan solubilization was performed with 40% (*m*/*m*) sodium hydroxide. Alternatively, the authors applied an enzymatic xylan solubilization that resulted in 22.10%. Xylan residual lignin was higher for solubilization from bagasse, external fraction, and leaf, with 14.55%, 12.47%, and 15.28%, respectively, using 10% sodium sulfite (Table 1). Xylan solubilized from the internode and node showed lower content of residual lignin, i.e., 8.22 and 5.29%, respectively. Xylan solubilized from sugarcane bagasse submitted to alkaline sulfite pretreatment was reported as 10.50% of residual lignin [29]. In order to increase the reaction time and the reagent concentration in the delignification, an alternative could decrease the amount of residual lignin in the solubilized xylan; however, it may depend on the material.

Table 1. Xylan solubilization from sugarcane biomasses partially delignified with sodium sulfite.

Sugarcane Biomass	Sodium Sulfite (%, *m*/*m*)	Solubilization Yield (%)	Residual Lignin (%)	Conductivity (µS/cm at 25 °C)	Solubility (%, *m*/*v*)
Bagasse	5	68 ^{a}	18 ^{a}	18	94
	10	77 ^{b}	15 ^{b}	19	77
	20	53 ^{c}	12 ^{c}	14	92
Internode	5	62 ^{d}	14 ^{b}	21	94
	10	30 ^{e}	8 ^{d}	19	84
	20	41 ^{f}	6 ^{e}	11	70
Node	5	56 ^{c}	6 ^{e}	20	94
	10	79 ^{b}	5 ^{e}	18	87
	20	55 ^{c}	10 ^{d}	13	87
External Fraction	5	79 ^{b}	18 ^{a}	21	96
	10	98 ^{g}	12 ^{c}	19	79
	20	51 ^{c}	16 ^{b}	11	76
Leaf	10	91 ^{f}	15 ^{b}	19	84
	20	66 ^{a}	15 ^{b}	12	81
Birchwood Xylan	-	-	-	12	70
Sodium Acetate Buffer pH 4.8	-	-	-	10	-

(-) Not detected/determined. The same letter in a column represents a statistically similar result (*p*-value lesser than 0.05).

The solubility of the xylan provided from all the conducted treatments was shown to be better than commercial xylan, having a minimum value of 70.30% of solubility of the xylan from the internode delignified with 20% of sodium sulfite. A higher solubility (over 90%) was observed for delignified materials with 5% of sodium sulfite (Table 1).

The conductivity of xylan solutions from all treatments was higher than the control. The xylan solution from the biomasses delignified with sodium sulfite 20% (*m*/*m*) presented lower values, i.e., around 12 µS/cm at 25 °C, against 10.46 µS/cm at 25 °C for the control solution (buffer) (Table 1). The xylan extracted from the materials delignified with sodium sulfite at 5 and 10% presented higher values of conductivity; for example, 18.26 46 µS/cm at 25 °C for the bagasse. The salt content in the xylan can be avoided by increasing the number of washing steps in the xylan solubilization process [11]. For the xylan samples that had biomasses delignified with sodium sulfite 20%, the results were close to commercial xylan (12.04 µS/cm at 25 °C), with a conductivity of 10.50 µS/cm at 25 °C.

3.3. Delignification with Sodium Chlorite

The xylan solubilization from sodium chlorite partially delignified biomass varied according to the material (biomass). Delignification with 5% of sodium chloride and subsequent xylan solubilization resulted in low yield. The delignification with 10 and 20% resulted in better subsequent xylan solubilization. The higher xylan yields were 97.84, 62.87, 49.26, 99, and 90.36%, respectively, from bagasse, internode, node, external fraction, and leaf (Table 2). Naran et al. [16] obtained xylan from stover and aspen with yields of around 39% and 31%, respectively. These results show that sugarcane biomass is more suitable for the delignification procedure. Xylan solubilization from internode and node resulted in lower yields when compared to bagasse. Possibly, the delignification of these materials affected the hemicellulose content and/or structure [16]. A study reported yields between 21 and 28% of xylan solubilization (based on total mass) for several biomasses, such as pine tree, poplar, and switchgrass, whose biomass is treated with the purpose of generating energy [30]. The residual lignin was lower than 10% for all the solubilized xylan (Table 2). The lowest value of residual lignin came from one of the xylans from the internode delignified with sodium chlorite 20%, with 3.94% (Table 2).

Table 2. Xylan solubilization from sugarcane biomasses partially delignified with sodium chlorite.

Sugarcane Biomass	Sodium Chlorite (%, *m*/*m*)	Extraction Yield (%)	Residual Lignin (%)	Conductivity (µS/cm at 25 °C)	Solubility (%, *m*/*v*)
Bagasse	5	40 ^{a}	9 ^{a}	20	90
	10	98 ^{b}	9 ^{a}	17	85
	20	85 ^{c}	7 ^{b}	18	75
Internode	5	8 ^{d}	9 ^{a}	20	84
	10	22 ^{e}	8 ^{b}	15	90
	20	63 ^{f}	4 ^{c}	19	77
Node	5	41 ^{a}	5 ^{c}	20	84
	10	42 ^{a}	4 ^{c}	17	87
	20	49 ^{g}	7 ^{b}	18	76
External Fraction	5	22 ^{e}	6 ^{d}	20	91
	10	99 ^{b}	7 ^{d}	17	88
	20	85 ^{c}	6 ^{d}	19	74
Leaf	10	90 ^{h}	9 ^{a}	17	82
	20	46 ^{g}	7 ^{b}	19	81
Birchwood Xylan	-	-	-	12	70
Sodium Acetate Buffer pH 4.8	-	-	-	10	-

(-) Not detected/determined. The same letter in a column represents a statistically similar result (*p*-value lesser than 0.05).

The conductivity of the xylan solutions from all of the fractions/biomasses partially delignified with sodium chlorite 5% presented values around 20 µS/cm at 25 °C, with a slight decrease as sodium chlorite increases. The conductivity of the xylan solution varied from 15 to 18 µS/cm at 25 °C for materials partially delignified with sodium chlorite at 10 and 20%. Based on the control—the buffer acetate of 10.46 µS/cm at 25 °C—all the solubilized xylan solutions presented some salt content (Table 2).

The solubility tests of the xylan extracted from the material previously delignified with sodium chlorite showed results above the commercial xylan (69.7%) in all treatments conducted with the xylan extracted from all the sugarcane biomasses (Table 2). The lowest result found was for the xylan extracted from the external fraction delignified with sodium chlorite 20% (*m*/*m*), with values of 74.40% solubility. The highest result found was also for xylan from the external fraction; however, its lignocellulosic material was delignified with sodium chlorite at 5% m/m. The value obtained for it was 91.4% of solubility (Table 2).

3.4. Delignification with Hydrogen Peroxide

Hydrogen peroxide in an alkaline medium is a reagent used for xylan solubilization. Considering a step of partial delignification, hydrogen peroxide was applied with a low charge (5 to 20% mass of peroxide per mass of material, equivalent to 0.5 to 1% mass/volume). It was 6–12 times lower compared to the standard method (6% *m*/*v*) for xylan solubilization [12]. Even with a lower hydrogen peroxide charge, some xylan could be solubilized in the step of partial delignification.

Xylan solubilization resulted in low yields (35.55% maximum) with biomass delignified with hydrogen peroxide at 5% (*m*/*m*) (Table 3). The xylan solubilization yield increased for the material partially delignified with hydrogen peroxide at 10 and 20% (*m*/*m*). The better yields were 92.83, 97.33, 98.54, 99, and 97.56% with bagasse, internode, node, external fraction, and leaf, respectively. Both the 10 and 20% (*m*/*m*) hydrogen peroxide applied to the partial delignification resulted in good subsequent xylan solubilization with close yield values for the respective biomasses. Cashew apple bagasse was pretreated with 4.3% (*v*/*v*) hydrogen peroxide at 35 °C for 6 h, resulting in 36% xylan solubilization [31]. Corn stover and Aspen showed results of 15.9% and 28.2% of xylan, respectively, after peroxide hydroxide delignification treatment of 3% (*v*/*v*) at 95 °C for 3 h [16].

Table 3. Xylan solubilization from sugarcane biomasses partially delignified with hydrogen peroxide.

Sugarcane Biomass	Hydrogen Peroxide (%, *m*/*m*)	Solubilization Yield (%)	Residual Lignin (%)	Conductivity (µS/cm at 25 °C)	Solubility (%, *m*/*v*)
Bagasse	5	12 ^{a}	13 ^{a}	17	86
	10	93 ^{b}	13 ^{a}	18	87
	20	84 ^{c}	10 ^{b}	18	74
Internode	5	27 ^{d}	10 ^{b}	18	87
	10	97 ^{e}	7 ^{c}	17	90
	20	98 ^{e}	4 ^{d}	19	82
Node	5	36 ^{f}	7 ^{c}	17	93
	10	90 ^{b}	7 ^{c}	16	88
	20	99 ^{e}	3 ^{d}	17	94
External fraction	5	19 ^{g}	7 ^{c}	16	74
	10	88 ^{h}	10 ^{b}	18	83
	20	99 ^{e}	12 ^{a}	19	80
Leaf	10	98 ^{e}	10 ^{c}	17	85
	20	98 ^{e}	17 ^{e}	20	75
Birchwood xylan	-	-	-	12	70
Sodium acetate buffer pH 4.8	-	-	-	10	-

(-) Not detected/determined. The same letter in a column represents a statistically similar result (*p*-value lesser than 0.05).

In the solubility tests, the values obtained for the xylan solubilized after biomass delignification with hydrogen peroxide varied from the maximum of 94% for node xylan treated after biomass delignification with hydrogen peroxide at 20% m/m to the minimum of 74.40% found for bagasse xylan from the same pretreatment. These values were superior to the ones found for commercial xylan, which presented a solubility of 69.7%, thus showing evidence that the samples obtained by extraction were more soluble than commercial xylan. In most cases, the conductivity results also displayed values higher than commercial xylan, showing values ranging from 15.90 to 19.74 µS/cm at 25 °C (Table 3). For the sake of comparison, the conductivity of the commercial xylan remained at 12.04 µS/cm at 25 °C.

3.5. Delignification of the Extracted Xylan

Aiming to reduce the amount of residual lignin in the obtained xylan, a strategy for xylan delignification was evaluated. The delignification agents used were hydrogen peroxide, sodium chlorite, and sodium sulfite in the external fraction (better extraction yields) in a concentration of 10% (*m*/*m*). This concentration of the delignifying agent was chosen to present the best result in the sugarcane biomass delignification essays (Tables 1–3). With the xylan delignification process, it was observed that the color of the material becomes lighter according to the reduction of the lignin content.

The mass recovery for xylan delignification was 78.40%, 79.03%, and 87.08%, using sodium sulfite, hydrogen peroxide, and sodium chlorite, respectively (Table 4). However, the mass recovery of xylan was statistically similar. Regarding the amounts of initial and residual lignin, the hydrogen peroxide resulted in better delignification, with a reduction of 58.44% of the initial lignin content, dropping from the initial 6.04% to 2.51%. The delignification assays with sodium chlorite and sodium sulfite presented statistically similar results with respect to residual lignin.

Table 4. Delignification of the xylan extracted from the external fraction and its amount of residual lignin, solubility, and conductivity.

Delignification Reagent (10%, *m*/*m*)	Mass Recovery (%)	Initial Lignin (%)	Residual Lignin (%)	Solubility (%)	Conductivity (uS/cm at 25 °C)
Hydrogen peroxide	79 ± 6^{a}	6 ± 2	3 ± 0^{a}	96 ± 3^{a}	14 ± 0^{a}
Sodium chlorite	87 ± 3^{a}	6 ± 2	6 ± 0^{b}	95 ± 0^{a}	14 ± 0^{a}
Sodium sulfite	78 ± 9^{a}	6 ± 2	6 ± 1^{b}	94 ± 1^{a}	21 ± 0^{b}
Birchwood xylan	-	-	-	70	12
Sodium acetate buffer pH 4.8	-	-	-	-	10

(-) Not detected. The same letter in a column represents a statically similar result (*p*-value lesser than 0.05).

The solubility of the delignified xylan presented statistically similar values: the material treated with hydrogen peroxide resulted in 95.95% solubility, followed by the sodium chlorite (95.05%) and the sodium sulfite (94.2%) (Table 4). The solubility of the delignified xylan was superior to commercial xylan (69.70%) and to the non-delignified xylan (80.67%). As the xylan precipitated once more with ethanol, there could be salt formation, and for this reason the conductivity in solution was determined. The xylan delignified with hydrogen peroxide presented 13.68 µS/cm at 25 °C, while the one delignified with sodium chlorite presented 13.57 µS/cm at 25 °C. The xylan delignified with sodium sulfite presented a higher value, with 20.67 µS/cm at 25 °C (Table 4). However, none of the xylan solutions were discrepant in relation to the value of the commercial xylan solution, which was 12.04 µS/cm at 25 °C. It is important to highlight that the salt formation can be solved with ethanol wash; however, it is desirable to reduce the number of washes because of operational costs [11].

3.6. Xylanase Enzymatic Activity Using the Solubilized Xylan

The results obtained in the enzymatic activities conducted with commercial xylanase are presented in Table 5. For these essays, the enzymatic extract Celluclast (Noyozymes) was used, since it is an enzymatic cocktail with higher xylanase content. The substrates used were the external fraction of the xylan obtained in this study.

Table 5. Determination of the enzymatic activity of the commercial xylanase by using the xylan extracted from the external fraction as substrate.

Xylan Origin	Activity (UI/mL)	Xylan Solubility (%)	Lignin Content (%)
Birchwood xylan–Sigma Aldrich	106 ± 10^{a}	70^{a}	-
From the biomass delignified with 5% hydrogen peroxide	175 ± 17^{b}	86^{b}	7 ± 0^{a}
From the biomass delignified with 10% hydrogen peroxide	166 ± 23^{b}	87^{b}	10 ± 0^{b}
Xylan delignified with 10% (*m*/*m*) hydrogen peroxide	137 ± 22^{c}	96^{c}	3 ± 0^{c}
Xylan delignified with 10% (*m*/*m*) sodium chlorite	58 ± 30^{d}	95^{c}	6 ± 0^{d}
Xylan delignified with 10% (*m*/*m*) sodium sulfite	85 ± 12^{d}	94^{c}	6 ± 1^{d}

(-) Not detected. The same letter in a column represents a statically similar result (*p*-value lesser than 0.05).

Among the results obtained, except for the xylan that went through delignification with sodium chlorite 10% and sodium sulfite 10% (58.30 and 84.60 UI mL^{-1}, respectively), all of them presented higher enzymatic activity than commercial xylan (106.37 UI mL^{-1}). It is important to highlight the xylan that had substrates suffered previous delignification with hydrogen peroxide with 5 and 10% (174.97 and 166.12 UI mL^{-1}) presented higher enzymatic activity. A higher lignin content could decrease the enzymatic activity due to a higher difficulty for the action of the enzyme in the substrate. The xylan chain could probably be protected, in some regions, by the lignin presence. Another negative effect that could occur due to the presence of lignin is unproductive adsorption, making the enzyme unavailable for the reaction. However, the highest activities were determined with the substrates with higher lignin content (Table 5). The lignin contents in this study do not seem to have affected the determination of the enzymatic activity. The enzymatic activity is determined with an excess of substrate, and, in this case, the lignin probably was not an interferent factor as the content was low.

The xylan chain presented a certain complexity in its hydrolysis, needing a great diversity of xylanolytic/accessory enzymes with different specificities, i.e., physical, chemical, and biochemical characteristics, for their complete hydrolysis [15]. The length of the molecule and its degree of substitution are probably the main factors that influence the efficiency of the xylan hydrolysis. Moreover, a characteristic studied here which could influence the activity was solubility. This characteristic was better for the xylan obtained in this study than for commercial xylan. It is likely that this factor contributed to the xylan in this study obtaining better enzymatic activity compared to commercial xylan.

4. Conclusions

The partial delignification processes of the biomasses for posterior xylan solubilization (macromolecule) indicated hydrogen peroxide as a better agent. Considering all of the evaluating parameters of extraction (yield without and with previous delignification, lignin content, solubility, and conductivity), the hydrogen peroxide in an alkaline medium resulted in higher lignin removal. The hydrogen peroxide in an alkaline medium was also the best reagent for the partial delignification of solubilized xylan, resulting in higher lignin removal. Furthermore, the xylan partial delignification resulted in some mass loss, and this strategy also resulted in a material with low residual lignin content. The solubilized xylan from the previous biomass delignification and from xylan partial delignification was shown to be appropriate for the determination of xylanase enzymatic activity, displaying higher action compared to commercial xylan. A substrate for enzymatic activity determination requires great substrate quality. The low residual lignin content was not an influencing factor in the xylanase activity; this may be a suppressed effect by the high solubility of the xylan. The present study determined a protocol for xylan production with possible control of the contaminant content and residual lignin removal. The characteristics of the xylan can thus be chosen according to the application quality required.

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