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Research Article

Ethanol-Based Organosolv Pretreatment for Enhancing Enzymatic Hydrolysis of Corn Straw, Corn Cob, Sugarcane Straw, and Sugarcane Bagasse

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Abstract

Organosolv pretreatment is an attractive approach to disrupt the recalcitrant structure of lignocellulosic biomass from agroindustrial wastes, aiming to facilitate its bioconversion into valuable bioproducts. This study investigated the ability of an ethanol/water (1:1 v/v) organosolv pretreatment to improve the enzymatic digestibility of sugarcane bagasse (SB), sugarcane straw (SS), corn straw (CS), and corn cob (CC) for the production of reducing sugars and monosaccharides, aiming at future applications in bioethanol production and integrated biorefineries. After pretreatment, substantial hemicellulose and partial lignin removal were observed, with high preservation of the cellulosic fraction, mainly in SB and CC (56.11% and 55.27%, respectively). Morphological and structural alterations, such as increased porosity

and crystallinity, were also detected and attributed to the removal of amorphous regions from the pretreated biomasses, with the most prominent changes observed in CC. These modifications demonstrated the key role of organosolv pretreatment in enhancing enzymatic saccharification, thereby increasing conversion yields. The maximum release of TRS and glucose was recorded in CC (44.58 and 40.14 g/L, respectively), with high bioconversion rates of cellulose to glucose (65.42%) and hemicellulose to xylose (61.86%) after 48 h of hydrolysis. These findings highlight the potential of ethanol-based organosolv pretreatment as a sustainable, environmentally friendly, and effective strategy for enhancing biomass conversion. Furthermore, the Life Cycle Assessment (LCA) of the pretreatment and enzymatic hydrolysis processes revealed relatively moderate environmental impacts, with energy consumption as the major contributor, driven by the energy source adopted in the system.

Keywords
Biomass; pretreatment; organosolv; saccharification; life cycle assessment

1. Introduction

In Brazil, crops occupy extensive cultivated areas, and annually, their harvests generate tons of lignocellulosic residues, such as sugarcane bagasse, sugarcane straw, corn straw, and corn cob, among others. In the search for renewable energy resources, the reuse of these widely available biomasses represents a low-carbon and cost-effective alternative. These residues are composed of carbohydrates, which, when fractionated, release sugars that can be converted into second-generation (2G) ethanol and other bioproducts within the biorefinery value chain [1, 2]. For example, sugarcane bagasse can be reused to produce steam, electrical energy, second-generation (2G) ethanol, and furfuraldehyde, accounting for approximately 20% of the Brazilian energy matrix in 2020 [2, 3]. Although there are numerous environmental and technological advantages associated with the valorization of these residues, the efficient deconstruction of lignocellulosic biomass remains a bottleneck, especially in the production and commercialization of second-generation (2G) ethanol on a large scale [4]. Due to the recalcitrant nature of lignocellulosic biomass, mainly associated with the complex structure of lignin, the action of hydrolytic enzymes is limited during the bioconversion of cellulose and hemicellulose. To overcome this recalcitrance, a biomass pretreatment step is generally required. These methods allow lignin removal and reduce biomass recalcitrance, thereby improving enzymatic saccharification and increasing the release of fermentable monosaccharides, mainly glucose and xylose, which can be converted into 2G ethanol and other value-added chemical products [5].

A variety of physical, chemical, and biological pretreatment methods, as well as combinations thereof, can be applied to disrupt the recalcitrant structure of lignocellulosic biomass. The most widely used processes are alkaline, organosolv, kraft, sulfide, and steam explosion [6]. Among them, the organosolv process, which involves the fractionation of biomass using organic solvents at high temperatures, has demonstrated remarkable efficiency in enhancing enzymatic hydrolysis by promoting effective delignification [7-13]. It also promotes hemicellulose solubilization, preserves cellulose, and enables the recovery of high-purity lignin [1]. According to Wei-Kit et al. [14], the

fractionation of lignocellulosic biomass by the organosolv process is carried out with a combination of organic solvents and water at high temperatures. The most commonly used solvents include ethanol, acetone, and methanol, alone or in combination with organic acids such as acetic and formic acid [15]. The use of ethanol in organosolv pretreatment stands out for its relatively low cost, low pollutant generation, and easy recovery via distillation, which contribute to sustainability and the circular economy [16]. In summary, ethanol-based organosolv pretreatment stands out for its high efficiency in the selective removal of lignin, preserving cellulose and significantly increasing biomass enzymatic accessibility. Compared with acidic processes, it generates fewer fermentation-inhibiting compounds; compared with alkaline processes, it reduces chemical effluent generation and allows for solvent recovery. Compared with hydrothermal methods, it promotes more effective delignification and higher enzymatic hydrolysis yields, while outperforming mechanical and biological processes by reducing biomass recalcitrance more efficiently and within shorter processing times. Furthermore, it enables the recovery of high-quality lignin for high-value applications and the reuse of ethanol, making the process more sustainable and attractive for integrated biorefineries. In this context, this study investigated the ethanol:water (1:1 v/v) organosolv pretreatment of sugarcane bagasse (SB), sugarcane straw (SS), corn straw (CS) and corn cob (CC) to remove lignin and hemicellulose while preserving cellulose, aiming to enhance the enzymatic digestibility of the biomasses and their consequent bioconversion into fermentable sugars. Finally, a cradle-to-gate Life Cycle Assessment (LCA) was conducted to assess the environmental impacts of the enzymatic hydrolysis process using biomass pretreated with organosolv. Sugarcane bagasse was selected as the representative biomass for LCA because the process configuration was identical for all evaluated biomasses. The results were also compared with those reported in similar studies to identify potential opportunities for improving the environmental performance of the proposed process.

2. Materials and Methods

2.1 Lignocellulosic Biomass

Sugarcane bagasse and sugarcane straw were provided by Bionergética Aroeira S/A, located in Tupaciguara (MG, Brazil). Corn cob was kindly provided by JC Rações and Insumos Siderúrgicos Ltda, located in Uberlândia (MG, Brazil). Corn straw was obtained from Bom Jardim Colombo Farm (Araguari, MG, Brazil). Sugarcane bagasse was milled, had an initial moisture content of 51% and was dried (under ambient conditions, exposed to sunlight and air) before being subjected to organosolv pretreatment. After drying, corn and sugarcane straws were crushed in a blender until particle sizes of less than 4 mm were obtained. The corn cob was crushed in a fodder crusher, sifted, and the particles smaller than 2 mm were selected. The moisture content of the samples was measured using a moisture analyzer (Ohaus, model MB 200).

2.2 Organosolv Pretreatment

The organosolv pretreatment was carried out at the Institute of Chemistry, University of São Paulo (São Carlos, SP, Brazil). The reactions were performed in a 7 L high-pressure reactor equipped with agitation and temperature control. For the pretreatment, 500 g (dry weight) of each biomass and 5 L of an ethanol/water (1:1, v/v) solution were used under the following conditions: 180°C,

static conditions for 2 h, with a solid-to-liquid ratio of 1:10 (m/v). Afterwards, the obtained pulps were dispersed in a defibrator and vacuum-filtered to remove the liquor. Then, the pulps were washed with abundant water until no visible residues remained, vacuum-filtered, and stored for subsequent analyses. The mass yield of the pretreatment was determined by Equation 1:

$$R = \frac{M_f}{M_i} * 100 \quad (1)$$

Wherein, *R* is the pretreatment yield (%), M_f is the final dry mass of the pretreated biomass, and M_i is the initial dry mass of the raw biomass (*in natura*).

2.3 Chemical Characterization

Raw and organosolv pretreated SB, SS, CS, and CC samples were chemically characterized according to the standard methods of TAPPI (Technical Association of the Pulp and Paper Industry) and NREL (National Renewable Energy Laboratory). Extractives were determined using cyclohexane (TAPPI T204 om-88); total Klason lignin (acid-soluble lignin (ASL) and acid-insoluble lignin (AIL)) was determined according to TAPPI 222 om-88; moisture content according to TAPPI T257 om-85; ash content according to TAPPI T211 om-93; and cellulose and hemicellulose contents according to NREL LAP-002. The contents of sugars, sugar degradation products, and organic acids were determined by high-performance liquid chromatography (HPLC), according to Ntimbani et al. [17]. Mass balance equations were used to convert the analyte concentrations determined by HPLC into cellulose and hemicellulose contents [18]. The chemical composition of the solid and liquid fractions was determined in triplicate and the results were averaged.

2.4 Crystallinity Index

The crystallinity index (CI) of raw and pretreated biomasses was evaluated by X-ray diffraction (XRD) analysis, according to Segal et al. [19]. The samples were dried at 50°C for 12 h in an oven and analyzed using a Shimadzu LabX XRD-6000 diffractometer, with an operating power of 40 KV/30 mA and λ (Cu Kα) = 1.5406 Å, over a 2θ range from 5 to 40°, with a scanning rate of 2° min^{-1} and a resolution of 0.02°. The *CI* was determined by Equation 2:

$$CI = \frac{(I_{200} - I_{am})}{I_{200}} * 100 \quad (2)$$

Wherein, *CI* is the crystallinity index, I_{200} is the maximum diffraction intensity, in arbitrary units, of the (200) plane, representing both the crystalline and amorphous regions at 2θ approximately 22.5°, and I_{am} is the diffraction intensity at 2θ = 18°, representing only the amorphous region.

2.5 Scanning Electron Microscopy (SEM)

Raw and pretreated biomasses were analyzed using a Hitachi low-vacuum scanning electron microscope (Model TM3000). Before analysis, the samples were fixed on carbon tape mounted on aluminum stubs and then coated with an ultra-thin layer of gold using a sputter coater. SEM photomicrographs were recorded with a magnification of 500× at an acceleration voltage of 5.0 kV [20].

2.6 Fourier Transform Infrared Spectroscopy (FTIR)

The infrared absorbance spectra of raw and pretreated SB, SS, CS, and CC were obtained using a Shimadzu IRPrestige 21 spectrometer. For this purpose, the samples were pelletized with potassium bromide (KBr) at a concentration of 1:100 (w/w) and analyzed over the spectral range of 4000 to 400 cm^{-1}, with a spectral resolution of 4 cm^{-1} and 32 scans [20].

2.7 Enzymatic Hydrolysis

Raw and organosolv-pretreated solid fractions (cellulosic pulps) of SB, SS, CS, and CC were submitted to enzymatic hydrolysis using the commercial enzymatic cocktail Cellic CTec3® (Novozymes). The assays were carried out in 50 mL Erlenmeyer flasks containing sodium citrate buffer (0.05 mol/L, pH 4.8), an enzyme loading of 10 FPU/g of cellulose, and 10% (w/v) total solids, in a final volume of 25 mL. The saccharification assays were performed in triplicate at 150 rpm and 50°C for 72 h. The liquid fractions (hydrolysates) containing the released sugars were centrifuged at 10,000 rpm (8760 G) for 10 min and membrane-filtered (0.20 µm, Chromafil® Xtra CA-20/25). The released Total Reducing Sugars (TRS) were quantified by the 3,5-dinitrosalicylic acid (DNS) method, using 100 µL of the hydrolysate and 100 µL of DNS solution, which were heated to 100°C for 10 min in a water bath [21]. Then, 800 µL of distilled water was added to the reaction mixture, and the absorbance was measured using a UV-Vis spectrophotometer (Metash, model UV-5100) at a wavelength of 540 nm.

The concentrations of sugars (cellobiose, glucose, xylose, and arabinose), organic acids (formic acid and acetic acid), furfural (FF), and 5-hydroxymethylfurfural (HMF) were determined by High Performance Liquid Chromatography (HPLC). The hydrolysates were diluted with the mobile phase (0.1% aqueous phosphoric acid (H_3PO_4)), filtered through a 0.20 µm membrane (Chromafil® Xtra CA-20/25) and injected into the chromatographic system (Shimadzu™ model LC-20A Prominence), using a Supelcogel™ C-610H column, equipped with ultraviolet (UV) and refractive index (RI) detectors. The UV detector was used to quantify organic acids at 210 nm, whereas the RI detector was used to quantify HMF, FF, and sugars. The analyses were carried out using 0.1% (v/v) H_3PO_4 as the mobile phase, with a flow rate of 0.5 mL/min at 32°C and detection by a refractive index detector [22]. Enzymatic bioconversions (CE) were calculated, respectively, according to Eq. (3) and (4):

$$CE\%_{glucose} = \left(w_{glucose} f_{hg} / w_{sample} y_{ic}\right) * 100 \quad (3)$$

$$CE\%_{xylose} = \left(w_{xylose} f_{hx} / w_{sample} y_{ix}\right) * 100 \quad (4)$$

Where $w_{glucose}$ and w_{xylose} (g) are the amounts of glucose and xylose released after the enzymatic hydrolysis of glucan (cellulose) and xylan (hemicelluloses), respectively; w_{sample} (g) is the amount of substrate used in the enzymatic assays; f_{hg} (f_{hg} = 0.9) and f_{hx} (f_{hx} = 0.88) are the conversion coefficients related to the conversion of glucose to glucan and xylose to xylan, respectively; and y_{ic} and y_{ix} (%) represent the glucan and xylan contents, respectively, in the substrate before enzymatic hydrolysis.

2.8 Data Analysis

Analysis of variance (ANOVA) was performed, followed by the Scott-Knott test, to identify significant differences among the samples at the 5% significance level. The analyses were performed using Action 2.9 (ANOVA) and Sisvar 5.6 (Scott-Knott).

2.9 Life Cycle Assessment

Life cycle assessment (LCA) is a systematic tool that evaluates the environmental impacts of a product throughout its life cycle, including those from its production process. LCA plays a fundamental role in establishing sustainable strategies and supports decision-making through a standardized methodology that aims to minimize environmental impacts [23, 24]. The Cradle-to-Gate LCA framework was applied in this study according to the ISO 14040/14044 standards [25, 26], using Sphera's LCA for Education Database 2020 software (GaBi 9.2.1.68) [27] and the Ecoinvent database v3.8. The study consisted of four phases: (i) definition of the system's objective and scope; (ii) life cycle inventory covering all materials, inputs, energy, and environmental emissions; (iii) life cycle impact assessment using characterization factors developed under the ReCiPe 2016 environmental model, converting inventory results into potential impacts; and (iv) interpretation of the results to support decision-making regarding the evaluated system.

2.9.1 Defining Objective and Scope

The objective of this analysis was to evaluate the environmental impacts of hydrolysates produced from lignocellulosic biomass (corn cob, corn straw, sugarcane bagasse, and sugarcane straw) and identify potential improvements. The functional unit of this LCA was 1 kg of lignocellulosic hydrolysate (xylose and glucose). The system boundary was assessed from a cradle-to-gate life-cycle perspective, beginning with biomass preparation, followed by the pretreatment and enzymatic hydrolysis processes (Figure 1). The environmental contribution of the acquisition and transportation of agro-industrial residues (sugarcane bagasse and straw, corn cob and corn straw) was excluded from the analysis, considering that these materials were treated as waste streams with no environmental burdens or credits associated with their generation. According to Ro et al. [28], by adopting the concept of "waste" within the defined system boundary, the environmental burden associated with waste generation and primary management becomes the responsibility of the generator, exempting the subsequent user from these impacts.

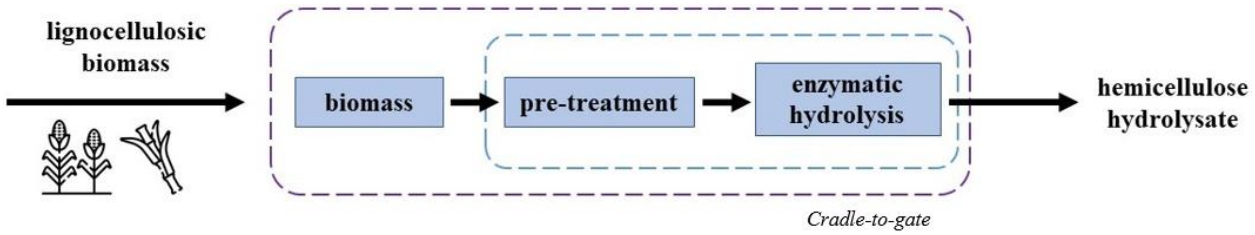

Figure 1 System boundaries for lignocellulosic hydrolysate production (Own authorship, 2026).

2.9.2 Life Cycle Inventory

The first step consisted of biomass preparation, which involved particle size reduction by grinding. This was followed by the organosolv pretreatment step, using water and ethanol in a pressure- and temperature-controlled reactor. After pretreatment, the solid fraction (cellulosic pulp) was subjected to enzymatic hydrolysis, during which cellulolytic enzymes converted structural polysaccharides, mainly cellulose, into fermentable sugars, primarily glucose. The electricity required for each process was estimated based on the nominal power of each piece of equipment and its actual operating time. All water used for washing was considered, including both imput water and wastewater output. The energy and mass balance data were normalized to the functional unit of 1 kg of lignocellulosic hydrolysate, based on results from laboratory-scale experiments. The main source of background inventory data was obtained from the Ecoinvent v3.0 database. Missing inventory data were collected from the literature.

2.9.3 Impact Assessment and Interpretation

The LCA was performed using Sphera's LCA for Education (formerly GaBi) using the ReCiPe method, Midpoint Hierarchical V1.13.2016. The environmental impact categories analyzed were terrestrial acidification (A-te); climate change (CC); freshwater ecotoxicity (Eco-f); terrestrial ecotoxicity (Eco-te); freshwater eutrophication (Eu-f); human toxicity (HT); ionizing radiation (IR); natural land transformation (NL-t); fossil depletion (FD); and water depletion (WD). For interpretation, the data were normalized using the normalization factors provided in the corresponding Simapro Database 2023. Normalization converts results to dimensionless values by relating the calculated impact to a reference value, typically the average annual per capita impact of a region or the global population. This approach makes the data comparable and provides a clearer view of the relative contributions of each category, facilitating the identification of priority mitigation areas and providing a solid basis for scientific communication and decision-making [29, 30]. Another assessment in this study evaluated total emissions associated with lignocellulosic hydrolysate production. This analysis enabled quantification of the environmental burden from atmospheric, aquatic, and terrestrial pollutants throughout the production process, providing an integrated view of the potential environmental impact.

3. Results and Discussion

3.1 Characterization of Biomasses

In the present study, raw and organosolve-pretreated SB, SS, CS, and CC were characterized for lignin, hemicellulose, cellulose, and ash content, and the mass balance was calculated for each sample (Table 1). In the raw samples, the cellulose content ranged from 33.18 (CC) to 41.25% (SB), hemicellulose from 28.86 (SS) to 35.15% (CC), and lignin from 19.04 (CC) to 27.06% (SB), clearly indicating that cellulose was the major structural component of all evaluated biomasses. The obtained compositional values were consistent with those reported in the literature for these untreated biomasses [31-35]. After the organosolv pretreatment, the pulp yields (solid fractions) were: 65.95% for SB, 51.00% for SS, 52.37% for CS, and 49.41% for CC, corresponding to mass losses of 34.05, 49.00, 47.63, and 50.59%, respectively. These data indicate distinct pulp yields among the

investigated biomasses, with the highest yield observed for pretreated SB, while CC exhibited the lowest yield. The observed mass loss after the organosolv pretreatment suggests the efficient removal of non-cellulosic components, such as hemicelluloses and lignin, due to their solubilization, resulting in solid fractions rich in cellulose.

Table 1 Chemical composition of raw and organosolv-pretreated sugarcane bagasse (SB), sugarcane straw (SS), corn straw (CS), and corn cob (CC).

Parameter	Raw SB	PT SB	Raw SS	PT SS	Raw CS	PT CS	Raw CC	PT CC
Cellulose (wt.%)	41.25 ± 4.59	56.11 ± 4.07	38.30 ± 0.80	51.23 ± 7.48	31.97 ± 4.15	50.70 ± 3.98	33.18 ± 1.15	55.27 ± 2.13
Hemicelulose (wt.%)	29.30 ± 2.96	12.87 ± 1.07	28.86 ± 3.53	18.31 ± 6.21	34.30 ± 3.12	25.13 ± 0.77	35.15 ± 1.06	18.40 ± 1.19
Total Lignin (wt.%)	27.03 ± 1.01	21.05 ± 2.04	25.70 ± 1.58	22.42 ± 2.38	21.80 ± 1.29	17.51 ± 0.42	19.54 ± 0.85	17.74 ± 1.39
Ash (wt.%)	2.87 ± 0.59	UN	1.37 ± 0.34	UN	3.91 ± 1.50	UN	6.84 ± 0.73	UN
Celullose/Lignin ratio	-	2.66	-	2.28	-	2.89	-	3.11
Cellulose/Hemicellulose ratio	-	4.36	-	2.8	-	2.01	-	3.00
Mass Balance (wt.%)	100.45 ± 2.18	90.03 ± 2.39	94.23 ± 2.33	91.96 ± 5.35	91.99 ± 2.51	93.34 ± 1.72	94.71 ± 0.94	91.41 ± 1.34

UN: Undetected.

After the organosolv pretreatment, the highest hemicellulose removals were observed in SB and CC (56.07 and 47.65%, respectively). These significant reductions in hemicellulose content are particularly interesting, since hemicelluloses are known to hinder the efficiency of biomass conversion processes due to the formation of undesirable by-products and competition for enzymatic activity [36]. Thus, organosolv pretreatment is recognized as an effective technique for removing unwanted components from lignocellulosic biomass, such as hemicelluloses and lignin, thereby increasing cellulose accessibility for subsequent biochemical conversion processes [6]. Previous studies on different biomasses, such as SB and CC, have reported similar results, corroborating the findings of the present study [13, 37, 38].

Regarding lignin, the greatest reduction was also observed in SB (22.12%). These results indicate that applied the organosolv pretreatment was more efficient at removing hemicellulose than lignin, probably because no alkaline washing step was performed after pretreatment. Thus, lignin may have been fragmented during organosolv pretreatment, only to be redeposited onto the cellulose fibers. These results agree with those of Sun et al. [9], who investigated the effect of organosolv pretreatment on the lignin and biomass composition of sugarcane bagasse. On the other hand, the cellulose content increased in all pretreated biomasses, especially in CC (66.57%), indicating the preservation of this polysaccharide. These results agree with those of Rabelo et al. [13], who demonstrated that organosolv pretreatment effectively preserves cellulose in the solid pulp, which is crucial for the effectiveness of the pretreatment process and the subsequent saccharification of cellulose.

Based on the cellulose/lignin and cellulose/hemicellulose ratios, the efficiency of ethanol-based organosolv pretreatment for each biomass was demonstrated. The cellulose/lignin ratio was higher in CC (3.11), while the cellulose/hemicellulose ratio was higher in SB (4.36), indicating greater cellulose accessibility to enzymatic attack in SB. These results also suggest that organosolv pretreatment promoted the fragmentation and reduction of lignin and hemicellulose, while the cellulosic fraction was preserved. Park et al. [31] applied organosolv pretreatment to corn straw under optimized conditions (56.6% ethanol and a reaction temperature of 187.5°C) and also achieved high lignin removal and a hemicellulose removal of 59.82%. Furthermore, Gurgel et al. [18] obtained solid fractions with high degrees of delignification (82.5%) at 140°C through organosolv pretreatment of sugarcane bagasse using a 1:1 (v/v) ethanol/water mixture assisted by high-pressure carbon dioxide. These data indicate that the use of organic solvents such as ethanol in organosolv pretreatment promotes the cleavage of lignin-lignin and carbohydrate-lignin linkages and, consequently, represents an effective strategy for the deconstruction of lignocellulosic biomass [7]. During the process, hemicellulose and lignin are fragmented and dissolved into the liquor, while cellulose is retained in the solid pulp. This high hemicellulose solubilization and partial lignin removal increase pore volume and cellulose surface area, thereby enhancing enzyme accessibility during the subsequent saccharification step, demonstrating that the organosolv method is a promising and effective pretreatment strategy [16].

3.2 FTIR Analysis

Infrared spectroscopy was employed to investigate alterations in the chemical structure and functional groups of raw and organosolv-pretreated biomasses. The FTIR vibrational spectra of the raw samples revealed typical characteristics of lignocellulosic biomass, such as the presence of an

absorption band associated with the stretching vibrations of hydroxyl (OH) groups in cellulose between 3600 and 3200 cm^{-1}, as well as a band in the range of 3000 to 2700 cm^{-1}, which indicates the presence of aliphatic carbon-hydrogen (C–H) stretching vibrations (Figure 2). Additionally, absorption bands in the range of 1700 to 1500 cm^{-1} were observed, suggesting the presence of aromatic structures characteristic of lignin and other phenolic groups. Absorption bands in the range of 1300 to 800 cm^{-1}, associated with hemicellulosic functional groups and other lignocellulosic components, were also identified [39]. After the organosolv pretreatment, the resulting spectra showed structural changes, as evidenced by changes in the intensity or disappearance of characteristic absorption bands, which can be attributed to the removal of lignin and hemicelluloses (Figure 2). The absorption band at 3437 cm^{-1}, attributed to the stretching vibration of the hydroxyl (OH) group in cellulose, was observed in all samples, indicating limited degradation of this component after pretreatment [39]. Furthermore, the absorption bands at 2918 cm^{-1} and 2852 cm^{-1}, attributed to aliphatic C–H stretching vibrations, were observed to increase in the CS and CC samples due to the higher preservation of cellulose and hemicelluloses [39]. These results suggest that the organosolv process was effective in maintaining the integrity of these components, possibly because it solubilizes lignin and other undesirable compounds without causing significant degradation of the carbohydrate fractions. On the other hand, the pretreated SS sample showed a decrease in the intensity of these absorption bands, indicating a more pronounced degradation of cellulose and hemicelluloses during the pretreatment. Pongchaiphol et al. [40] applied the ethanosolv pretreatment to different types of lignocellulosic biomass, such as sugarcane bagasse, sugarcane straw, and corn cob, and reported similar results.

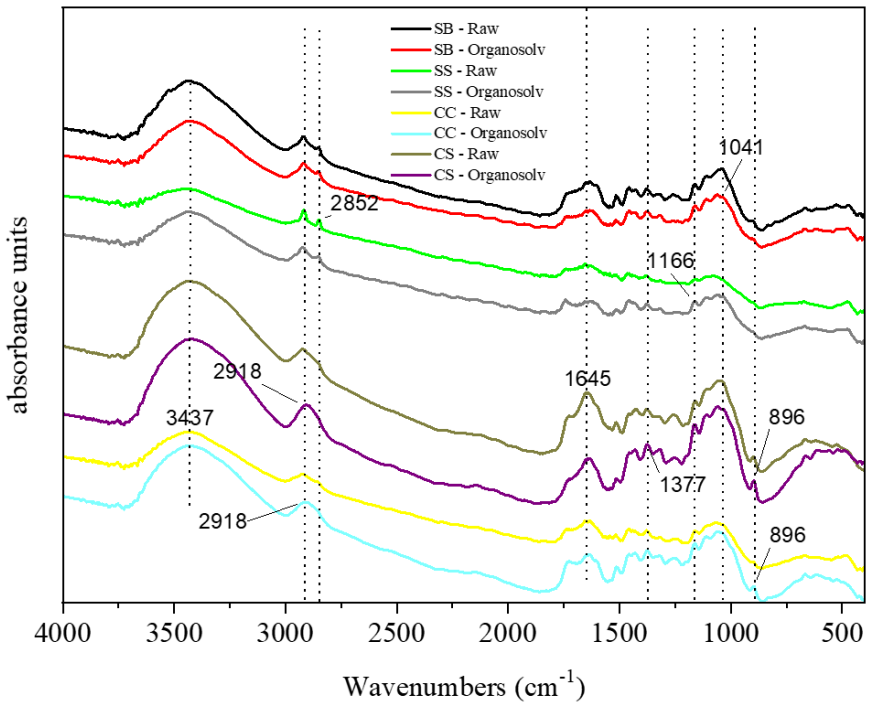

Figure 2 FTIR spectra of raw and organosolv-pretreated sugarcane bagasse (SB), sugarcane straw (SS), corn straw (CS), and corn cob (CC).

The vibration peak at 1645 cm^{-1} is characteristic of the presence of aldehyde groups (C=O) in lignin. After organosolv pretreatment, a decreasing trend in the intensity of this peak was observed,

especially in SB and SS. This intensity reduction indicates that the organosolv pretreatment was effective in modifying or removing the aldehyde groups from lignin. According to Sun et al. [9], aldehyde groups in lignin can be highly reactive and susceptible to degradation or modification reactions during pretreatments. Thus, the decrease in the intensity of this peak suggests that the organosolv method was able to reduce the presence of these groups, possibly due to partial solubilization or modification of lignin. Specifically, the more pronounced decrease observed for this peak in SB can be attributed to the specific characteristics of this biomass, such as the chemical composition and lignin structure. It is possible that lignin from SB is more susceptible to modification or removal of aldehyde groups during organosolv pretreatment in comparison to other biomass sources. Tang et al. [41] reported similar results with a decrease in the intensity of the peaks at 1603, 1510, and 1423 cm^{-1} (aromatic skeletal vibrations in lignin) after alkali-catalyzed organosolv pretreatment of corn straw.

The stretching vibration at 1377 cm^{-1} in CS represents the C–H groups of aliphatic chains, characteristic of cellulose and hemicelluloses. The presence of this peak is associated with the integrity and preservation of these biomass components after the organosolv pretreatment. Since aliphatic groups are less susceptible to degradation reactions, the maintenance of the intensity of this peak suggests the effectiveness of the pretreatment in preserving the structures of cellulose and hemicelluloses [9]. On the other hand, the stretching vibration of the peak at 1166 cm^{-1} in SS, attributed to ester groups of lignin (C=O), suggests the presence of lignin, and the in-plane deformation band at 1041 cm^{-1} (C–O) indicates the removal of part of the hemicelluloses, especially in SB and CS. Besides, the reduction in the intensity of this peak suggests a decrease in the amount of hemicelluloses in the samples, which is consistent with the removal of these components during the organosolv pretreatment. These results agree with those reported by Tang et al. [41], who also observed similar changes in alkali-catalyzed organosolv-pretreated cornstalks, reinforcing the effectiveness of the organosolv method in modifying and preserving the constituents of lignocellulosic biomass.

The stretching vibration observed at the absorption peak around 896 cm^{-1} in pretreated CC and CS is characteristic of the β-1,4 glycosidic bond, indicating that the cellulose structure was preserved during the pretreatment in these samples [39]. This finding is consistent with Qing et al. [42], who detected a peak at 899 cm^{-1} after the organosolv pretreatment of corn straw and attributed it to β-1,4-glycosidic linkages between sugar units in cellulose, indicating the preservation of cellulose. These results indicate that the changes that occurred in the structures of these pretreated biomasses are associated with the removal and modification mainly of the lignin and hemicelluloses fractions. These structural alterations align with the chemical composition and respective percentages of lignin, hemicellulose, and cellulose in each evaluated biomass after organosolv pretreatment (Table 1) and allow the formation of fibers more suitable for the subsequent enzymatic saccharification step.

3.3 X-Ray Diffraction (DRX)

Crystallinity is a measure of the molecular organization of cellulose, which influences the accessibility of enzymes during the hydrolysis and, consequently, affects ethanol production efficiency. X-ray diffraction (XRD) was employed to examine the crystallinity level of the investigated lignocellulosic biomasses. The diffractograms were analyzed to determine the crystallinity index (CI)

in the organosolv-pretreated biomasses and to compare them with the raw samples (Figure 3). The results showed that CI increased in the pretreated samples in comparison with the raw biomasses. According to Zhang and Wu [43], an increase in crystallinity indicates a more organized and compact cellulosic structure. Besides, the use of organic solvents in organosolv pretreatment partially removes lignin and hemicellulose, resulting in a more concentrated cellulose fraction, which leads to an increase in crystallinity and greater exposure of cellulose chains.

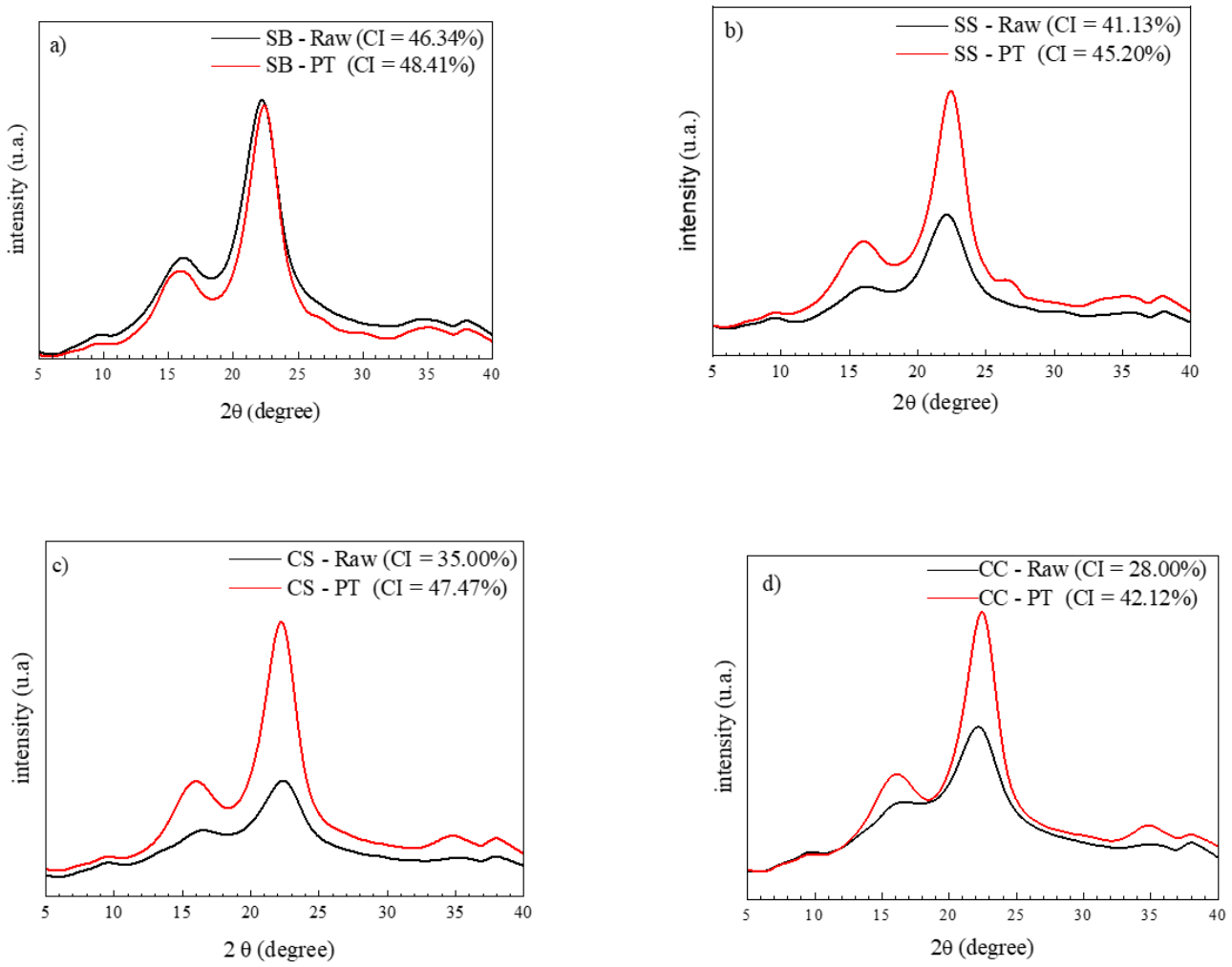

Figure 3 X-ray diffractograms and crystallinity index of raw and pretreated (a) sugarcane bagasse (SB), (b) sugarcane straw (SS), (c) corn straw (CS), and (d) corn cob (CC).

After the organosolv pretreatment, SB exhibited the highest crystallinity index (48.41%), followed by CS (47.47%), SS (45.20%), and CC (42.12%). Although all samples increased their CI after pretreatment, sample CC showed the greatest increase in CI, rising from 28.00% to 42.12%. This indicates that the greatest structural change observed occurred in this biomass. These findings are consistent with the characterization of the biomass (Table 1), which revealed that CC exhibited the highest mass loss after the pretreatment. In addition, these results agree with those of Sannigrahi et al. [44], who reported that the degree of crystallinity increases under more severe organosolv pretreatment conditions and is attributed to the preferential degradation of less structured regions of cellulose. In this context, the increase in crystallinity may be associated with the removal of amorphous fractions present in the pretreated biomass, particularly hemicellulose, resulting in an

increase in the cellulose content, as described in Table 1. Zhang and Wu [43] pretreated sugarcane bagasse by the organosolv method with ethanol at 180°C and also observed an increase in the crystallinity, with a CI value of 57.32% in the pretreated SB compared with 37.24% in the raw sample.

According to Zhang and Wu [43], delignification and removal of amorphous hemicellulose during ethanol-based organosolv pretreatment are the main contributors to the increase in the crystallinity index of the pretreated solids. These authors calculated the crystallinity index based on the crystalline cellulose content of pretreated sugarcane bagasse. Furthermore, when the pretreatment temperature increased from 160 to 200°C, the CI gradually increased, indicating that greater amounts of lignin and hemicellulose were removed at higher temperatures. Therefore, the increase in crystallinity or the prevalence of more crystalline regions in pretreated samples from the current study is consistent with the significant reduction in the hemicellulosic fraction, as well as the partial removal of lignin, suggesting that the organosolv pretreatment preserved the cellulose fraction, thereby facilitating enzymatic hydrolysis.

3.4 Morphological Changes in Solid Fractions

Scanning electron microscopy (SEM) has been widely used in studies of lignocellulosic waste characterization, as it allows visualization of changes in the matrix structure after pretreatments [45-47]. The SEM analysis of raw biomasses exhibited dense, smooth, and compacted structures (Figure 4 and Figure 5). In contrast, organosolv-pretreated samples revealed more relaxed structures, with the presence of loosening, fissures, and fiber delamination, thereby allowing greater exposure of cellulose. Similarly, the emergence or increase in the number of small pores was also observed (Figure 4 and Figure 5).

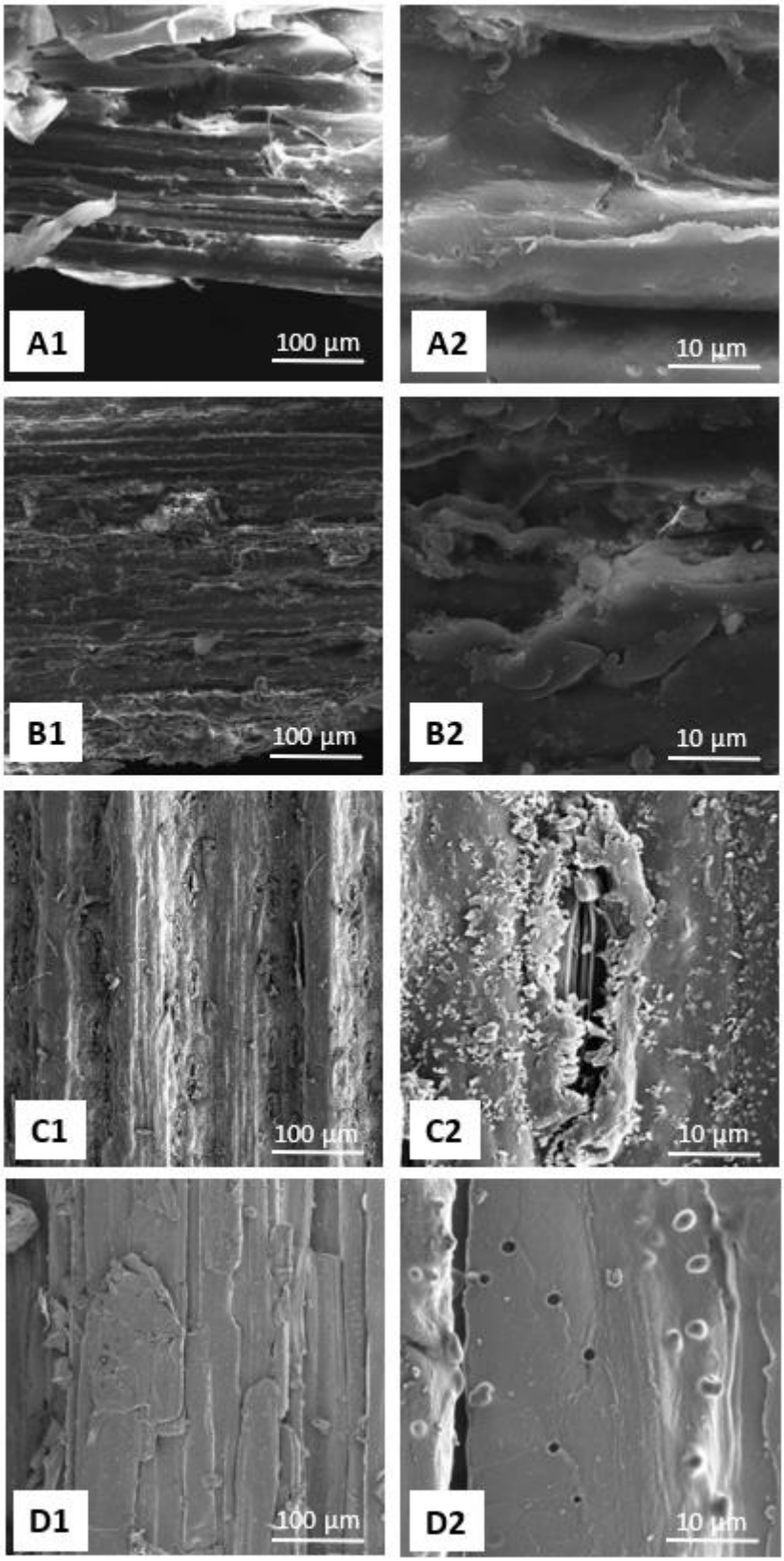

Figure 4 Scanning electron micrographs of raw sugarcane bagasse (A1 and A2), organosolv-pretreated sugarcane bagasse (B1 and B2), raw sugarcane straw (C1 and C2), and organosolv-pretreated sugarcane straw (D1 and D2). (1: Magnification of 500× and 2: 5000×).

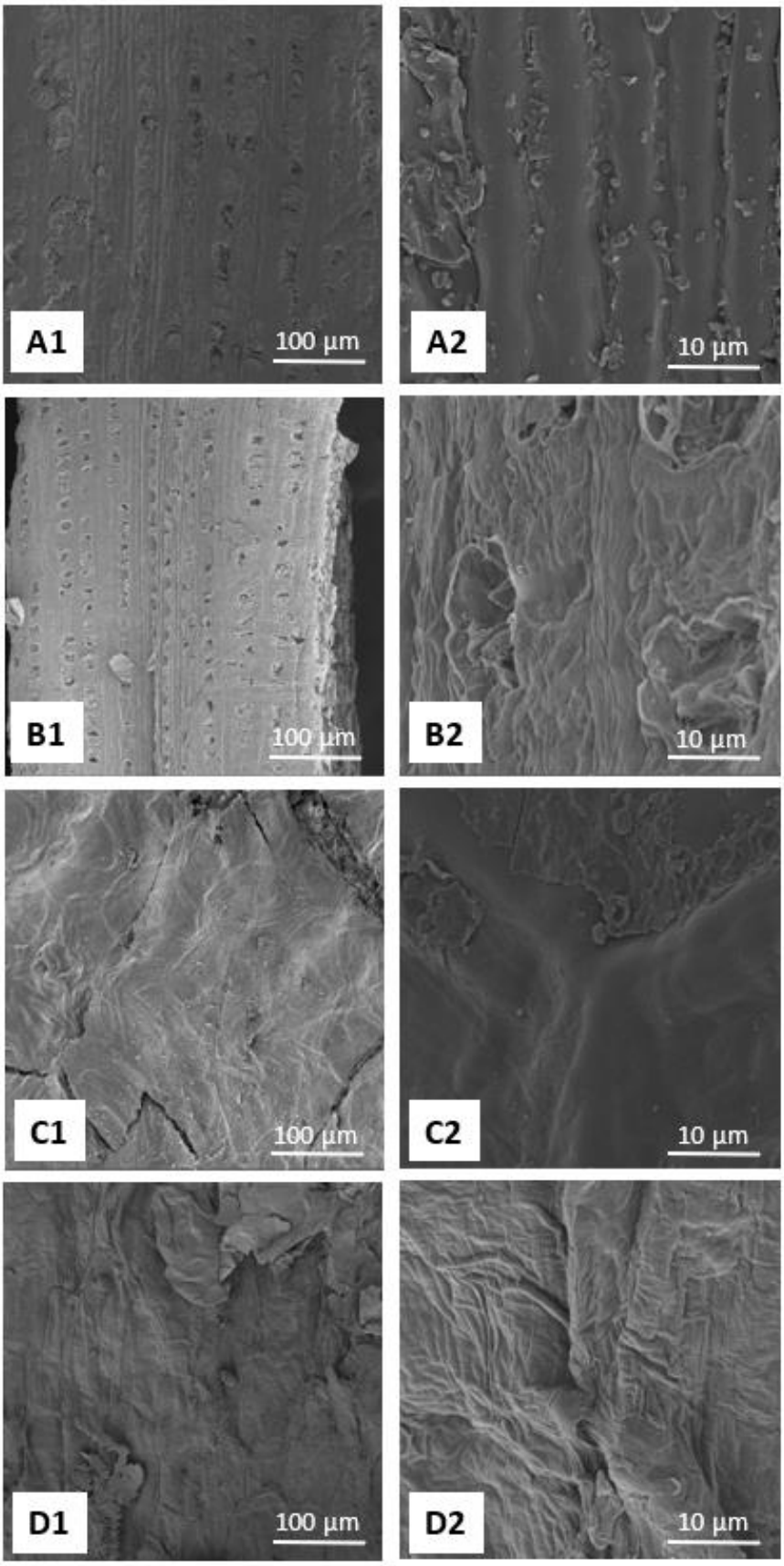

Figure 5 Scanning electron micrographs of raw corn straw (A1 and A2), organosolv-pretreated corn straw (B1 and B2), raw corn cob (C1 and C2), and organosolv-pretreated corn cob (D1 and D2). (1: Magnification of 500× and 2: 5000×).

This observation suggests that the organosolv pretreatment significantly altered the biomass structure, making it more accessible to subsequent processes, such as cellulose conversion into bioproducts. These morphological changes highlight the influence of organosolv pretreatment on the biomass structure, facilitating the enzymatic attack during the subsequent saccharification stage and leading to higher conversion yields [44, 47]. Zhang and Wu [43] compared the structure of native and ethanol-based organosolv-pretreated sugarcane bagasse and observed similar results. They found that the raw material exhibited an intact, smooth fibrillar structure, without fissures or fragments, whereas the pretreated SB displayed considerable surface roughness, indicating a significant defibration process. The present results are also consistent with those reported by Nantnarphirom's et al. [48], who observed an analogous phenomenon after the organosolv pretreatment of sugarcane bagasse with ethanol/water at various concentrations at 180°C. This process increased material porosity due to structural damage from partial removal of hemicelluloses and lignin, leading to changes in its structure, including the formation of pores and fissures. These structural alterations can enhance the accessibility of enzymes to cellulose for subsequent industrial applications. Li et al. [10] also verified the dissolution of the rigid structure formed by the lignin-hemicellulose-cellulose interactions after the organosolv pretreatment catalyzed by NaOH at 205°C, which resulted in an improvement in the performance of the biomass conversion into energy and chemical products. Based on these data, the modifications observed in the SEM analysis in the present study can be attributed to partial removal of hemicelluloses and lignin, indicating that the ethanol-based organosolv pretreatment contributed to the degradation of the recalcitrant lignocellulosic matrix of the evaluated samples.

3.5 Enzymatic Hydrolysis of Raw and Pretreated SB, SS, CS, and CC

The pretreated biomasses were hydrolyzed to determine the effect of organosolv pretreatment on cellulose accessibility to enzymatic attack. After saccharification, the hydrolysates were analyzed for total reducing sugars (TRS) (Table 2), potential inhibitors (organic acids, HMF, and furfural) (Table 3), and free sugars (glucose, xylose, and arabinose) (Table 4). The saccharifications were performed at different time intervals to investigate the optimal release of TRS (Table 2). In the raw hydrolysates, the highest TRS production was reached after 72 h. However, no statistical differences were observed between 48 and 72 h for raw SB, SS, and CC. In addition, TRS values were not noteworthy in the raw samples due to their inherent recalcitrant nature.

Table 2 Total reducing sugars (g/L) after enzymatic saccharifications of raw and pretreated sugarcane bagasse (SB), sugarcane straw (SS), corn straw (CS), and corn cob (CC). Assays were carried out at 50°C, with 10% solids and an enzymatic load of 10 FPU/g of cellulose. Aliquots were taken after 24, 48, and 72 h.

	Time (h)	TRS (g/L)			
		SB	**SS**	**CS**	**CC**
Raw	24	9.70 ± 0.76	15.15 ± 3.56	6.18 ± 1.86	9.54 ± 0.23
	48	12.75 ± 1.51	15.96 ± 0.88	10.19 ± 0.25	10.07 ± 0.89
	72	13.12 ± 1.19	16.67 ± 1.10	15.50 ± 0.87	10.61 ± 1.71
Pretreated	24	18.63 ± 3.38	12.75 ± 0.27	13.71 ± 1.47	27.46 ± 0.99
	48	20.00 ± 0.35	14.71 ± 1.75	21.32 ± 1.84	33.95 ± 1.13
	72	25.43 ± 1.79	19.95 ± 1.48	22.61 ± 1.02	44.85 ± 1.91

Table 3 Concentrations (g/L) of organic acids, HMF, and FF after enzymatic saccharification of raw and pretreated sugarcane bagasse (SB), sugarcane straw (SS), corn straw (CS), and corn cob (CC). * PT = Pretreated.

Inhibitors (g/L)	**Time (h)**	**Raw SB**	**PT SB**	**Raw SS**	**PT SS**
Formic Acid	24	0.07 ± 0.07	0.05 ± 0.001	0.42 ± 0.36	0.06 ± 0.003
	48	-	0.12 ± 0.001	0.11 ± 0.002	0.07 ± 0.001
	72	0.06 ± 0.005	0.07 ± 0.006	0.09 ± 0.01	0.07 ± 0.005
Acetic Acid	24	0.31 ± 0.004	0.18 ± 0.04	0.63 ± 0.41	0.19 ± 0.004
	48	0.13 ± 0.01	0.09 ± 0.007	0.71 ± 0.02	0.22 ± 0.02
	72	0.17 ± 0.09	0.13 ± 0.02	1.14 ± 0.34	0.18 ± 0.01
HMF	24	0.12 ± 0.002	-	0.07 ± 0.003	-
	48	0.24 ± 0.02	0.05 ± 0.006	0.09 ± 0.02	-
	72	0.23 ± 0.002	0.05 ± 0.003	0.11 ± 0.004	-
FF	24	-	-	-	-
	48	-	-	-	-
	72	-	-	0.12 ± 0.005	-
Inhibitors (g/L)	**Time (h)**	**Raw CS**	**PT CS**	**Raw CC**	**PT CC**
Formic Acid	24	0.06 ± 0.002	0.0 ± 0.009	0.11 ± 0.02	0.09 ± 0.003
	48	0.45 ± 0.13	0.06 ± 0.006	0.49 ± 0.47	0.37 ± 0.03
	72	0.17 ± 0.01	0.07 ± 0.004	0.46 ± 0.22	0.22 ± 0.06
Acetic Acid	24	0.50 ± 0.01	0.21 ± 0.04	0.83 ± 0.02	0.74 ± 0.03
	48	1.63 ± 0.44	0.22 ± 0.004	0.69 ± 0.16	1.52 ± 0.05
	72	0.08 ± 0.01	0.19 ± 0.11	0.67 ± 0.26	0.97 ± 0.14
HMF	24	0.08 ± 0.01	0.08 ± 0.003	0.05 ± 0.003	0.05 ± 0.003
	48	0.07 ± 0.004	0.08 ± 0.004	0.06 ± 0.001	0.05 ± 0.003
	72	0.08 ± 0.02	0.08 ± 0.005	0.07 ± 0.001	0.05 ± 0.001
FF	24	-	-	-	-
	48	-	-	-	-
	72	-	-	-	-

Table 4 Concentrations (g/L) of monosaccharides and cellobioses after enzymatic saccharification of raw and pretreated sugarcane bagasse (SB), sugarcane straw (SS), corn straw (CS), and corn cob (CC). * PT = Pretreated.

Sugars (g/L)	Time (h)	Raw SB	PT SB	Raw SS	PT SS
Glucose	24	4.12 ± 0.08	9.60 ± 2.44	6.78 ± 2.04	12.67 ± 0.06
	48	4.76 ± 0.89	19.15 ± 1.15	8.28 ± 0.02	14.55 ± 0.91
	72	4.60 ± 1.01	19.17 ± 1.26	8.74 ± 0.31	14.44 ± 0.55
Xylose	24	1.868 ± 0.36	3.55 ± 1.22	1.52 ± 0.45	2.71 ± 1.12
	48	1.697 ± 0.00	3.26 ± 0.30	2.24 ± 0.20	2.20 ± 0.18
	72	2.85 ± 0.30	4.579 ± 1.57	2.17 ± 0.03	2.59 ± 0.08
Arabinose	24	0.75 ± 0.02	-	0.52 ± 0.14	-
	48	0.11 ± 0.001	-	0.77 ± 0.03	-
	72	0.03 ± 0.003	-	0.87 ± 0.07	-
Cellobiose	24	1.23 ± 0.015	0.01 ± 0.12	-	0.02 ± 0.00
	48	0.01 ± 0.00	0.21 ± 0.05	-	0.09 ± 0.00
	72	-	0.18 ± 0.03	-	0.05 ± 0.00
Sugars (g/L)	**Time (h)**	**Raw CS**	**PT CS**	**Raw CC**	**PT CC**
Glucose	24	4.13 ± 0.39	21.18 ± 1.25	4.07 ± 0.30	22.48 ± 0.70
	48	2.80 ± 0.05	21.51 ± 0.24	3.64 ± 1.13	38.32 ± 1.09
	72	7.19 ± 3.18	25.69 ± 1.55	2.58 ± 1.69	40.14 ± 1.54
Xylose	24	2.36 ± 0.38	7.92 ± 0.37	3.31 ± 0.15	8.68 ± 0.69
	48	2.13 ± 0.11	8.06 ± 0.24	1.19 ± 1.12	12.94 ± 0.20
	72	3.71 ± 0.16	5.67 ± 0.31	3.74 ± 1.56	11.49 ± 0.15
Arabinose	24	0.39 ± 0.02	0.25 ± 0.02	0.29 ± 0.03	-
	48	0.45 ± 0.08	0.34 ± 0.06	0.17 ± 0.12	-
	72	0.57 ± 0.16	0.29 ± 0.02	0.48 ± 0.07	-
Cellobiose	24	-	0.86 ± 0.18	-	1.85 ± 0.15
	48	-	0.63 ± 0.12	-	2.86 ± 0.08
	72	-	0.79 ± 0.16	-	2.13 ± 0.19

On the other hand, the beneficial effect of organosolv pretreatment on enhancing enzymatic saccharification was evidenced in the pretreated biomasses by increased TRS concentrations. According to Xu et al. [3], the pretreatment of lignocellulosic biomass is essential to overcome the biomass recalcitrance, which is the natural resistance to degradation due to its complex and rigid structure. Organosolv pretreatment disrupts this structure, removing or altering lignin and hemicellulose, and exposing cellulose, the primary target for the production of fermentable sugars. In the pretreated samples, the maximum TRS concentrations were also recorded at 72 h of saccharification, with the highest TRS release (44.85 g/L) achieved in CC (Table 2). This result demonstrates the positive impact of ethanol-based organosolv pretreatment on saccharification efficiency, significantly enhancing sugar release from the pretreated biomasses. Li et al. [49] examined the effect of alkaline organosolv pretreatment (NaOH-ethanol) on the saccharification of different parts of sorghum stalk and described similar results. The enzymatic hydrolysis was conducted at 50°C for 72 h in the presence of Cellic® CTec2 cellulase (15 FPU/g substrate) and β-

glucosidase (30 CBU/g substrate). The authors reported that the pretreatment enhanced the TRS release, corroborating the effectiveness of organosolv pretreatment in improving sugar availability.

In the present study, all the evaluated organosolv-pretreated biomasses exhibited improved TRS production in comparison with raw samples, but the pretreated corn cob (CC) stood out for its superior hydrolytic performance (Table 2). This higher TRS release in CC than in the other pretreated biomasses can be attributed to the alterations in its composition and structure induced by the organosolv pretreatment. The organosolv-pretreated CC exhibited notable hemicellulose removal and delignification (Table 1), factors that may have improved enzymatic accessibility to the cellulose fraction, enabling greater TRS release in the resultant hydrolysate. Previous studies have reported that biomasses with lower hemicellulose and lignin contents can be more easily hydrolyzed, since the presence of these components can be a limiting factor for hydrolytic effectiveness [7, 15, 46, 50]. In addition, pretreated CC also displayed morphological and structural alterations that could have favored bioconversion effectiveness and, consequently, TRS production (Figure 3 and Figure 5).

The biomass pretreatments are generally carried out at high temperatures and can generate inhibitory compounds during saccharification, such as furfural (FF), which is formed from the degradation of pentoses (xylose and arabinose) derived from hemicelluloses, and hydroxymethylfurfural (HMF), which can be generated from the degradation of hexoses (glucose, mannose, and galactose) [51, 52]. The presence of FF and HMF can hinder enzymatic activity and the fermentative process, negatively affecting bioconversion efficiency [53]. These compounds can act as inhibitors of hydrolysis and fermentation when their concentrations exceed 0.5 g/L [54]. In this study, FF was detected only in the raw SS hydrolysate after 72 h of enzymatic hydrolysis (0.12 g/L). On the other hand, HMF was detected in all hydrolysates, with the highest concentration detected in raw SB (0.24 g/L). However, after organosolv pretreatment, HMF was not detected in the 24 h hydrolysate of pretreated SB or in pretreated SS hydrolysates. Furthermore, the detection of this compound decreased in hydrolysates of the other pretreated biomasses, with negligible concentrations (Table 4). These data demonstrated the outstanding and advantageous effect of ethanol-based organosolv pretreatment on reducing FF and HMF, which were detected at levels much lower than the critical inhibitory limit of 0.5 g/L. Li et al. [55] performed mild organosolv pretreatment of sugarcane bagasse with acetone, phenoxyethanol, and water to enhance the enzymatic hydrolysis. However, these authors reported that the presence of FF and HMF in pretreated samples negatively affected the efficiency of enzymatic saccharification, resulting in lower reducing sugar concentrations.

Regarding organic acids, formic and acetic acids were detected at very low concentrations in the evaluated biomasses. Compared with raw samples, acid concentrations decreased in the pretreated hydrolysates, except for acetic acid, which increased in the pretreated CC after 48 and 72 h of hydrolysis (Table 3). These results suggested that the organosolv pretreatment may have triggered the degradation of organic acids, resulting in insignificant concentrations in the pretreated hydrolysates. Furthermore, the obtained values were considerably lower than the critical inhibitory concentrations of acetic and formic acids in enzymatic hydrolysis, which are reported to above 2.0 g/L [56, 57]. These data indicated that the ethanol-water organosolv pretreatment was effective in modifying the composition of the hydrolysates, reducing the presence of inhibitors, but increasing the availability of reducing sugars. Among the investigated samples, pretreated CC revealed the best performance, with the highest TRS release and the lowest inhibitor formation.

Concerning the release of free sugars, the results followed the same pattern found for total reducing sugars, with high concentrations of monosaccharides in the organosolv-pretreated hydrolysates. In all the reactions, the remarkable presence of xylose and glucose was recorded. Additionally, low levels of arabinose and cellobiose were detected (Table 4). These results are consistent with previous studies that investigated the organosolv effect on the production of fermentable sugars from lignocellulosic biomass [6, 11, 17]. The significant presence of xylose and glucose was expected, as these monosaccharides are the main components of the plant cell wall polysaccharides xylan and cellulose, respectively. Additionally, the presence of arabinose, although in smaller quantities, is consistent with the typical composition of the plant cell wall, where arabinose is primarily present as a component of arabinoxylan. The detection of cellobiose in the pretreated biomasses is also expected, as this disaccharide is a common intermediate product of cellulose hydrolysis. Among the detected monosaccharides, glucose and xylose are particularly relevant for ethanol production. This is due to the ability of yeasts to efficiently metabolize these sugars to produce ethanol under anaerobic conditions [58].

Among the evaluated samples, the maximum glucose release was observed in the pretreated CC hydrolysates, reaching 40.14 g/L after 72 h of saccharification (Table 5). The data also indicated that there were no significant differences between the hydrolysates after 48 and 72 h of reaction (glucose concentrations of 38.32 and 40.14 g/L, respectively). Regarding xylose, the maximum concentration was also observed in the pretreated CC after 48 h of hydrolysis, at 12.94 g/L, with no significant difference at 72 h (11.49 g/L). Since hydrolytic processes of industrial interest can achieve minimal sugar concentrations of 20 g/L [59], these results are promising since SB, CS, and CC pretreated hydrolysates presented concentrations around or above this value, with pretreated CC exhibiting the highest glucose concentration (Table 4). These findings demonstrated that the organosolv pretreatment positively influenced the enzymatic saccharification, reaffirming the importance of this step for efficient lignocellulose bioconversion. In addition, the preservation of cellulose, the partial removal of lignin, and the lower percentage of hemicellulose in pretreated CC favored enzyme access to the cellulosic fraction, leading to a considerable increase in glucose concentration in this hydrolysate compared with the other biomasses. These data are in agreement with Arumugam et al. [60], who also highlighted the superior sugar release from corn cobs compared with corn straw after enzymatic digestion of sulfuric acid-pretreated samples.

Table 5 Bioconversion rate (%) of cellulose and hemicellulose after the enzymatic hydrolysis of raw and pretreated sugarcane bagasse (SB), sugarcane straw (SS), corn straw (CS), and corn cob (CC). * PT = Pretreated.

Monosaccaride (%)	Time (h)	Raw SB	PT SB	Raw SS	PT SS	Raw CS	PT CS	Raw CC	PT CC
Glucose	**24**	8.98	15.4	15.93	22.25	11.62	37.6	11.04	36.61
	48	10.37	30.72	19.45	25.56	7.88	38.18	9.86	65.35
	72	10.04	30.74	20.54	25.36	20.24	45.6	7.0	62.39
Xylose	**24**	10.64	12.77	4.0	13.02	6.05	27.73	8.28	41.5
	48	9.77	11.6	6.83	10.56	5.45	28.23	2.97	61.86
	72	8.54	31.3	7.89	10.42	9.51	19.84	9.36	54.96

This study also indicated that the cellulose bioconversion rate significantly increased after ethanol-based organosolv pretreatment, reaching 65.35% in the hydrolysate of pretreated CC against only 9.86% in raw CC, reinforcing the outstanding effect of this pretreatment (Table 5). Regarding xylose, the highest bioconversion rate was also observed in the CC hydrolysate, reaching 61.86% after 48 h of reaction. The improvement in cellulose and hemicellulose bioconversion rates after the organosolv pretreatment is consistent with other studies. For example, Luo et al. [8] reported a significant increase in the cellulose and hemicellulose bioconversion of corn straw after organosolv pretreatment at 180°C with 80% glycerol. The glucose bioconversion rate increased from 19 to 58%, and xylose conversion increased from 10 to 40% after enzymatic hydrolysis carried out with 3.0 FPU/g substrate. Similarly, Park et al. [61] found that organosolv pretreatment of corn straw with an ethanol-water mixture at 170°C, followed by enzymatic hydrolysis using CTec2 at 33 FPU/g dry biomass, resulted in a maximum glucose bioconversion of 56.9%, compared with 39.3% for untreated biomass. In addition, xylose digestibility also increased from 16.9 to 39.1%. These results corroborate the findings of this study, demonstrating the positive effect of organosolv pretreatment on enhancing enzymatic accessibility and bioconversion efficiency. Among the evaluated biomasses in the present study, pretreated corn cob (CC) showed the highest saccharification performance, with its superiority evidenced by the significant release of TRS, particularly glucose, reaching 40.14 g/L after 72 h of hydrolysis. Furthermore, the results showed no significant difference in sugar concentration between the hydrolyses performed after 48 and 72 h of reaction, indicating optimal release of fermentable sugars within 48 h. These results emphasize the remarkable role of ethanol-based organosolv pretreatment in enhancing bioconversion processes and highlight the potential of corn cob as a highly promising feedstock for bioethanol production.

3.6 Life Cycle Impact Assessment (LCIA)

3.6.1 Total Emissions

As can be seen in Table 6, the equivalent emissions assessment method based on ReCiPe 2016 v1.1 (E) includes biogenic carbon (person equivalents) and specifies all emissions generated during the process of obtaining lignocellulosic hydrolysate. It can be seen that the most relevant items indicate the use of raw materials and the discharge of emissions into freshwater. This result can be explained by the high energy consumption of the process, since the electricity flow considered was obtained from a hydroelectric source. This type of generation has significant environmental impacts, both due to the intensive use of water resources and the occupation of land intended for the construction of these plants. However, in industrial-scale applications, such impacts can be reduced by adopting energy-efficiency strategies, such as the generation and use of thermal energy within the process itself, thus reducing the external demand for electricity [62, 63].

Table 6 Emissions assessment using the ReCiPe 2016 v1.1 method (unit: EF 2.0 Global equivalents/method: EF 2.0 with tox categories).

Flows	BR: Enzymatic Hydrolysis
Resources	3.52E-13
Deposited goods	0
Emissions to air	7.55E-14
Emissions to fresh water	-3.43E-13
Emissions to sea water	3.06E-19
Emissions to agricultural soil	6.57E-18
Emissions to industrial soil	2.35E-20

Data obtained by the authors, 2026.

This energy transition has been a global highlight in the development of new technologies. In the study by Li et al. [64], given the accelerated progression of China's renewable energy layout, it is predicted that electricity generated from renewable sources, such as wind power, will gradually transform the traditional energy structure. In Brazil, the current advance comes from solar energy, with the country reaching 58.9 GW of installed capacity. This technology holds the second-largest share of the Brazilian electricity matrix, at 23%, behind only hydroelectric plants, which total 110 GW and account for 43.9% of the Brazilian generating fleet [65]. However, when comparing the Brazilian energy matrix with that of other countries, a significant emphasis is observed regarding the share of renewable sources. While the global average share of energy from renewable sources is only 14.3%, in Brazil, it reaches 47.4%, highlighting the country's relevance in the global low-carbon energy production [66].

Figure 6 clearly represents the data presented in the emissions table. It should be noted that emissions from agriculture, industry, and marine waters are not included and account for less than 1% of total emissions, making them insignificant relative to the process. Air emissions, despite being relatively low, can be reduced on an industrial scale by adding a solvent recovery and recycling step, such as the recovery of ethanol used in the organolsolv pretreatment.

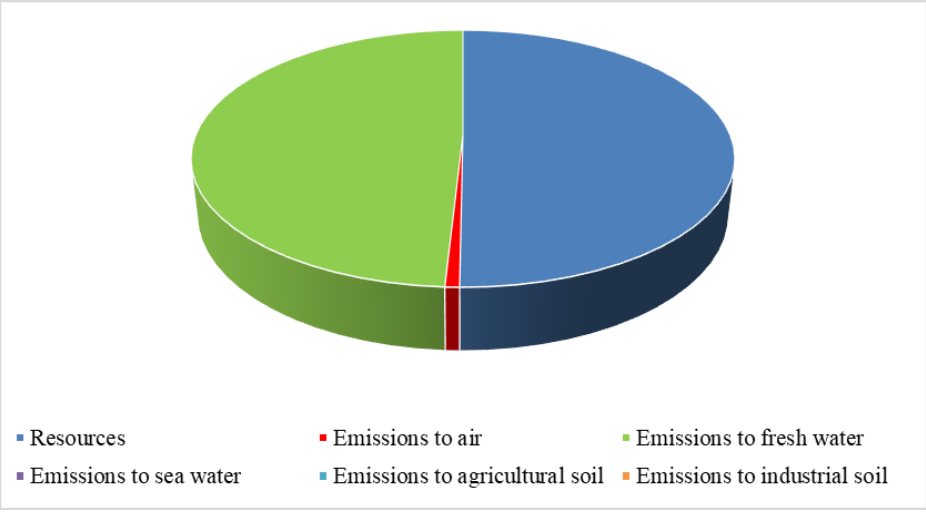

Figure 6 Emissions generated during the production of 1 kg of hydrolyzed lignocellulosic biomass.

3.6.2 Impact Assessments

The potential environmental impact was determined using the results of the inventory analysis (Table S1). Life cycle impact assessment (LCIA) allows interpretation of the inventory analysis, translating all relevant outputs and emissions into multiple environmental impact scores, particularly after normalization. Figure 7 shows the environmental profile of the lignocellulosic hydrolysate production process and provides a comparative assessment of the relevance of the environmental indicators (Table S2, Table S3).

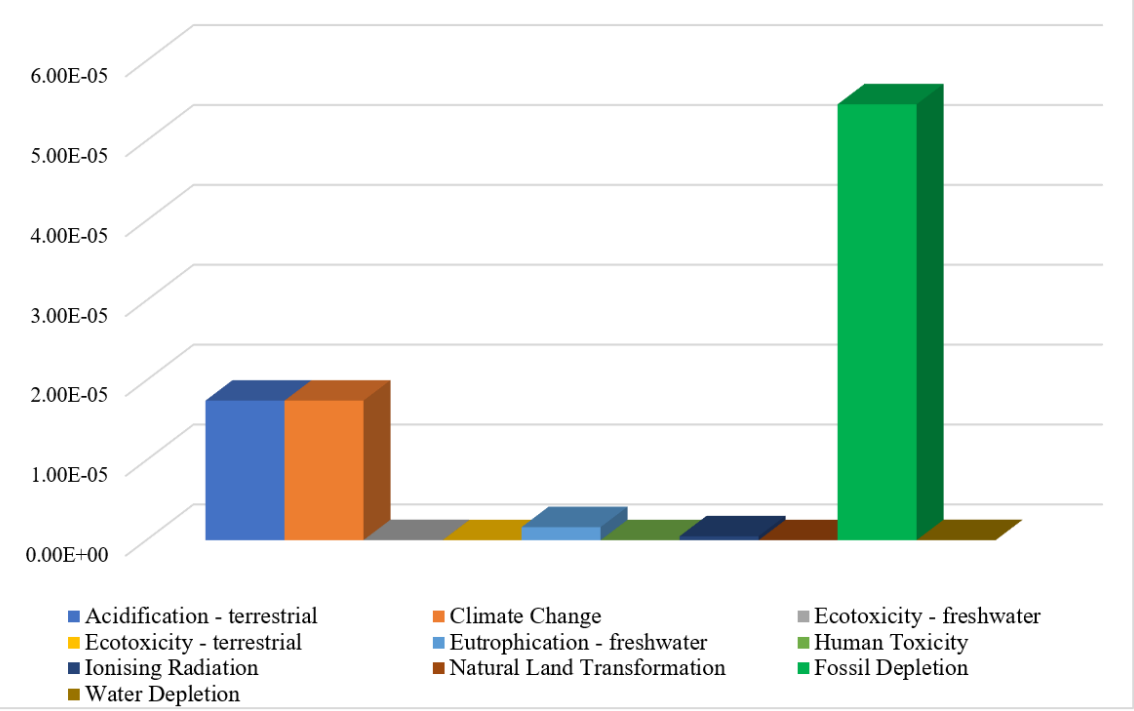

Figure 7 ReCiPe (2016) environmental indicators for the production of 1 kg of hydrolyzed lignocellulosic biomass.

4. Conclusions

In this study, ethanol-based organosolv pretreatment was successfully applied to effectivly fractionation of SB, SS, CS, and CC, leading to significant improvement in enzymatic hydrolysis. The remarkable release of total reducing sugars (TRS) and monosaccharides highlighted the potential of ethanol as a low-toxicity, low-cost, low-impact solvent for sustainable bioconversions. The pretreated corn cob (CC) exhibited superior sugar release, attributed to the effective removal of hemicellulose and lignin, thereby improving enzymatic accessibility to the cellulosic fraction. The maximum TRS release was reached after 72 h of enzymatic hydrolysis of pretreated CC, with optimal glucose and xylose production achieved within 48-72 h. Furthermore, FF, HMF, and organic acids were detected in very low concentrations, reinforcing the effectiveness of organosolv pretreatment in mitigating inhibitory effects in saccharification. These findings emphasize the potential of organosolv pretreatment as a key step in enhancing lignocellulosic bioconversion, especially for biorefineries, thereby advancing the prospects of a sustainable bioeconomy. Based on the results presented in the LCA, the most relevant factors are the use of raw materials and emissions into

freshwater, with relatively moderate impacts. Energy consumption from hydroelectric plants is particularly noteworthy. However, this impact can be improved through the transition to renewable energy sources, such as wind or solar energy, both of which are rapidly expanding in Brazil. Therefore, it is worth emphasizing the importance of a more detailed analysis of all lignocellulosic biomass valorization processes, as it provides a comprehensive overview of environmental impacts, from raw material extraction to final disposal. This approach allows the identification of areas for improvement and the comparison of different alternatives to make the process more sustainable.

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Author Contributions

N.D. Araújo: Methodology, Investigation. P.O. Rodrigues: Methodology, Investigation, Formal analysis, Visualization, Validation, Writing - original draft. M. F. Borges: Writing - review & editing. A.G. Corrêa: Writing - review & editing. G.C.S. Pompeu: Writing - review & editing. M.A. Baffi: Methodology, Investigation, Validation, Data curation, Writing - review & editing, Supervision. D. Pasquini: Conceptualization, Methodology, Investigation, Validation, Resources, Project administration, Funding acquisition, Writing - review & editing, Supervision.

Competing Interests

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.

AI-Assisted Technologies Statement

The authors declare that no generative AI or AI-assisted technologies were used in the creation, preparation, or editing of this manuscript. All content, data interpretation, and formatting were produced entirely and exclusively by the authors without any automated assistance.

Additional Materials

The following additional materials are uploaded at the page of this paper.

1. Table S1: Life cycle inventory (LCI) with input and output data for each process to obtain lignocellulosic hydrolysate.
2. Table S2: List of impact categories included, recommended characterization model and indicator.
3. Table S3: ReCiPe midpoint impact indicators (FU: 1 kg product of lignocellulosic hydrolysate).

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