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# Effective Mild Ethanol-Based Organosolv Pre-Treatment for the Selective Valorization of Polysaccharides and Lignin from Agricultural and Forestry Residues

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**Abstract:** Organosolv pre-treatments aiming to selectively remove and depolymerise lignin and hemicellulose and yield an easily digestible cellulose fraction are one of the potential options for industrial implementation within the biorefinery concept. However, the use of high temperatures and/or high catalyst concentrations is still hindering its wide adoption. In this work, mild temperature organosolv processes (140 °C) that were either non-catalysed or catalysed with sulphuric or acetic acid were compared to standard similar conditions using ethanol-based organosolv for both wheat straw (WS) and eucalyptus wood residues (ERs) as agricultural and forestry-derived model raw materials, respectively. The experimental results demonstrated that high cellulose purities could be obtained for the catalysed ethanol-based processing of the WS, which resulted in high saccharification yields (>80%), conversely to the non-catalysed process, which only reached values close to 70%. For eucalyptus residues (ERs), the pulp yields obtained were lower than the values obtained for the WS, suggesting that the ERs were a more reactive material. Cellulose purity was higher than that obtained for the corresponding treatment for the WS, with the highest cellulose purity being obtained for the ethanol-based process catalysed with sulphuric acid. Both materials presented high lignin yield recovery in the liquid stream.

**Keywords:** acetic acid; acid catalysis; biorefinery; enzymatic hydrolysis; eucalyptus residues; glucan-enriched solids; lignin-derived products; lignocellulosic biomass fractionation; organosolv; wheat straw



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## 1. Introduction

Lignocellulosic biomass, a source of renewable raw material and an alternative to fossil resources that is used to produce a wide range of fuels, chemicals, and materials, is expected to play a relevant role in the transition to a bio-based economy. However, its natural recalcitrance makes its processing difficult [1], and implies the use of fractionation/deconstruction processes to make full use of it.

The efficient and economic processing of biomass has been one of the main research goals in this area in the last decade [2], but the approaches already available impose, in general, the use of unattractive and/or high energy and catalyst costs. Thus, the search for energy-efficient processes using non-hazardous catalysts and/or green solvents for the selective separation of polymeric components (cellulose, hemicellulose, and lignin), remains one of the main objectives and one of the key challenges for biorefineries [3].

Lignin represents up to one-third of the content of the lignocellulosic materials and is a promising resource for many biobased applications such as composites, carbon fibres, hydrogels, resin adhesives, and others [4,5]. However, it is still a majorly underutilised resource, not only because its extraction is difficult, but also because some pre-treatments do not yield lignin or lignin-derived products with the required purity/characteristics for

special applications, as some methods are more focused on (hemi)cellulose removal and/or recovery. These are the cases of lignins obtained as by-products of the pulp and paper industry, such as kraft lignin, lignosulfonates, and soda lignin [6]. Other types of lignin are those obtained as biorefinery by-products; i.e., after steam explosion or acid hydrolysis pre-treatments [6–8]. Furthermore, high-purity lignins should be sulphur-free and present a low carbohydrate and ash content.

Pre-treatments with organic solvents, called organosolv processes, enable highly pure lignin to be obtained [9,10]. In this approach, lignin is typically recovered earlier in the process, leaving cellulose saccharification as the last fractionation step in biomass processing. Together with lignin, hemicellulose is also depolymerised into either oligo- and/or monosaccharides, with the former more suitable for added-value applications, and the latter for fermentative purposes [11–13]. Typically, these processes have the potential to produce relatively clean streams of lignin, hemicellulose, and cellulose, and are one of the most promising options for industrial implementation when lignin valorisation is envisaged. This designation includes the use of a broad range of organic solvents, namely alcohols, organic acids, and ketones, although the most common solvents used are ethanol and methanol due to the low cost and low boiling point of each, which allow easy recovery. Comparably, acetone also offers an advantage of a higher solubility of lignin than in ethanol, and thus acetone also proved to be efficient both as a delignification solvent and in improving the enzymatic digestibility of cellulose [10,14,15]. Nevertheless, alcohols with high boiling points, such as glycerol or butanol, can potentially be used at lower temperatures and pressures [9], although their efficiencies at low temperatures still need to be proved/optimised. All these solvents have the advantage that they can be obtained from biomass, as they are simultaneously biorefinery raw materials and products.

Organosolv typically occurs in a temperature range of 160–210 °C, and although these processes can enable an integrated fractionation of biomass and may also benefit from a lower CAPEX (specifically if ethanol is the base solvent, as its recovery can be integrated into the most common biorefinery downstream processing concepts), the possibility to operate at moderate temperatures; i.e., around 140 °C, would benefit the process economics.

The development of low-temperature organosolv processes has been a relevant target, although to be efficient, catalysts are usually required. These include mineral acids such as sulphuric acid or hydrochloric acid; organic acids such as formic, acetic, or oxalic acid; and inorganic salts such as $FeCl_3$ [10,16–19].

This work aimed to develop an innovative option for an integrated biomass valorisation concept to improve the production of lignin derivatives for value-added applications and clean sugar streams. The organosolv approach, based on ethanol–water mixtures under non-catalysed conditions or low-catalyst concentrations (acetic or sulphuric acid), was studied. The process was applied to two model lignocellulosic residues, one of agricultural origin (wheat straw) and another of forest origin (eucalyptus), and was studied at conventional temperatures for this type of solvent (190 °C) and at a lower (milder) temperature (140 °C).

## 2. Materials and Methods

### 2.1. Raw Materials

The samples of wheat straw (WS) and eucalyptus residues (ERs) were kindly provided by TNO (Netherlands) and The Navigator Company from their paper mill in Cacia (Portugal), respectively. The physical characterization; i.e., the particle size distribution of the milled WS (<4 mm screen as received from TNO) and the ERs (sawdust) was carried out using a sieve shaker (Endecotts, England) using sieves with different-sized pores from 0.25 mm to 3.55 mm. The raw material (ca. 100 g) was screened for 20 min, and the material retained on each sieve was weighed to determine the respective mass fraction yields. These assays were carried out at least in duplicate.

### 2.2. Ethanol Organosolv Pre-Treatment

Ethanol-based organosolv processes were carried out in a 600 mL stirred batch reactor (Parr®, Moline, IL, USA) using ethanol–water mixtures (50%, *w*/*w*) and a liquid-to-solid ratio of 10 (*w*/*w*) for both feedstocks.

The first set of experiments was carried out with no catalyst added. The mixture was heated to 190 °C and kept at isothermal conditions up to a maximum reaction time of 2 h, under 150 rpm stirring. For comparison purposes, additional experiments using a low concentration of sulphuric acid (50 mM) or acetic acid (50 mM–1 M) as catalysts were also carried out at the same temperature.

To evaluate the organosolv fractionation at a lower temperature (140 °C), experiments were carried out without a catalyst and in the presence of sulphuric acid or acetic acid (50 mM in both cases).

Regardless of the tested conditions, the reaction mixtures were always collected after cooling to 50 °C, then the solid and liquid fractions were separated by pressing (up to 200 bar) in a benchtop press (Sotel, Portugal), followed by rapid filtration of the liquid phase using filter paper (Whatman no. 1) for recovery of total solids. The solid phase was washed twice with the same amount of the ethanol–water solution used in the organosolv process, followed by washing with water using double the amount of the initial liquid phase. Following that, the solid residue was dried at 45 °C for at least 48 h, then stored in closed plastic containers pending chemical characterization and enzymatic hydrolysis. The filtrate and first wash liquor were stored at 4 °C until subsequent analysis.

### 2.3. Lignin Precipitation

The dissolved lignin in the hydrolysates was precipitated using dilution with water (4:1 water:hydrolysate, *w*/*w*), incubated at 30 °C (2 h, 150 rpm), and collected via centrifugation at 6000× *g* for 15 min. The wet pellet was dried at 45 °C (48 h) and weighted.

### 2.4. Enzymatic Hydrolysis

The digestibility of pre-treated solids was evaluated using enzymatic hydrolysis with the Celli® CTec2 (200 FPU·mL$^{-1}$). Essays were carried out using an enzyme loading of 10% (*w*/*w* enzyme mixture/cellulose) and a 5% solids loading in a reaction mixture containing 0.05 M sodium citrate buffer pH 5.0 and 0.02% *w*/*v* sodium azide. The reaction was carried out in an orbital incubator (Comecta Ivymen®, Barcelona, Spain) at 50 °C and 180 rpm for 72 h. Proper substrate and enzyme blanks were assayed simultaneously.

After enzyme inactivation via incubation in a water bath at 100 °C for 10 min, the samples were filtered under a vacuum through 0.45 µm nylon membranes (Millipore®, Burlington, MA, USA), followed by filtration (using 0.22 µm syringe filters) before the HPLC analysis described below.

### 2.5. Chemical Analysis

Both biomasses and pre-treated solids were characterised to determine the total moisture, total lignin, and polysaccharide content using methods based on NREL protocols [20–22]. Protein in the feedstocks was also quantified according to NREL protocols [23]. Acid-insoluble (Klason) lignin was determined gravimetrically after correction for ash, while acid-soluble lignin was established using UV spectrophotometry with absorptivity constants of 30 and 25 L/g·cm at 320 nm and 240 nm, respectively, for WS and ER, as described in [20]. All analyses were conducted at least in duplicate and are presented as mean values.

The hydrolysates obtained from the pre-treatments, enzymatic hydrolysis, and quantitative acid hydrolysis of the feedstocks and pre-treated biomass were analysed using HPLC with Agilent 1110 series equipment (Waldbronn, Germany). In some cases, the hydrolysates containing high concentrations of lignin were previously filtered through Whatman GD/X, 0.45 µm RC w/GM (Whatman, Little Chalfont, Buckinghamshire, UK), before filtration through 0.22 µm syringe filters. Analyses were performed using a Bio-Rad Aminex HPX-87H column (Hercules, CA, USA). Analyses of biomass feedstocks and en-

zymatic hydrolysates were performed at 50 °C using 5 mM $H_2SO_4$ as the mobile phase at a flow rate of 0.4 mL·min$^{-1}$. The other samples were analysed at 0.6 mL·min$^{-1}$. The detection and quantification were performed using a refractive index detector (RID) for monosaccharides (glucose, xylose, and arabinose) and acetic acid and a diode array detector (DAD) set at 280 nm wavelength for furans (furfural and 5-hydroxymethylfurfural).

Quantification of oligosaccharides (OS) was performed using an indirect method based on the post-hydrolysis of the liquors according to [24], followed by HPLC analysis as described above. The concentrations of OS were calculated while considering the increase in sugar monomers and accounting for sugar degradation.

Total phenolic compounds in hydrolysates and washing solutions (ethanol–water solutions) were determined using the Folin–Ciocalteu colorimetric method according to an improved procedure described elsewhere [25] and adapted to a microplate format using spectrophotometric detection and microtiter 96-well plates. Absorbance was measured at 725 nm in a UV–Vis spectrophotometer (Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer, Waltham, MA, USA) after 40 min incubation in the dark at room temperature. Total phenolic content was converted to mg GAE (gallic acid equivalents)/g extract by employing a gallic acid standard curve. All experiments were carried out at least in triplicate.

Qualitative characterization of the phenolic profile was carried out using capillary zone electrophoresis (CZE) according to a method described before [25] using an Agilent Technologies CE equipment (Waldbronn, Germany) with a DAD detector. A fused-silica uncoated and extended light-path capillary from Agilent with an i.d. = 50 µm and a total length of 62 cm was used. The electrolyte was 15 mM sodium tetraborate decahydrate in 10% MeOH adjusted to pH 9.1. Phenolic compounds were identified using electrophoretic comparisons (migration times and UV spectra) with authentic phenolic standards in the Agilent ChemStation software (Rev B.04.01).

## 3. Results and Discussion

### *3.1. Biomass Characterisation*

The granulometric characterization of the samples of wheat straw (WS) and eucalyptus residues (ERs) used in this work are presented in Figure 1. For the WS, the most representative fraction, corresponding to 30% of the total mass, was the fraction with a particle size ranging between 1.6–1.0 mm, followed by a 2.36–1.60 mm fraction corresponding to 28%. A similar trend was found for the ERs, with the fraction corresponding to 1.6–1.0 mm also accounting for 30%, followed by 2.36–1.60 mm and 0.71–1.0 mm fractions corresponding to 20% and 19% of the total mass, respectively. This implied that these two feedstocks samples presented a similar granulometric distribution, and that the differences found (see Figure 1) may not be significantly attributed to their physical dimensions.

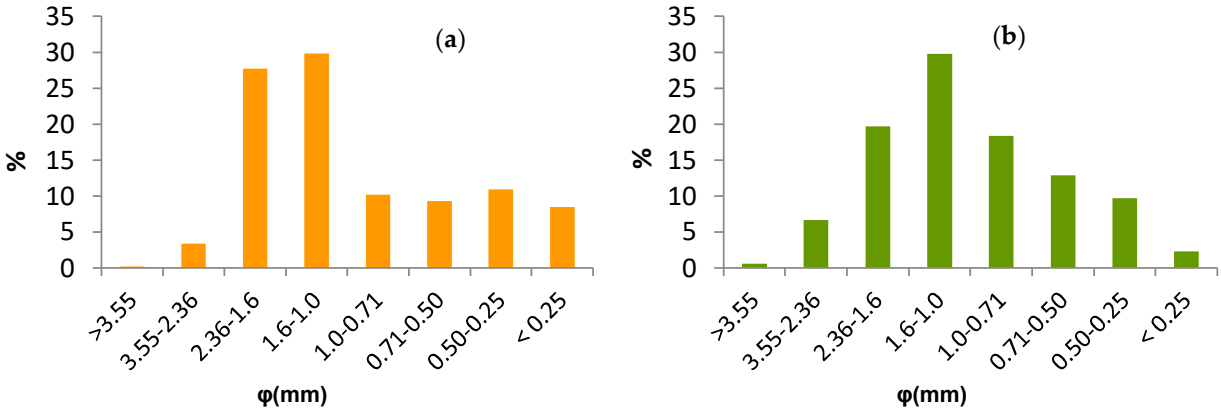



**Figure 1.** Particle size distribution (expressed in g/100 g of raw material) of the wheat straw (**a**) and eucalyptus residue (**b**) samples used in this work.

Biomass comminution depends on the specific structural characteristics, and therefore is influenced by the material anatomy [26]. In this case, a rather homogeneous physical comminution with a low amount of fine particles was found, in particular for the ERs, which was in agreement with the anatomical features of the feedstocks. As for both samples, no fraction exceeded 50% of the total, and the percentage of the fine particles was only residual, so all fractions were used in the subsequent treatments.

The chemical compositions of the two feedstocks are shown in Table 1. Although they showed some differences, both raw materials presented a relevant chemical composition for their upgrade within the biorefinery biochemical platform in processes leading to the recovery of lignin and sugars. Both materials presented a high polysaccharide content of approximately 63%, with cellulose being higher than hemicellulose. The ERs were distinguished by presenting a higher glucan content, as well as a higher content of lignin and acetyl groups.

**Table 1.** Chemical compositions of wheat straw and eucalyptus residues on a dry basis (%).

| Component | Wheat Straw (WS) | Eucalyptus Residues (ER) |
|---|---|---|
| **Glucan** | 35.9 | 44.1 |
| **Hemicellulose** | 26.7 | 19.6 |
| Xylan | 22.1 | 15.7 |
| Arabinosyl groups | 2.0 | 0.5 |
| Acetyl groups | 2.6 | 3.4 |
| **Lignin** | 16.7 | 33.8 |
| Acid-insoluble | 15.5 | 26.4 |
| Acid-soluble | 1.2 | 7.4 |
| **Ash** | 11.4 | 0.99 |
| **Extractives** | | |
| Water | 9.4 | 3.3 |
| Water (not ash) | 5.1 | 0.17 |
| Ethanol | 1.4 | 1.5 |

The cellulose content found for the WS was slightly higher than those reported in [17], but were slightly lower than the content found in [27]. The hemicellulose content was mainly above the values reported in the literature [17,27]. Conversely to hemicellulose, the lignin content was lower than the values presented by previous authors [17,27]. However, all the previous data were within the range of values presented in [28]. Regarding the ERs, the overall biomass composition was also similar to the previous values reported in the literature for eucalyptus wood (e.g., [15,29]). In contrast to the ERs, which had a relatively low ash content, the WS presented a high value (11.4%). This value was similar to that reported in [30], but was slightly higher than some data reported previously [17,27,28], and may have induced buffering effects during the biomass pre-treatment.

### *3.2. Non-Catalysed Organosolv Fractionation*

Table 2 shows the kinetic data for the ethanol–water organosolv process at 190 °C without the addition of a catalyst. Data related to the distribution of products obtained for the WS and ERs are shown in Figures 2 and 3, respectively.

**Table 2.** Fractionation of wheat straw (WS) and eucalyptus residues (ERs) biomass using ethanol-based organosolv process (no added catalyst, 190 °C).

| | Time (min) | pH | Solid Yield (%) | Glucan Solub. (%) | Xylan Solub. (%) | Delignification (%) |
|---|---|---|---|---|---|---|
| WS | 0 | 5.6 | 86.4 | 6.6 | 9.1 | 19.8 |
| | 15 | 5.2 | 78.8 | 9.8 | 28.0 | 34.3 |
| | 30 | 5.1 | 74.9 | 6.7 | 48.8 | 40.7 |
| | 60 | 4.9 | 66.7 | 18.9 | 46.0 | 46.8 |
| | 120 | 4.5 | 62.8 | 14.2 | 44.6 | 59.0 |
| ER | 0 | 5.0 | 87.6 | 11.3 | 14.1 | 26.1 |
| | 15 | 4.5 | 68.9 | 25.4 | 39.8 | 45.8 |
| | 30 | 4.0 | 68.6 | 22.3 | 59.3 | 58.8 |
| | 60 | 4.1 | 63.7 | 17.8 | 47.3 | 64.4 |
| | 120 | 4.0 | 58.4 | 12.5 | 51.5 | 70.9 |

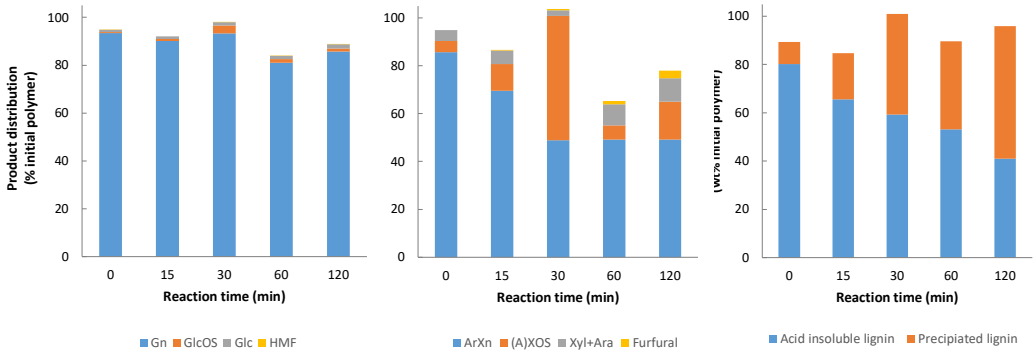



**Figure 2.** Product distribution obtained for wheat straw (WS) using ethanol-based organosolv fractionation (no catalyst added) for glucan (**a**), (arabino)xylan (**b**), and lignin (**c**). Gn, glucan; GlcOS, gluco-oligosaccharides; ArXn, (arabino)xylan; (A)XOS, xylo-oligosaccharides (arabinosyl-substituted); Glc, glucose; Xyl, xylose; Ara, arabinose; HMF, 5-hydroxymethylfurfural.

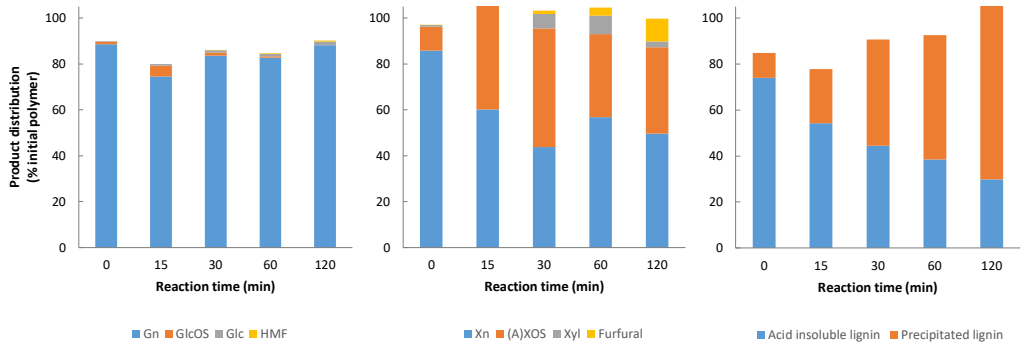



**Figure 3.** Product distribution obtained for eucalyptus residues (ERs) using ethanol-based organosolv fractionation (no catalyst added) for glucan (**a**), (arabino)xylan (**b**) and lignin (**c**). Abbreviations are as in caption of Figure 2.

For both residues, the solid yield decreased with an increase in treatment severity, reaching values of 62.8% and 58.4% for the WS and ERs, respectively, after 120 min of treatment. These decreases in solid yield can be explained by the delignification and partial hydrolysis of hemicellulose, which increased to reach values around 50% for both feedstocks, but were slightly higher for the ERs.

Delignification increased with process severity for both feedstocks, reaching 59.0% and 70.9%, respectively, for the WS and ERs after 120 min of isothermal reaction, a considerable increase compared to the less severe treatment (0 min), which only presented a modest delignification, with 80.2% and 73.9% of lignin still retained in the solid for the WS and ERs, respectively. The delignification yield obtained for the WS was slightly higher than the 48.8% reported previously [17] for the treatment of WS using a non-catalysed ethanol–water (*w*/*w*) organosolv process. The data obtained for the ERs also favourably compared to a delignification yield of 65.2% attained before the eucalyptus wood was treated at 200 °C for 70 min [15], and also was close to the range obtained for birch sawdust at 200 °C [31].

The solubilised lignin present in the liquid phase was easily recovered via water precipitation (Figures 2c and 3c). Under the best condition (120 min treatment), precipitated lignin could reach 54.8% and 83.3% of the feedstock acid insoluble lignin for the WS and ERs, respectively, corresponding to 93% and up to 100% recovery of solubilised lignin for those materials. A similar trend was also reported for the delignification of wheat straw using acetone-based organosolv fractionation without the use of mineral acids [14]. Furthermore, the total lignin recovery obtained suggested that lignin degradation was not relevant to the process conditions applied compared to that described previously [14], and it was even better than that obtained in [31] for birchwood sawdust.

Due to the hydrolysis of hemicellulose, important production of oligosaccharides was found, with arabinosyl-substituted xylo-oligosaccharides (A)XOS representing about 50% of the recovered hemicellulosic sugars (30 min) (Figures 2b and 3b). As no acid was added, the observed behaviour may have been associated with a higher buffer capacity of the WS as compared to the ERs, which may have been related to the higher ash content found in the WS together with the high acetyl group content in the ERs. In the 120 min treatment, the (A)XOS yield reached 15 and 34.1 g/100 g initial (arabino)xylan for the WS and ERs, respectively. These are potential added-value products that can be targeted to a very diverse range of applications, ranging from food and feed to nutraceutical applications, and were hence relevant to the process economic evaluation [32]. As such, it was important to observe its production and degradation kinetics. The (A)XOS reached a maximum concentration for a 30 min reaction time for both the ERs and WS, which respectively reached 6.7 g/L and 7.2 g/L. The production of pentose sugars was quite low, with the highest values attained for xylose, which had maximum values of 1.0 g/L and 1.3 g/L for the WS and ERs, respectively, which implied that the oligosaccharides could be produced selectively under these conditions. More severe conditions led to the increased production of pentoses, and at the severest conditions, monomeric pentoses from the WS presented a higher concentration, corresponding to 9.9 g/100 g xylan, which was very similar to the xylose yield found for the ERs, which was 9.4 g/100 g initial xylan obtained under the same treatment conditions. These values were even slightly higher than those previously reported for the non-catalysed acetone organosolv delignification of WS [14]. Hemicellulose hydrolysis also led to the hydrolysis of acetyl groups that were completely solubilised to produce acetic acid, and thus a decrease in the pH of the hydrolysate was observed (Table 2). The attained pH levels were always lower for the ERs in comparison with the WS pre-treatment in the corresponding conditions, which agreed with the higher content of acetyl groups in the ERs. In addition, associated with the increase in process severity, there was a decrease in the xylose and arabinose concentrations, mainly due to their degradation, leading to an increase in the furfural concentration, which reaching its highest values for the 2 h treatments for both materials (3.2% and 5.2% of degradation of the initial (arabino)xylan, respectively). However, the furfural production was practically negligible for the low-severity conditions tested as currently described both for organosolv and other processes, such as autohydrolysis [3,33].

The fact that the closure of mass balance decreased, in particular for the longer reaction times, suggested that a relevant part of the hemicellulosic sugars reacted to form unknown products. The reaction of lignin condensation products with aldehydes such as furfural has been described previously for ethanol organosolv delignification [19].

The complete chemical compositions of the hydrolysate obtained are shown in Table S1 (Supplementary Materials).

Cellulose (measured as glucan) was mainly retained in the solid phase for both feedstocks, showing in the severest condition tested a solubilisation of 12.5% and 14.2% for the WS and ERs, respectively. These were only slightly higher than those previously reported in [14] for WS and eucalyptus wood [15]. Consequently, GlcOS and glucose were produced, but in minimal amounts, as well as HMF (Figures 2b and 3b). The higher GlcOS yields were 3.3 g/100g glucan and 4.58 g/100 g initial glucan for the WS and ERs, respectively. In addition, glucose and HMF reached the highest concentrations after 120 min of treatment, although the HMF concentrations were practically negligible in hydrolysates obtained from both feedstocks. The solids obtained after 120 min of treatment mainly contained glucan (51.0% in the WS and 68.1% in the ERs), and are hence possible relevant raw materials for fermentative upgrade, i.e., for the production of advanced biofuels after enzymatic hydrolysis (see below). The full chemical characterization of the pre-treated solids is shown in Table S2 (Supplementary Materials).

### *3.3. Acetic Acid Catalysed Organosolv Fractionation*

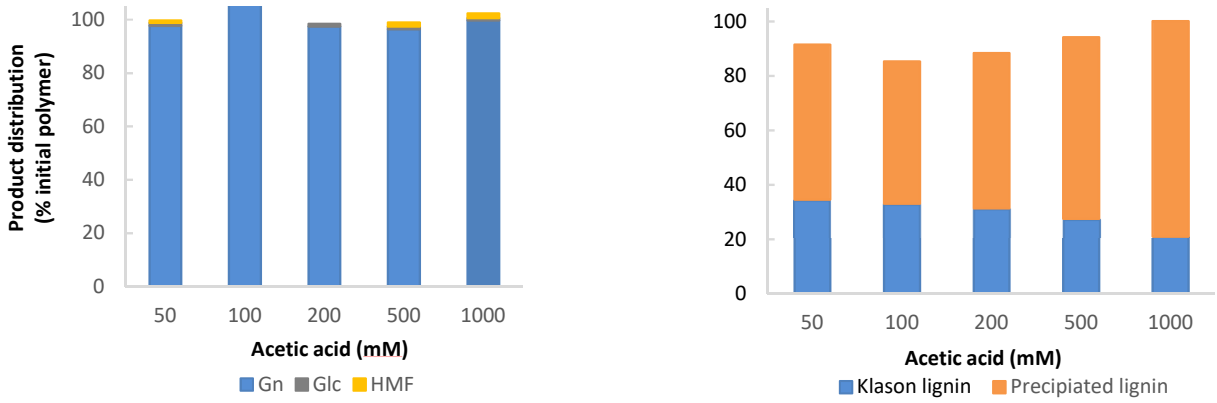
In order to evaluate the effect of low/non-corrosive catalyst concentrations on delignification yields and sugar production for the ethanol organosolv process, the addition of acetic acid was tested for both the WS and ERs. The results obtained regarding fractionation data are shown in Table 3.

**Table 3.** Fractionation of wheat straw (WS) and eucalyptus residues (ER) biomass using ethanol-based organosolv process (acetic acid added catalyst, 190 °C, 120 min).

| | Acetic Acid (mM) | pH | Solid Yield (%) | Glucan Solub. (%) | Xylan Solub. (%) | Delignification (%) |
|---|---|---|---|---|---|---|
| WS | 50 | 4.46 | 59.4 | 7.6 | 60.0 | 61.8 |
| | 100 | 4.43 | 59.0 | 14.2 | 68.5 | 58.8 |
| | 200 | 4.38 | 57.2 | 17.7 | 65.2 | 64.4 |
| | 500 | 4.07 | 56.7 | 21.4 | 65.2 | 61.2 |
| | 1000 | 3.93 | 55.1 | 12.7 | 65.2 | 55.0 |
| ER | 50 | 3.91 | 55.9 | 2.24 | 67.0 | 65.4 |
| | 100 | 3.93 | 56.3 | 0 | 100 | 66.9 |
| | 200 | 3.87 | 53.8 | 2.51 | 100 | 68.6 |
| | 500 | 3.70 | 52.6 | 3.50 | 100 | 72.5 |
| | 1000 | 3.57 | 51.1 | 0.23 | 100 | 79.0 |

The use of acetic acid (in a range of 50–1000 mM) had a minor effect on the increase in delignification, with 6% and 8% increases at the maximum for the WS and ERs, respectively, as compared to non-catalysed conditions. However, the most important effect of this organic acid was on xylan hydrolysis. The product distribution profile obtained for the WS using acetic acid as a catalyst was similar to that obtained without a catalyst, as these values increased from 49% (no catalyst added) to 68%, although this was more relevant for the ERs, for which a complete hydrolysis of xylan occurred for acetic acid concentrations of 100 mM or higher. However, it should also be mentioned that the mass balances for WS hemicelluloses ranged between 63% and 84%, quite lower than the values obtained for non-catalysed reactions, suggesting a higher degradation into unknown products (data not shown). The values obtained for the ERs, which were even lower than those obtained for the WS, suggested that in these cases, the degradation of xylan should have been high, which was demonstrated by the furfural concentrations found (data not shown), although additional degradation reactions may have occurred, yielding other products that could explain the mass balances obtained for hemicelluloses.

As an example, Figure 4 shows the product distribution profile (glucan and lignin) for the ERs.




(**a**) (**b**)

**Figure 4.** Product distribution obtained for eucalyptus residues (ER) using ethanol-based organosolv fractionation (acetic acid added as catalyst, 190 °C, 120 min) for glucan (**a**) and lignin (**b**). Abbreviations are as in caption of Figure 2.

The data obtained show that, in contrast to non-catalysed processes, higher cellulose purity fractions can be produced (particularly in the case of ER) together with a high recovery of soluble lignin. In the best conditions, these values can reach 67.6% and 79.2% of initial insoluble lignin, respectively for WS and ER.

The comparison of the data obtained with the literature was not easy, as organic acids such as acetic acid are the most commonly used as delignification solvents themselves, and not as catalysts added to a low-boiling point solvent such as ethanol. The strategy followed in this work had the advantage of using a non-corrosive acid that was easily recycled back into the process together with ethanol at relatively low concentrations, which favoured the hydrolysis and reactivity of feedstocks that by themselves have shown to provide relevant performance for the purpose of this work.

However, and despite the relevant delignification and lignin recovery, as well as the high cellulose purity of the resulting pulp, the data obtained for hemicellulose-derived products, namely the low concentrations of sugars and high degradation observed, did not encourage the intensive use of this catalysed process.

### *3.4. Sulphuric Acid Catalysed Organosolv Fractionation*

To evaluate the fractionation of these materials for the purposed targets, additional organosolv experiments at low temperature (140 °C) were carried out under non-catalysed conditions or assisted with a low concentration of acetic (50 mM) or sulphuric acid (50 mM). Table 4 shows the fractionation data obtained under those conditions.

**Table 4.** Fractionation of wheat straw (WS) and eucalyptus residues (ER) biomass using ethanol-based organosolv process (no catalyst, acetic acid or sulphuric acid added as catalysts at 50 mM concentration, 140 °C, 120 min).

| | Catalyst | pH | Solid Yield (%) | Glucan Solub. (%) | Xylan Solub. (%) | Delignification (%) |
|---|---|---|---|---|---|---|
| WS | - | 5.30 | 84.6 | n.a | n.a. | n.a. |
| | $CH_3COOH$ | 5.12 | 89.5 | 19.6 | 13.6 | 12.0 |
| | $H_2SO_4$ | 2.00 | 54.4 | 0 | 71.1 | 72.4 |
| ER | - | 4.60 | 92.5 | 6.6 | 0 | 14.1 |
| | $CH_3COOH$ | 4.17 | 91.6 | 7.2 | 0 | 20.3 |
| | $H_2SO_4$ | 1.70 | 49.8 | 5.5 | 100 | 74.4 |

The addition of an acid catalyst led to a reduction in the pH of the organosolv hydrolysates. However, the decrease in reaction temperature from 190 °C to 140 °C also led to a significant decrease in xylan hydrolysis and delignification, specifically when no catalyst was added or when a low acetic acid concentration was used. In these cases, the delignification achieved also was poor.

In contrast to acetic acid, sulphuric acid had a remarkable effect at 140 °C, promoting xylan hydrolysis up to 71% (WS) and 100% (ERs) and delignification yields of 72% (WS) and 74% (ERs). This agreed with the effect of an acid catalyst both on the kinetics of xylan hydrolysis and the chemical depolymerization of lignin. Although there are not many works on organosolv at low temperatures; i.e., at 140 °C as tested in this work, the results obtained showed that the addition of a catalyst such as sulphuric acid had a remarkable effect even at a low temperature, promoting both xylan hydrolysis and chemical depolymerization of lignin, as previously reported in [34]. Regarding the product distribution, it is important to highlight that in this case, the pentoses/sugar yield was higher than that obtained at higher temperatures. The same occurred to the recovered lignin, which reached a 67% and 95% yield for the WS and ERs, respectively, when using 50 mM sulphuric acid (data not shown).

### *3.5. Characterization of Phenolic Compounds*

As mentioned previously, the solubilised lignin produced a clean soluble lignin fraction that was easily recovered using water precipitation. In addition to this precipitated lignin's low molecular weight, phenolic compounds were also found in hydrolysates. Total phenolics concentrations, as analysed using the Folin–Ciocalteu method, increased with process severity for both feedstocks. For non-catalysed conditions, the highest value was obtained for the highest severity, respectively 9.8 g/L and 7.5 g/L for the WS and ERs (see Table S1 in the Supplementary Materials). Under catalysed conditions, phenolic concentrations tended to be lower (at least half of the concentration found under non-catalysed conditions), probably due to the precipitation of phenolics under acidic conditions.

The characterisation of the low-molecular weight phenolics present in the hydrolysates was carried out using capillary zone electrophoresis (CZE). As an example, Figure 5 shows an electropherogram for the WS hydrolysate obtained at 190 °C for 120 min without any catalyst. It was possible to find a good match with the standards available in the database, enabling us to identify vanillin, tricin, and *p*-coumaric acid, as well as quercetin derivatives. The variety of phenolic compounds found in the electropherograms met the characteristics of this herbaceous biomass, the lignin of which is rich in all hydroxyphenyl, guaiacyl, and syringyl monomers [35].

The phenolic profiles of the eucalyptus residues' hydrolysates (hardwood with lignin essentially composed of syringyl and guaiacyl units) also revealed the presence of the characteristic lignin-derived phenolic compounds *p*-coumaric acid, vanillin, and syringaldehyde (data not shown), except for the flavone tricin, which was not present in the hardwoods. The recovery of added-value low-molecular-weight phenolic compounds could also be obtained from the washing solutions, as can be seen in Figure 6, which shows an example of the results obtained for the washing solution from the WS, in which syringaldehyde, vanillin, and tricin were identified.

Thus, lignin from agro-forest residues could originate value-added compounds with recognised biological activities (mainly antioxidant, but also antitumoral, antiviral, and immunopotentiation activities, as well as antibacterial and antiparasitic actions [25]) under specific pre-treatment conditions. Tricin from the WS in particular is a flavone with a wide spectrum of health-promoting effects, and is considered one of the most potent anticancer agents tested, most probably due to the stability of its structure, since it was previously detected undegraded in the faeces of tricin-fed rats [36].

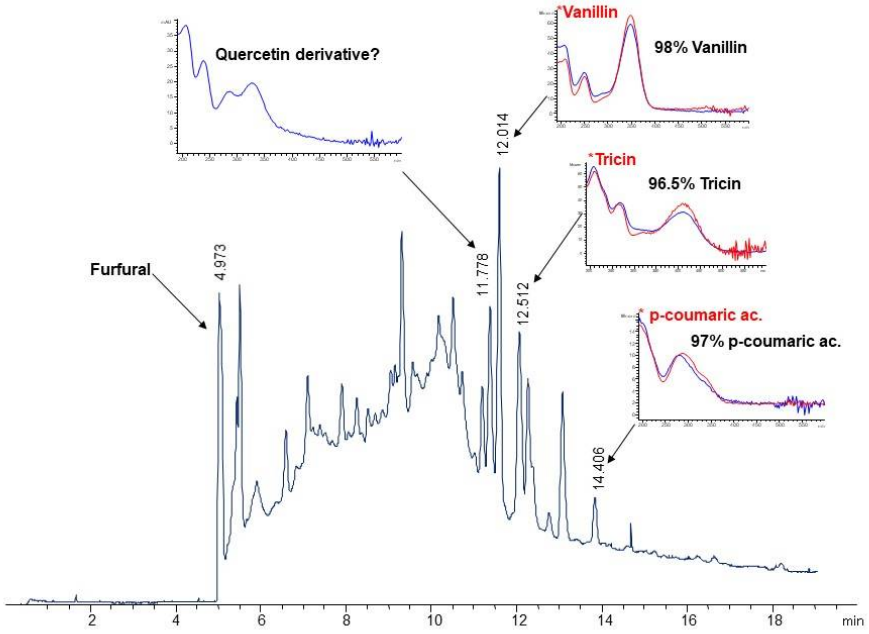



**Figure 5.** Electropherogram showing the phenolic profile for non-catalysed ethanol organosolv hydrolysates from wheat straw (190 °C, 120 min). Matching was obtained via comparison with authentic standards (*) run under the same conditions as the sample.

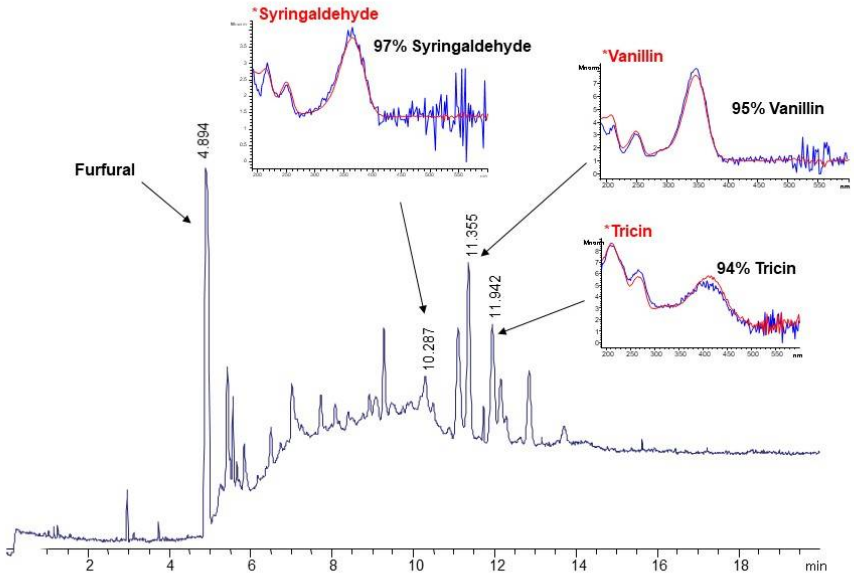



**Figure 6.** Electropherogram showing the phenolic profile for non-catalysed ethanol organosolv washing solution from wheat straw (190 °C, 120 min). Matching was obtained via comparison with authentic standards (*) run under the same conditions as the sample.

### *3.6. Enzymatic Saccharification of Pre-Treated Solids*

The effectiveness of the ethanol-based pre-treatment conditions on the enzymatic hydrolysis of the remaining cellulose in pre-treated the WS and ERs biomass was evaluated using the Celli® CTec2. The saccharification yields obtained for the pulps obtained at different reaction times at 190 °C without catalyst addition and using an enzyme dose of 10% based on available glucan are shown in Figure 7.

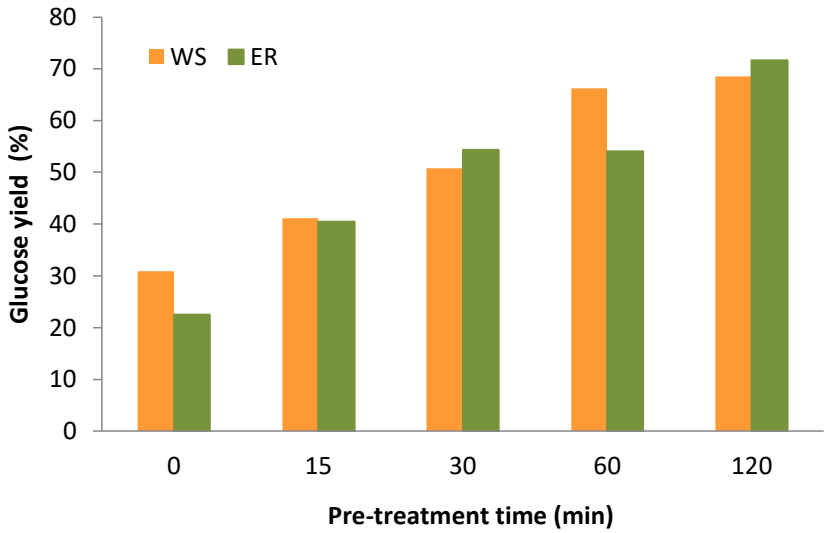



**Figure 7.** Enzymatic saccharification of ethanol–water organosolv pulp obtained at 190 °C (no catalyst added) for wheat straw (WS) and eucalyptus residues (ERs).

Under these conditions, enzymatic cellulose conversion to glucose increased with the pre-treatment time to reach the highest values for a 2 h reaction, which were 68.5% for the WS and 72% for the ERs. These values represented near to 5-fold and 7-fold increases as compared to the original feedstocks; i.e., 10.6% and 14.2% for the WS and ERs, respectively (data not shown). The saccharification yields obtained here were close to the values found for acetone organosolv delignification and higher than those reported for ethanol organosolv delignification of WS at this temperature [10,14], although slightly lower than some reported for birch wood [31], but higher than those reported for pine under similar conditions [37].

The addition of acetic acid as a catalyst at 190 °C further increased the enzymatic saccharification yields of the WS, even at low concentrations (50 mM, Figure 8), leading to an increase in the saccharification yield from 68.5% to 86.9%. This trend was maintained with the increase in the acetic acid concentration up to 500 mM (30 g/L), reaching the highest saccharification yield obtained; i.e., 99.5% (data not shown). This could have been related to a slight increase in the delignification yield obtained under these conditions, but also to the increase in the xylan depolymerisation and hydrolysis at this temperature, probably enabling easier access to cellulose. In the case of the ERs, the addition of acetic acid did not induce a higher saccharification yield, as the values obtained were close to the values previously found without a catalyst, even though complete hydrolysis of xylan occurred for acetic acid concentration above 100 mM. These data agreed with the previous findings that the application of catalysts in organosolv was particularly effective for WS [10], and could partially be related to the higher concentration of acetyl groups in the feedstocks in the sample of ERs.

The reduction in the pre-treatment temperature also reduced the saccharification yields obtained for both feedstocks. In fact, when no catalyst was added, the saccharification yields were only slightly higher than those obtained directly in the untreated feedstock samples. At 140 °C, only sulphuric acid was effective in promoting cellulose hydrolysis, and saccharification values of 82.6% and 54.4% were obtained for the WS and ERs, respectively (Figure 9).

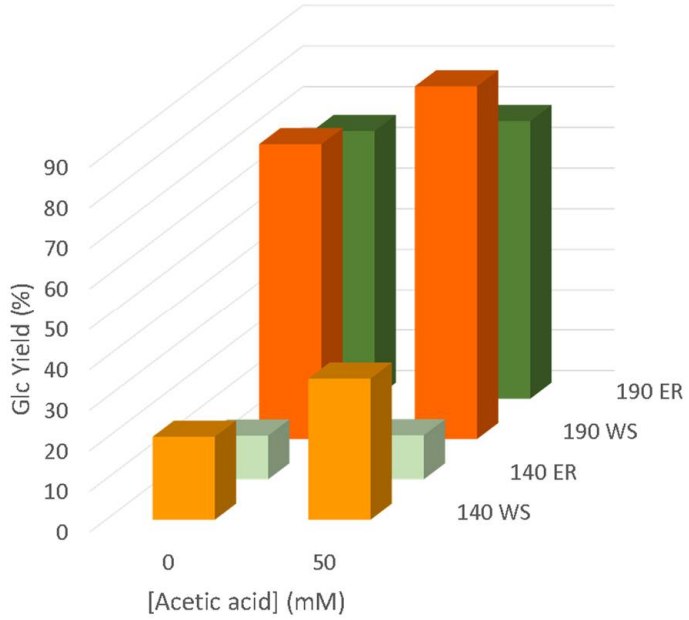



**Figure 8.** Comparison of enzymatic saccharification of ethanol–water organosolv pulp obtained at 140 °C and 190 °C without (0) or with 50 mM acetic acid (50) as catalyst for wheat straw (WS) and eucalyptus residues (ERs).

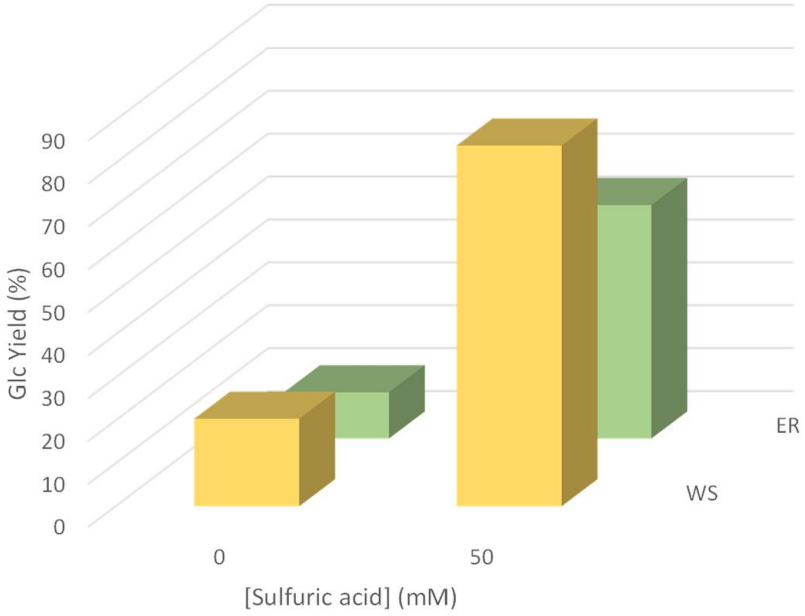



**Figure 9.** Comparison of enzymatic saccharification of ethanol–water organosolv pulp obtained at 140 °C without (0) or with 50 mM sulphuric acid (50) as catalyst for wheat straw (WS) and eucalyptus residues (ERs).

To further optimise enzyme utilization, an additional set of experiments was carried out under more stringent conditions; i.e., a decrease in the enzyme dose to 5% (Figures 10 and 11). As described in other studies [38], this reduction induced a decrease in the saccharification yield for both feedstocks. At the maximum, this reaction could reach up to 50% for WS treated using acetic acid as catalyst at 190 °C.

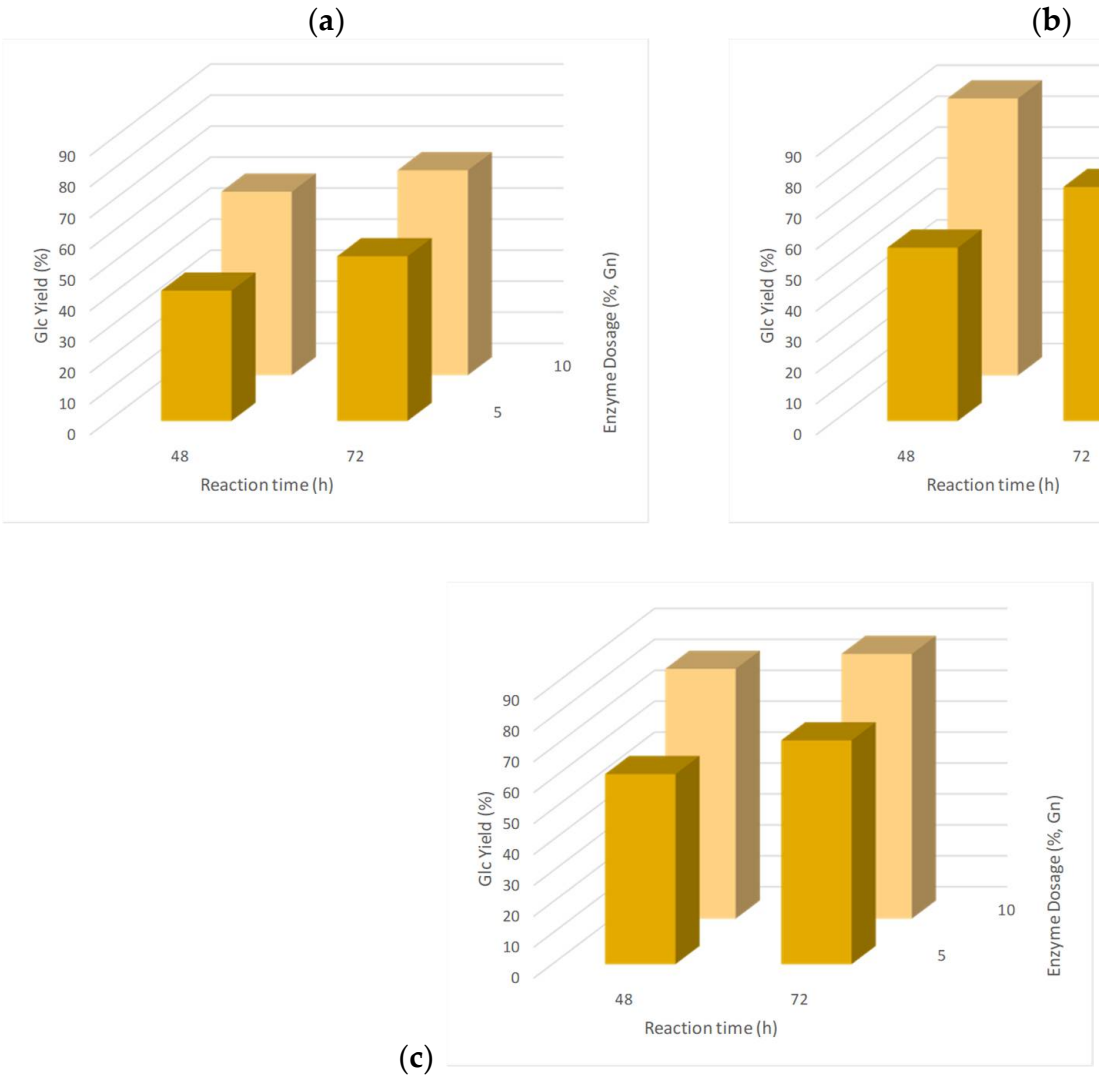



**Figure 10.** Effect of enzyme dosage and enzymatic reaction time on the enzymatic saccharification of wheat straw ethanol–water organosolv pulp obtained at 190 °C without (**a**) and with 50 mM acetic acid (**b**), or at 140 °C and with 50 mM sulphuric acid as catalyst (**c**).

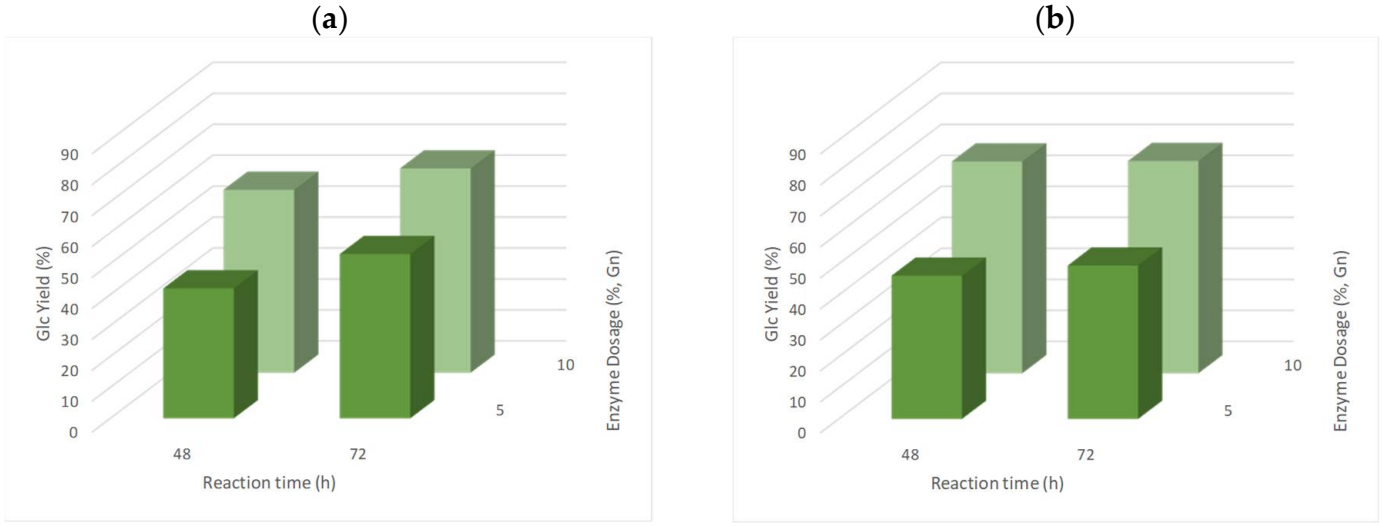
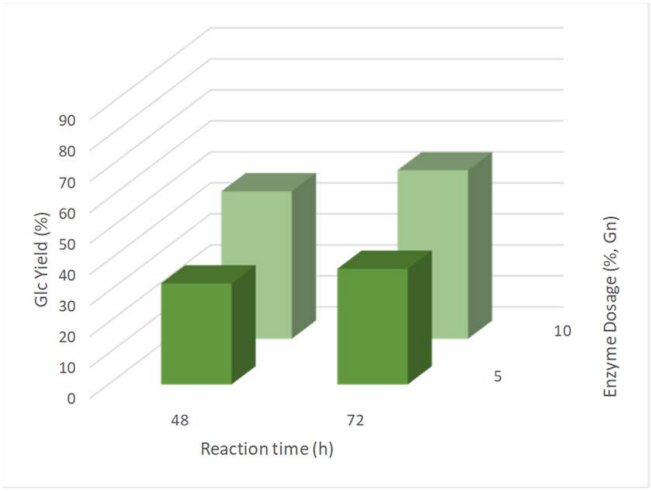



**Figure 11.** *Cont.*




(**c**)

**Figure 11.** Effect of enzyme dosage and reaction time on the enzymatic saccharification of eucalyptus residues ethanol–water organosolv pulp obtained at 190 °C without (**a**) and with 50 mM acetic acid (**b**) or 140 °C and with 50 mM sulphuric acid as catalyst (**c**).

However, it was also interesting to note that the data obtained for 48 h and 72 h were quite similar, suggesting that saccharification time could be significantly reduced without a relevant decrease in the saccharification yield. This effect was especially relevant in the case of the ER pulps, indicating that saccharification time could be reduced without a significant decrease in saccharification yield, which may have relevant impacts in terms of CAPEX and OPEX.

### *3.7. Mass Balances*

The mass balances obtained at lab-scale conditions for the WS and ERs for the standard (190 °C) and the optimal mild conditions identified in this work (140 °C, 50 mM sulphuric acid) for eucalyptus residues are presented in Figures 12–14.

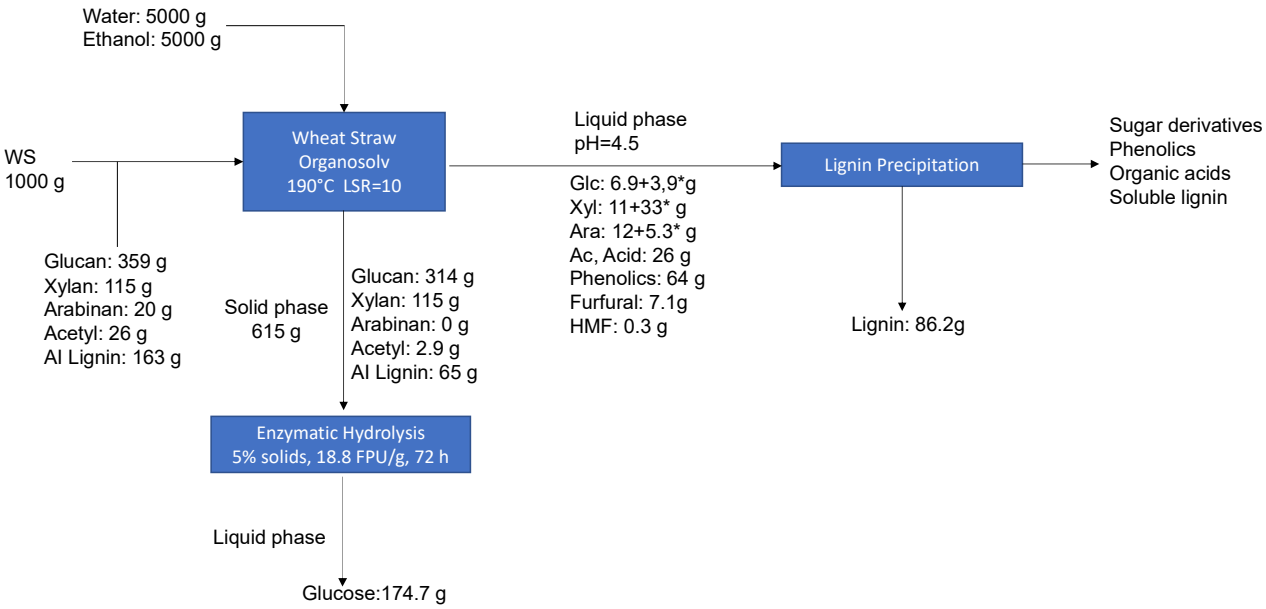



**Figure 12.** Mass balance of the main constituents for the ethanol organosolv processing of wheat straw at 190 °C (120 min) without catalyst, followed by enzymatic hydrolysis and lignin precipitation of the organosolv liquid stream. AI Lignin, acid-insoluble lignin; * Denotes sugars in oligomeric form.

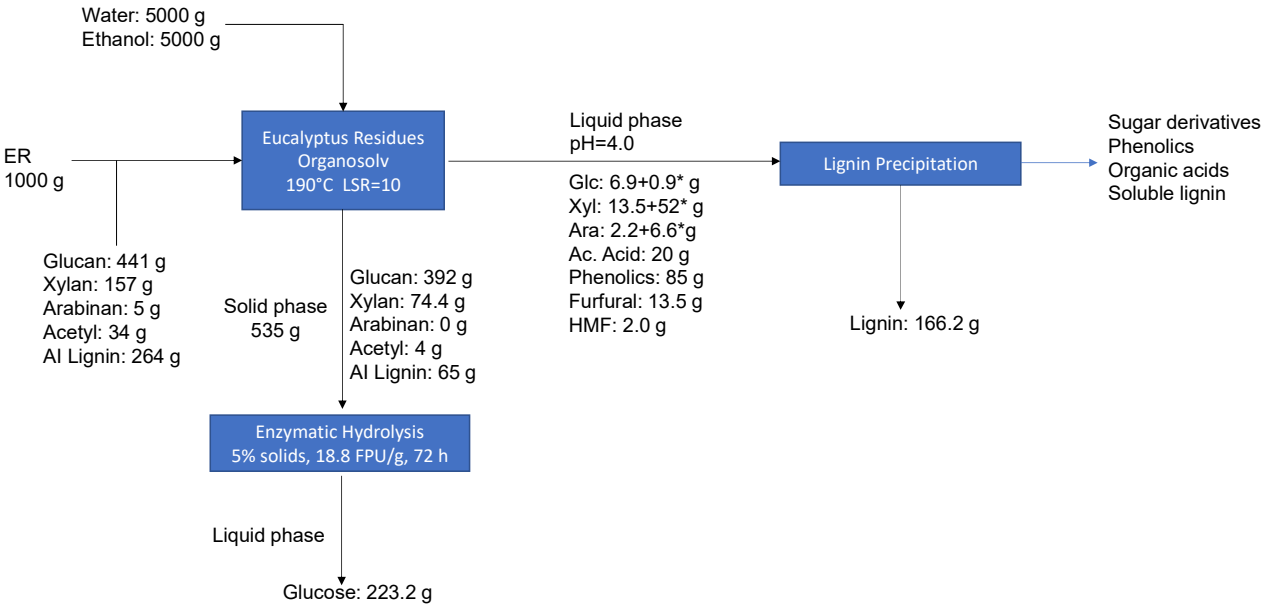



**Figure 13.** Balance and flow of the main constituent processing of eucalyptus residues using ethanol-organosolv at 190 °C (120 min) without catalyst and enzymatic hydrolysis. * Denotes sugars in oligomeric form.

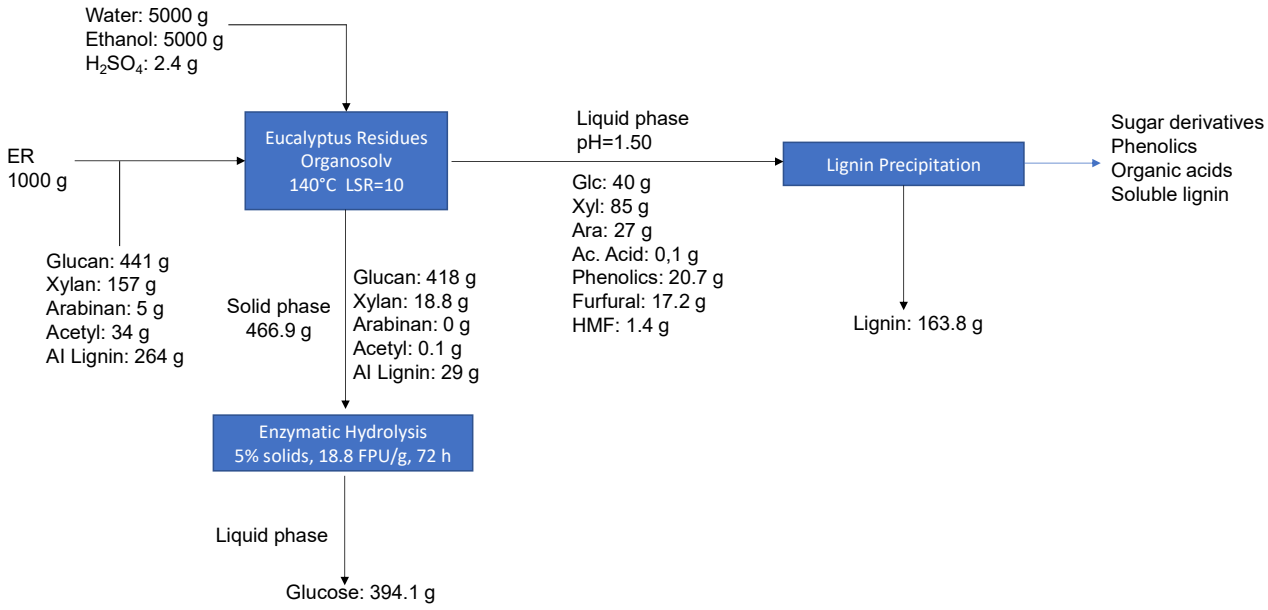



**Figure 14.** Balance and flow of the main constituent processing of eucalyptus residues using ethanol organosolv at 140 °C (120 min) catalysed with sulphuric acid and enzymatic hydrolysis.

Organosolv pre-treatments produced cellulose (glucan)-enriched pulps that, after saccharification with enzymes, provided a glucose-rich stream that can be easily converted into advanced biofuels. Under non-catalysed conditions, glucan (and also potential glucose) amounts were higher for the ERs than for the WS (Figures 12 and 13). These amounts were higher if sulphuric acid (50 mM) was added to the reaction, even at a lower temperature (140 °C) (Figure 14). Under these conditions, glucose could reach 394.1 g/kg ER, accounting for 80% of the initial glucose of the feedstock. Similarly, the residual lignin that was still retained in the solid phase (AIL) decreased in the same order to reach the minimum content of 29 g/kg ER, around 10% of the initial lignin. Together with delignification and cellulose

enrichment of the pre-treated solids, hemicellulose was partially depolymerised, and a liquid stream containing hemicellulosic sugars together with soluble lignin was obtained.

Under non-catalysed conditions, hemicellulosic sugars production was lower, but with a relevant content of (potentially added-value) oligomeric sugars. The highest value of oligomers was obtained for the ERs, from which 52 g of the xylo-oligosaccharides/kg of the initial biomass was obtained. Even under a very low catalyst concentration, catalysed process did not produce oligomeric pentoses, but instead a maximum of 85 g xylose/kg ERs. Under all conditions, the production of degradation compounds was always relatively low, with a maximum production of furfural of 17.2 g/ kg ERs (Figure 14).

The production of low-molecular-weight (potentially added-value) phenolics was also relevant, with the highest production of 85 g/kg, followed by 65 g/kg, respectively found for the ERs and WS under non-catalysed conditions (Figures 12 and 13). The highest recovery of lignin in the liquid stream was also obtained for the ERs, corresponding to the best conditions of 166 g/kg ER, which was very similar to the value obtained under catalysed conditions (164 g/kg ER) (Figure 14).

## 4. Conclusions

The present study showed that the ethanol organosolv pre-treatments tested were relevant options to selectively remove and depolymerise lignin and hemicellulose, producing an easily digestible cellulose-enriched fraction. Non-catalysed processes were more efficient at high temperatures, leading to relevant delignification yields, together with a high recovery of lignin, particularly in the case of eucalyptus residues. These processes also produced hemicellulose-derived products, with the most noteworthy being oligomeric sugars with potential added value. Under these conditions, moderate saccharification yields were obtained. Catalysed processes enabled us to obtain high cellulose purities and higher saccharification yields. Furthermore, it also allowed us to operate at lower temperatures, which also provided a high delignification yield if the process was assisted with a low concentration of sulphuric acid. The eucalyptus residues' biomass tended to be more reactive, producing lower pulp yields than the WS, although both materials presented high lignin yield recovery in the liquid stream.

Future studies using a techno-economic analysis will enable us to further ascertain the best pre-treatment conditions in face of the (dis)advantages between targeting the main production of oligosaccharides and lignin-derived products or the optimization of the subsequent cellulose hydrolysis to obtain concentrated glucose syrups for advanced biofuel production via fermentation.

**Supplementary Materials:** Extra supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/en15155654/s1. Table S1: Composition of the liquid phase (g/L) obtained after the organosolv processing of wheat straw (WS) and eucalyptus residues (ER) biomass, at 190 °C and without catalyst addition; Table S2: Solid yield (%) and composition of the solid phase obtained after the organosolv processing of wheat straw (WS) and eucalyptus residues (ER) biomass at 190 °C and without catalyst addition.

**Author Contributions:** Conceptualization, F.C., L.C.D. and F.G.; methodology, F.C., L.C.D., F.P., V.V.-D., L.S. and L.B.R.; validation, F.C. and L.C.D.; formal analysis, F.C., L.C.D. and F.P.; investigation, F.C., F.P., V.V.-D. and L.S.; resources, F.C. and F.G.; data curation, F.C., L.C.D., F.P., L.S. and L.B.R.; writing—original draft preparation, F.C., F.P. and L.C.D.; writing—review and editing, F.C., L.C.D., F.P., V.V.-D., L.S., L.B.R. and F.G.; visualization, F.C., L.C.D. and F.G.; supervision, F.C. and F.G.; project administration, F.C. and F.G., funding acquisition, F.C. and F.G. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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