biomass

MDPI

Article

Lignin from Residual Sawdust of *Eucalyptus* spp.—Isolation, Characterization, and Evaluation of the Antioxidant Properties

Débora Tavares [1], Matheus Cavali [2,*], Valcineide de Oliveira Andrade Tanobe [1], Luis Alberto Zevallos Torres [1], Anderson Steyner Rozendo [1], Arion Zandoná Filho [3], Carlos Ricardo Soccol [1] and Adenise Lorenci Woiciechowski [1]

[1] Department of Bioprocess Engineering and Biotechnology, Federal University of Paraná, Curitiba 81531-908, Brazil
[2] Department of Sanitary and Environmental Engineering, Federal University of Santa Catarina, Florianópolis 88040-970, Brazil
[3] Department of Chemical Engineering, Federal University of Paraná, Curitiba 81531-908, Brazil
* Correspondence: cavali.matheus@gmail.com

Abstract: Lignin is an abundant biopolymer, as well as cellulose and hemicellulose. Thus, this work aimed at isolating and characterizing the lignin from *Eucalyptus* spp. Sawdust—a lignocellulosic waste generated in large amounts in sawmills—to evaluate its antioxidant capacity. A biorefinery perspective was utilized: the biomass was fractionated using a sequential acid-alkaline treatment to recover the hemicellulosic carbohydrates, preserving the cellulose-rich solid fraction and isolating the lignin. The physicochemical characterization of isolated lignin was carried out using thermogravimetric (TGA), Fourier-transform infrared spectroscopy (FTIR), and nuclear magnetic resonance (NMR) analyses, while the antioxidant property was evaluated employing electron transfer and using DPPH and ABTS assays. After sequential acid-alkaline treatment, 68.15% of the hemicellulosic carbohydrates were recovered using mild acid treatment. The specific yield of lignin was 69.38%, and the remaining solid fraction contained 60.42% of cellulose. The antioxidant activity of lignin was evaluated using a DPPH radical test, and it showed an inhibition of 81.58% and IC_{50} of 60 µg/mL. For the ABTS test, the inhibition was 99.86%, and the IC_{50} was 7.39 µg/mL. Therefore, the lignin isolated from residual eucalyptus sawdust using sequential acid-alkaline treatment presented interesting antioxidant properties, which should be further investigated and evaluated for different applications.

Keywords: biorefinery; lignocellulosic waste; acid-alkaline treatment; biomass valorization; DPPH assay; ABTS assay

Citation: Tavares, D.; Cavali, M.; Tanobe, V.d.O.A.; Torres, L.A.Z.; Rozendo, A.S.; Zandoná Filho, A.; Soccol, C.R.; Woiciechowski, A.L. Lignin from Residual Sawdust of *Eucalyptus* spp.—Isolation, Characterization, and Evaluation of the Antioxidant Properties. *Biomass* **2022**, 2, 195–208. https://doi.org/10.3390/biomass2030013

Academic Editor: Lasse Rosendahl

Received: 9 August 2022
Accepted: 7 September 2022
Published: 11 September 2022

1. Introduction

Lignocellulosic wastes are renewable feedstocks for several processes. These materials, composed of cellulose, hemicellulose, and lignin, can be exploited to produce biofuels and other high-value chemicals. Lignocellulosic wastes are plentifully available at a low cost as agro-industrial and forest residues [1–4]. For instance, it is estimated that the forest processing industry generates approximately 180 million m^3 of lignocellulosic waste globally and about 75 million m^3 only from sawing process (sawdust) [5]. The European Organization of the Sawmill Industry reported that the sawdust generation in 2020 was approximately 13.5 million m^3, and the amount was projected to reach almost 14 million m^3 in 2021 [6]. In Brazil, the annual generation of wastes from mechanical wood processing was estimated to be greater than 48 million m^3 [7], and 77% of the total area of planted trees in the country is composed of *Eucalyptus* spp. [8]. *Eucalyptus* spp. is the most planted wood due to its remarkable adaptability to different climatic conditions, rapid growth, and superior wood properties [9].

Regarding sawdust generation, the high amounts might become an environmental problem when improperly managed. Burning sawdust in an open space contributes to

atmospheric pollution, and its application on soil leads to negative effects, such as increasing acidity [10]. Therefore, in order to avoid environmental concerns related to improper disposal of sawdust, this residue can be better used by valorization of its main fractions: cellulose, hemicellulose, and lignin. Additionally, sawdust utilization has the advantage of not requiring a physical pre-treatment to reduce the particle size of biomass [5].

From a biorefinery perspective, cellulose, hemicellulose, and lignin can be converted into marketable valuable products [11,12]. However, although carbohydrates have received large attention regarding their utilization in several processes [13], lignin has been less exploited. The industrial application of lignin is still considered a challenge, mainly because of the structural modifications caused by the different extraction processes. Thence, lignin is generally burnt for energy generation [14,15].

This scenario emphasizes the importance of lignin valorization. After cellulose, lignin is the most abundant biopolymer found in nature, corresponding to about 30% of the organic carbon content of the biosphere. Lignin is a natural high molecular weight phenolic component made of phenylpropanoid units linked through ether and carbon–carbon bonds, such as β-O-4′, 4-O-5′, β-β′, β-1′, β-5, and 5-5. The three basic phenylpropanol monomers are known as monolignols, which are named p-coumaril, coniferyl, and synapyl alcohols. When incorporated into the lignin polymer, the units from monolignols are called p-hydroxyphenyl (H), guayacyl (G), and syringyl (S), respectively [16–20].

Lignin presents diverse biological properties, such as antioxidant activity [21]. As a natural antioxidant, lignin can be placed in the group of antioxidants polyphenols as well as flavonoids and phenolic acids [22,23]. The characteristics of lignin (e.g., high phenolic content, ability to interact with radicals, and diversity of functional groups) make it suitable for applications as polymer additive, in UV tolerance and antimicrobial applications, and as the basis for various nanomaterials [24]. Nevertheless, the lignin properties are strongly influenced by the recovery method utilized, such as deep eutectic solvents [25] organosolv [26], mild acid [27], steam explosion [28], and sequential acid-alkaline treatments [29,30].

Therefore, as previously mentioned, this work aimed at evaluating the recovery of lignin from residual sawdust of *Eucalyptus* spp. using a sequential acid-alkaline treatment to also obtain hemicellulosic carbohydrates and cellulose. Lignin's characteristics and its antioxidant properties were evaluated as well.

2. Materials and Methods

2.1. Material

Residual sawdust of eucalyptus (*Eucalyptus* spp.) was obtained from a sawmill located in Santa Catarina state (Brazil). It is the residue from primary wood processing. The sawdust was air-dried at 70 °C to reduce the moisture content, and it was subsequently sieved. The biomass with particle size between 0.35 and 0.85 mm was selected for the experiments using sieves No. 20 and No. 45 (ASTM) as employed in other work [30]. All the reagents used in the experiments were of analytical grade.

2.2. Biomass Composition

Residual sawdust of eucalyptus was characterized in terms of the lignocellulosic content according to National Renewable Energy Laboratory (NREL) procedures [31]. For the soluble acid lignin analysis, a wavelength of 205 nm and molar absorptivity of 110 L/g.cm was chosen. Carbohydrate analysis was performed using high-performance liquid chromatography (HPLC) on a Shimadzu chromatograph equipped with an Aminex column HPX-87H operating at 60 °C with 5 mM H_2SO_4 as mobile phase at a flow rate of 6 mL/min. After the treatment stages, the resulting biomass composition was characterized again. All experiments were performed in triplicate.

2.3. Sequential Acid-Alkaline Treatment

Residual sawdust of eucalyptus was submitted to sequential acid-alkaline treatment to extract hemicellulosic carbohydrates, recovering lignin, and preserving the cellulose-rich solid fraction. For acid treatment, a 2^3 experimental design was utilized to evaluate the carbohydrate recovery, varying pretreatment time (20, 40, and 60 min), temperature (100, 115, and 130 °C), and H_2SO_4 percentage (1.5%; 3.0%; 4.5% *w*/*w*). The response variable was the total amount of carbohydrates in g/L. All experiments were developed in an autoclave. Subsequently, a higher temperature was evaluated in a stirred reactor (Parr® Series 4530) at 140 °C for 20 min with 1.5% H_2SO_4 *w*/*w* and 10% *w*/*w* of biomass load in 300 g of total reaction. The test was performed with a triplicate in the central point. The analysis of sugars in the hydrolysate was carried out in duplicate using HPLC. The hemicellulose recovery from the final treatment condition was calculated using Equation (1):

$$Y_H = \frac{AHO \times 0.88}{HC} \quad (1)$$

where Y_H is the yield of hemicellulose, AHO is the percentage of hemicellulosic carbohydrates present in the hydrolysates, 0.88 (132/150) is an anhydrous correction factor used for C_5 sugars due to the release of a water molecule to form the glyosidic bond, and HC is the hemicellulose content present in untreated residual sawdust of eucalyptus.

The alkaline treatment was evaluated in two stages. In the first stage, NaOH concentrations were assessed (10%, 20%, and 30% *w*/*w*) in an autoclave at 121 °C for 90 min with 10% *w*/*w* of dry biomass load. The second stage was performed in a stirred reactor (Parr® Series 4530) increasing the temperature to 130 °C to improve the lignin yield. After each treatment, the structural carbohydrates from the resulting solid fractions were analyzed according to NREL procedures aforementioned. The lignin was recovered from the liquid fraction obtained after alkaline treatment by decreasing the solution pH to 2 with 72% *w*/*w* H_2SO_4. The lignin was precipitated overnight in the dark and separated using vacuum filtration with No. 4 Whatman filter paper. Samples were air-dried at 55 °C [2], and the global yield (Gy) and the specific yield (Sy) of lignin extracted were calculated using Equations (2) and (3):

$$G_Y = \frac{M_l}{M_t} \times 100 \quad (2)$$

$$S_y = \frac{M_l}{M_{lt}} \times 100 \quad (3)$$

where M_l is the mass of lignin recovered, M_t is the total sawdust mass, and M_{lt} is the acid insoluble lignin mass present in sawdust after acid treatment. The fractions obtained were characterized as follows.

2.4. Crystallinity Index

In order to evaluate de modifications in its lignocellulosic structure, the crystallinity of eucalyptus sawdust was estimated using X-ray diffraction (XRD). For this analysis, samples of eucalyptus *in natura*, the biomass obtained after acid treatment and the biomass obtained after alkaline treatment were analyzed. Wide-angle X-ray scattering patterns of the samples were obtained in the reflection mode with an XRD 700 MAXIMA (Shimadzu, Tokyo, Japan) diffractometer operating with Ni-filtered copper radiation (CuKα, λ = 1.5418 Å). The diffractograms were obtained with scans of 2°/min in a range of 5 to 35°, and the generator was operated at 40 kV and 20 mA. The crystallinity index (CI) was calculated according to Equation (4):

$$\mathrm{CI\%} = 100 \times \left(\frac{\mathrm{I_{200}} - \mathrm{I_{am}}}{\mathrm{I_{200}}} \right) \quad (4)$$

where I_{200} is the maximum intensity 2θ between 22–23° for cellulose type I and between 20–22° for cellulose type II; I_{am} is the peak of minimum intensity in the amorphous region at 2θ between 15° and 17° for cellulose type I and 12° and 13° for cellulose type II [32].

2.5. Lignin Characterization

2.5.1. Thermal Analysis

Thermal stability of lignin was evaluated using thermogravimetric analysis in a TGA 400 (Perkin-Elmer, Waltham, MA, USA), under synthetic air from 30 to 900 °C with a heating rate of 10 °C/min and gas flow of 50 mL/min.

2.5.2. Fourier-Transformed Infrared Spectroscopy

Recovered lignin was characterized using FTIR spectroscopy in a VERTEX 70 equipment (Bruker), with the accessory DRIFTS (diffuse reflectance) with 64 scans, 4 cm^{-1} resolution, without the elimination of atmospheric compensation. The samples were previously dried at a temperature of 100 °C for 24 h. Then, samples were grounded, mixed with potassium bromide (KBr) to homogeneity and placed in DRIFTS accessory for the acquisition of the spectra.

2.5.3. Nuclear Magnetic Resonance

The 2D HSQC-NMR (Two-Dimensional Heteronuclear Single Quantum Correlation-Nuclear Magnetic Resonance) spectrum was recorded using an NMR spectrometer (Bruker) at 400 MHz. The 2D HSQC NMR analysis was carried out through a correlation between ^{1}H and ^{13}C NMR spectra acquired at 30 °C. The sample was prepared by dissolving 80 mg of lignin in 0.5 mL of dimethylsulfoxide (DMSO-d6). The chemical shift for ^{13}C NMR was calibrated with reference to the DMSO-d6 standard peak at 39.51 ppm and 2.50 ppm for ^{1}H NMR spectra. The acquisition times were 0.24 and 0.002 s for ^{1}H and ^{13}C dimensions, respectively. The spectral widths were 4261.36 and 22,137.02 Hz for the ^{1}H and ^{13}C dimensions, respectively. The filter width was 125 MHz, and the receiver gain was 203.

2.6. Total Phenolic Content

The total phenolic content (TPC) was carried out using the Folin-Ciocalteu method [21]. A lignin solution of 200 μg/mL was prepared by dissolving lignin in alkaline water and then neutralizing it with sulfuric acid. The neutralized solution was completed with distilled water. For the phenolic content analysis, 500 μL of the sample was added to the reaction tube containing 2.5 mL of the Folin-Ciocalteu solution (1/10 *v*/*v*). After 5 min, 2 mL Na_2CO_3 (7.5% *w*/*v*) was added. The mixture was held at room temperature in the dark for 1 h, and then, the absorbance was read at 740 nm in a spectrophotometer (Spectrumlab 22PC) using a quartz cuvette. Distilled water was submitted to the same procedure to obtain the blank sample. The TPC was determined using the equivalent in micrograms of gallic acid, for which a curve of 0–100 μg/mL of gallic acid was previously prepared (R^2 = 0.997). The tests were performed in triplicate.

2.7. Antioxidant Assays

Antioxidant activity was determined from the capability to inhibit the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical. For this, 0.1 mmol/L DPPH (dissolved in methanol) and lignin solutions of 5, 50, 100, 150, 200, and 250 μg/mL were prepared. Lignin performance was evaluated in comparison to a standard high-strength antioxidant substance-Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). Trolox concentrations of 2 to 50 μg/mL were evaluated for the DPPH assay. In the reaction, 0.8 mL of DPPH solution was added to 0.2 mL of lignin solution, the mix was stirred and allowed to react for 30 min in the dark at room temperature. For the control sample, distilled water was used instead of lignin. Absorbance was measured at 517 nm in a spectrophotometer (Spectrumlab 22PC). The blank was adjusted with water and methanol. Equation (5) was used to calculate the percentage of free radical inhibition:

$$I(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \quad (5)$$

where $A_{control}$ is the absorbance of the sample containing 0.8 mL of the DPPH solution and 0.2 mL of distilled water, and A_{sample} is the absorbance of the sample with lignin solution.

The antioxidant capacity of the lignin was also assessed by inhibiting the 2,2′-Azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical. The assay was performed as described in another work [33]. Lignin concentrations evaluated were 5; 7.5; 10; 12.5; 15; 20; 25; and 30 μg/mL. For comparison, a Trolox curve was prepared and ranged from 2 to 12 μg/mL. Absorbance was measured on a microplate reader (Biotek). Both assays (DPPH and ABTS) were performed in triplicate.

2.8. Statistical Analysis

In order to evaluate the influence of time, temperature and acid percentage on the carbohydrate recovery, the analysis of the 2^3 experimental design was carried out using Minitab 15.0 software (Minitab, State College, PE, USA). ANOVA tests were employed in order to evaluate significancy of the influence of an independent variable such as percentage of NaOH on the lignin recovery.

3. Results and Discussion

3.1. Lignocellulosic Composition

The composition of eucalyptus sawdust may vary depending on the species, planting location, and age of the wood. Herein, in this work, the content of cellulose, hemicellulose, ethanol extractives, water extractives, acid insoluble lignin (AIL), acid soluble lignin (ASL), and ash (%) was 42.67 ± 0.8; 16.05 ± 0.24; 1.50 ± 0.10; 3.31 ± 0.34; 21.99 ± 0.50; 1.74 ± 0.05; 1.02 ± 0.04, respectively. Other work using *Eucalyptus grandis* sawdust presented similar content of cellulose and hemicellulose [34]. Regarding lignin, the content of AIL was close to the results found in other studies for different eucalyptus species, such as *Eucalyptus camaldulensis* (21.4%) and *Eucalyptus urophydis* (21.0%) [35]. Additionally, higher percentages of AIL were also reported for *Eucalyptus globulus* wood (25.7%) [36], *Eucalyptus grandis* (27.0%) [34], and sawdust from *Eucalyptus* spp. (28.6%) [37].

3.2. Acid Treatment

Acid treatment was used to recover the hemicellulosic carbohydrates from residual sawdust from *Eucalyptus* spp. The results of the experimental design performed for each test are presented in Table 1.

Table 1. Total sugar recovered (g/L) at different conditions of acid pretreatment of *eucalyptus* sawdust.

Test	Independent Variables			Total Recovered Carbohydrates (g/L)
	Time (min)	Temperature (°C)	H_2SO_4 (% *w*/*w*)	
1	20 (−)	100 (−)	1.5 (−)	0.40
2	60 (+)	100 (−)	1.5 (−)	0.96
3	20 (−)	130 (+)	1.5 (−)	11.93
4	60 (+)	130 (+)	1.5 (−)	14.90
5	20 (−)	100 (−)	4.5 (+)	0.95
6	60 (+)	100 (−)	4.5 (+)	5.98
7	20 (−)	130 (+)	4.5 (+)	15.09
8	60 (+)	130 (+)	4.5 (+)	14.60
9	40 (0)	115 (0)	3 (0)	9.97
10	40 (0)	115 (0)	3 (0)	11.69
11	40 (0)	115 (0)	3 (0)	10.40

(+) Maximum level taken by the independent variable. (−) Minimum level taken by the independent variable. (0) Medium level taken by the independent variable.

According to analysis of the 2^3 experimental design (ANOVA), temperature was the only factor with significant influence on carbohydrate recovery (p value < 0.05), presenting a positive effect. Time and acid concentration, as well as the interactions between

the factors, had negligible effects on carbohydrate recovery (p value > 0.05). When low acid concentrations are used, as those evaluated in this study, the pretreatment generally demands high temperature [38]. This explains the positive effect of temperature. Under acidic conditions, however, a short reaction time is advisable to reduce the dehydration of carbohydrates from lignocellulosic biomass into furfural and hydroxymethylfurfural [39].

Considering the positive effect of temperature, a new treatment condition to recover hemicellulosic carbohydrates was evaluated at 140 °C. In this test, named HER140, both time and acid concentration were fixed at their lower levels (20 min and 1.5% H_2SO_4 w/w), and the total mass of the reaction was scaled up to 300 g. Accordingly, the total recovered carbohydrates increased to 17.06 ± 1.32 g/L, (2.88 ± 0.88 g/L of glucose, 13.32 ± 0.37 g/L of xylose, and 0.86 ± 0.15 g/L of arabinose). In comparison with test 3 (Table 1), in which time and acid concentration were also at the lowest level, an increase of almost 43% in the total recovered carbohydrates was obtained by treating residual eucalyptus sawdust at 140 °C. It is advantageous because shorter hydrolysis time and lower acid concentrations reduce the possibility of corrosion of the reactor and contribute to a more environmentally friendly process [40].

In order to examine the influence of temperature on the lignin recovery yields, the resulting solid fractions from test 7 (HER130) and the test performed at 140 °C (HER140) were submitted to alkaline treatment.

3.3. Alkaline Treatment and Lignin Recovery

The samples HER130 and HER140 were submitted to alkaline pretreatment in an autoclave at 121 °C for 90 min at three different NaOH concentrations. The lignin yields are shown in Figure 1.

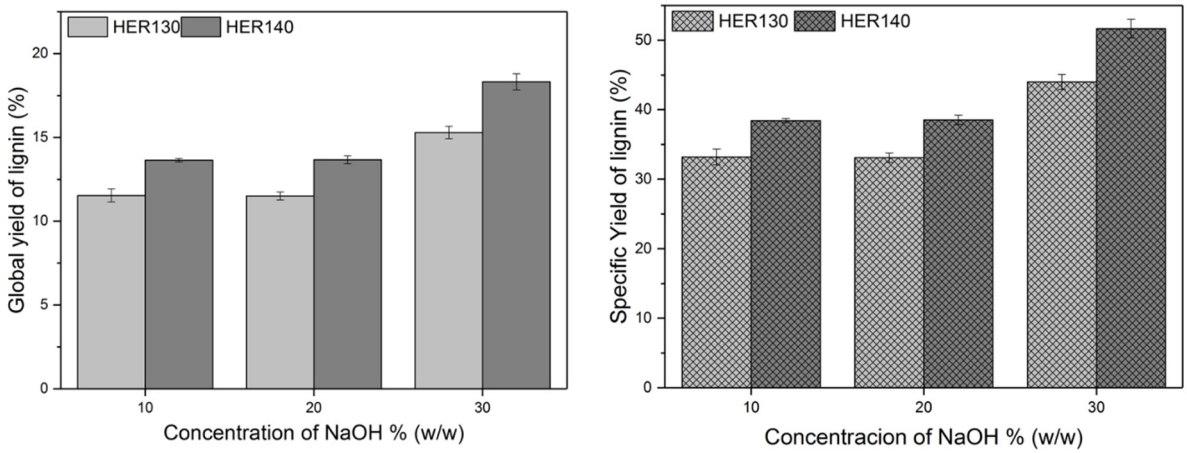

Figure 1. Global (left) and specific (right) lignin yields after alkaline treatment of HER130 and HER140.

HER140 showed higher lignin recovery at all NaOH concentrations compared to HER130. For the same NaOH concentration and reaction time, the temperature variation will govern the lignin recovery by affecting the break of its chemical bonds. High temperatures promote better delignification [30]. Therefore, only HER140 was utilized in the subsequent tests.

When using 10% w/w and 20% w/w NaOH, there was no significant difference in the lignin yields. These data were statistically confirmed using ANOVA (p value > 0.05). A significant increase in the lignin yields occurred with 30% w/w NaOH, in which a specific yield greater than 50% was achieved. However, although it could be a high yield, 30% w/w NaOH is considered to be a high concentration, and it is environmentally unsuitable. Thus, to decrease the NaOH concentration and enhance the lignin yield, the temperature of the alkaline treatment was increased from 121 to 130 °C, and the NaOH concentration was reduced from 30% w/w to 10% w/w. The reaction was then performed in a stirred reactor (Parr® Series 4530) for 90 min at 120 rpm. The global and specific yields of lignin

for this process were 22.99% and 69.38%, respectively. By increasing temperature and using agitation, the obtained yields exceeded all yields previously achieved. Additionally, the yield obtained in this work was superior to that from *Eucalyptus globulus* (63.4%) by the organosolv process [41].

After alkaline treatment, the remaining lignin in the resulting solid fraction, named NER130, was 10.60% *w*/*w*. Thence, it emphasizes the efficiency of the alkaline treatment adopted to recover the lignin from residual eucalyptus sawdust. Indeed, alkaline treatment is one of the main alternatives used in many integrated biorefineries, where biofuels, platform chemicals, and value-added compounds are obtained from lignocellulosic materials [42].

The lignocellulosic composition of the residual eucalyptus sawdust, H_2SO_4-treated fraction (HER140), NaOH-treated fraction (NER130), and the recovered lignin (LIRES) after each treatment were analyzed following the NREL procedures (Table 2). A decrease in the hemicellulose content was observed, as well as an increase in the lignin and cellulose contents, after acid treatment. Mild acidic conditions are more efficient to remove hemicellulose than cellulose and lignin.

Table 2. Lignocellulosic composition of the residual eucalyptus sawdust and the derived fractions after acid-alkaline treatments.

	SAWDUST	HER140	NER130	LIRES *
ASL (%)	1.74 ± 0.05	0.71 ± 0.06	0.98 ± 0.04	3.83 ± 0.24
AIL (%)	21.99 ± 0.05	33.13 ± 0.57	10.60 ± 0.79	89.64 ± 1.31
Hemicellulose (%)	16.05 ± 0.24	6.54 ± 0.07	7.23 ± 0.01	1.61 ± 0.02
Cellulose (%)	42.67 ± 0.80	47.58 ± 0.80	60.45 ± 2.32	1.96 ± 0.02

* LIRES: Lignin isolated from residual eucalyptus sawdust after sequential acid-alkaline treatment.

During alkaline treatment, the cleavage of the β-O-4 and α-O-4 bonds occurs, resulting in many phenolic hydroxyl groups, and due to the lack of hydrophilic groups, alkali solubilization of lignin in the liquor takes place [39]. Therefore, the lignin content decreased from 33.13% *w*/*w* to 10.60% *w*/*w*. The remaining solid fraction is considered to be a cellulose-rich fraction, since it presented around 60% of cellulose. Regarding lignin recovery, almost 90% was AIL and 4% ASL in the LIRES fraction. This result agrees with other works. The insoluble and soluble lignin extracted from *Eucalyptus tereticornis* using the organosolv treatment was 89.2% and 0.9%, respectively [43], whereas the willow lignin isolated using deep euthetic solvents was 92.57% insoluble and 1.89% soluble [25]. The carbohydrate content—reported as cellulose and hemicellulose—present in LIRES was less than 4%.

Therefore, these results demonstrate that sequential acid-alkaline treatment was efficient to fractionate eucalyptus sawdust. The acidic treatment removed 68.15% of hemicellulose whose carbohydrates can be used in other processes, such as fermentation of monomeric sugars. For instance, xylose can be converted into furfural, which is a platform chemical. L-xylose can also be hydrogenated or enzymatically transformed into xylitol [36,44]. The alkaline treatment recovered lignin with purity higher than 90%, presenting a specific yield of 69.38%. Lignin can be applied in carbon fibers, thermoplastics, and emerging potential applications as nanomaterials and antioxidants. Regarding the remaining solid fraction, it is rich in cellulose (60.42%), which can be used in other processes (e.g., ethanol production, polymers, and nanocomposites). Accordingly, the approach of preserving the main fractions of lignocellulosic biomass truly agrees with the biorefinery concept [36,44,45]. The biomass fractions obtained from Eucalyptus sawdust before and after sequential acid-alkaline treatment are exhibited in Figure 2.

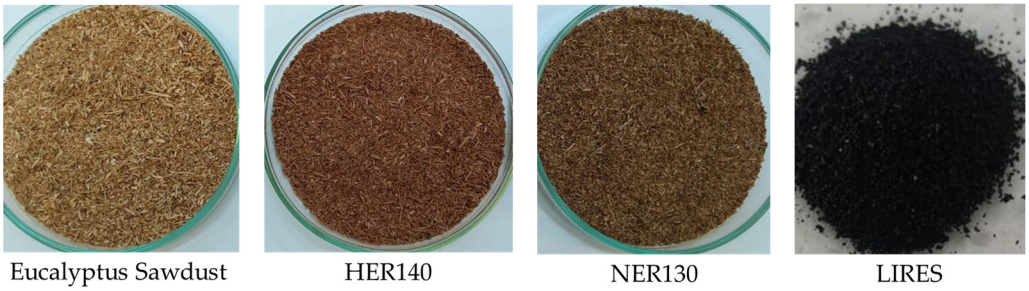

Figure 2. Eucalyptus sawdust before and after sequential acid-alkaline treatment. HER140 represents the biomass fraction obtained after acid pretreatment, NER130 is the biomass obtained after sequential acid-alkaline treatment, and LIRES is the lignin isolated from residual eucalyptus sawdust.

3.4. Crystallinity Index

The solid fractions obtained after different treatments were submitted to X-ray diffraction (XRD) analysis, and the CI was calculated, as shown in Figure 3. A slight change in the CI of the residual eucalyptus sawdust was observed *in natura*, increasing from 45.72% to 49.97% after acid treatment (HER140). It is related to the removal of amorphous material, mainly hemicellulose and amorphous cellulose [46]. After alkaline treatment for lignin recovery, the CI of the remaining solid fraction (NER130) was 47.82%. The lower CI of the NER130 than HER140 might be attributed to the solubilization of lignin and hemicellulose, causing cellulose swelling and solubilization of the crystalline fraction [47]. Furthermore, the appearance of other peaks indicates that the treatment also caused a structural change in cellulose, evidencing polymorphic transformation of cellulose type I into cellulose type II [30]. Cellulose type I is the natural cellulose present in bacteria, algae, and plants. Cellulose type II can be produced from cellulose type I after NaOH treatment through solubilization and re-crystallization [13].

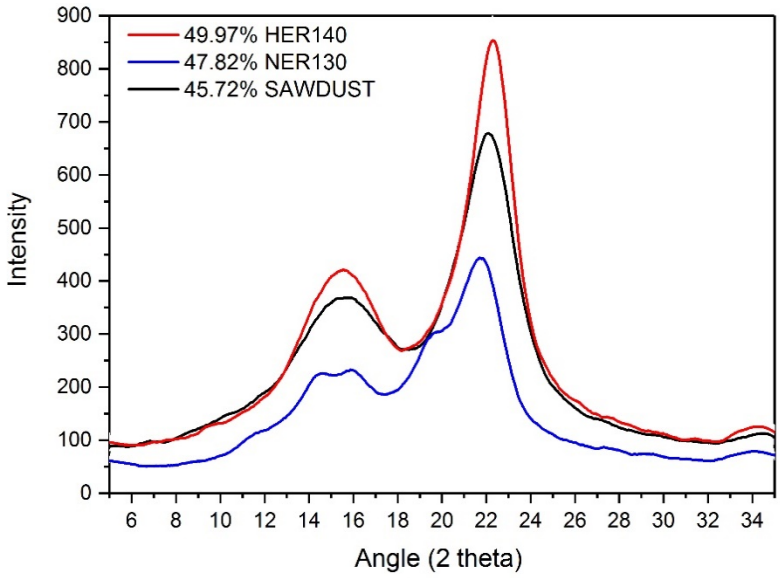

Figure 3. X-ray diffractograms of residual eucalyptus sawdust *in natura* and after acid (HER140) and alkaline (NER130) treatments.

3.5. Lignin Characterization

3.5.1. Thermogravimetric Analysis

Thermogravimetric analysis (TGA) is used to study the properties of materials with the change in temperature. TGA serves as a monitor of the weight loss of a lignin sample as it is heated [48]. TGA and dTG curves for lignin are shown in Figure 4.

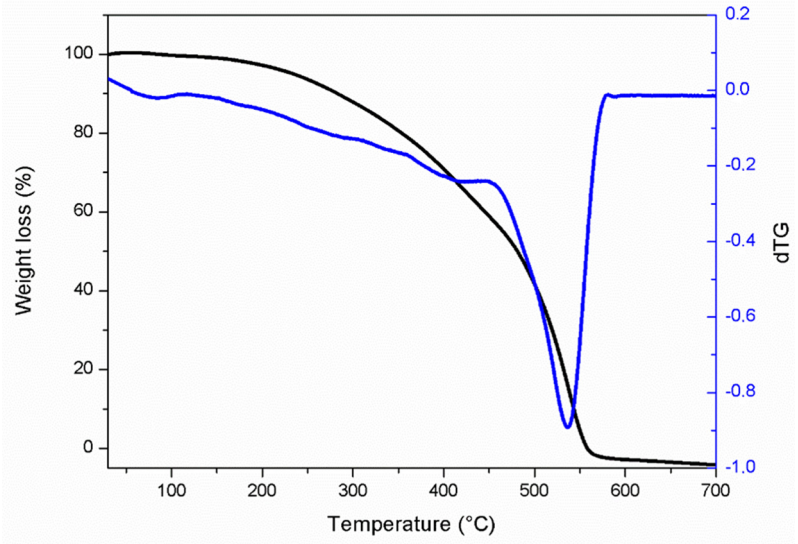

Figure 4. Thermogravimetric analysis for lignin recovered from eucalyptus.

During TGA, the utilization of synthetic air supplied an oxidative atmosphere, in which the sample generally decomposes faster compared to a nitrogen atmosphere [49]. In the first thermal stage, between 100 and 200 °C, the mass loss (3.01%) was due to the elimination of adsorbed water and low molecular weight compounds [50,51]. The second stage occurred between 200 and 400 °C, in which mass loss was 26.76%. In this stage, hemicellulose and cellulose are degraded, and some aryl ether bonds in lignin can be cleaved at 310 °C [50,52]. From 400 °C, complete degradation of lignin started. It was possible to observe that at 560 °C lignin was completely charred. At this stage, the mass loss observed was 70.24%.

3.5.2. Fourier-Transformed Infrared Spectroscopy

The Fourier-transformed infrared spectroscopy (FTIR) analysis revealed the main functional groups present in lignin. The band found at 1712 cm^{-1} is attributed to carbonyl and carboxyl groups [53]. At 1500 cm^{-1}, the vibration of the aromatic skeleton is registered [53]. At 1600 cm^{-1}, the vibration of C=O conjugated to the aromatic ring was recorded [51]. Further, hardwood lignin possesses S units that are represented in the band at 1328 cm^{-1} [51]. G units were also observed at 1215 cm^{-1} [51]. A band at 1116 cm^{-1} was attributed to aromatic C-H in simple deformation of the S unit [54]. The band at 1030 cm^{-1} is assigned to the deformation of the C-O bond in primary alcohols [51]. At 2842 cm^{-1} and 2950 cm^{-1}, there is a C-H stretching from methoxy, methyl, or methylene groups of aromatic side chains [54]. The band between 3000 and 3350 cm^{-1} is attributed to hydroxyl groups [53].

3.5.3. Nuclear Magnetic Resonance

2D HSQC NMR spectra of lignin recovered from residual eucalyptus sawdust under different conditions are presented in Figure 5. The main attributions of crossed signals are listed in Table 3.

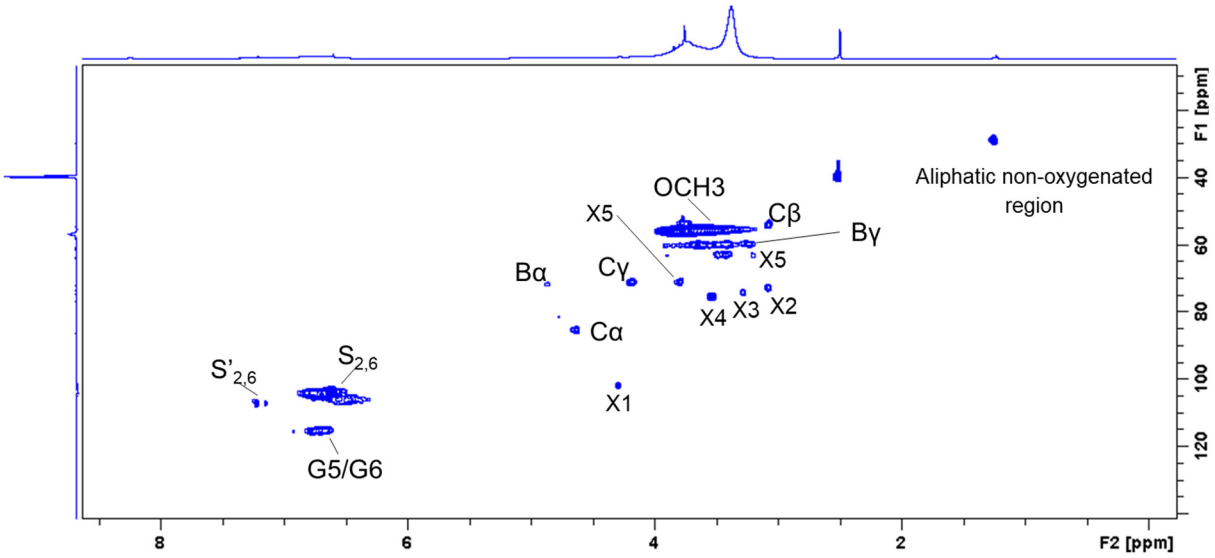

Figure 5. Nuclear Magnetic Resonance (NMR) spectrum of the lignin recovered from residual eucalyptus sawdust (LIRES). (Blue color represent the signs registered by the equipment).

Table 3. Correlation signals of 2D HSQC NMR spectra.

Legend	δ_C/δ_H ppm	Assignment	Reference
Cβ	53.5/3.07	C_β-H_β in β-β resinol substructures	[55]
OCH_3	54.5/3.7 and 56.8/3.75	C-H in methoxyl groups	[56]
Bα	59.3/3.23	C_α-H_α in β–O–4 linked to a syringyl units	[57]
X_5	62.7/3.20	C_5-H_5 in xylan	[57]
Cγ	70.7/3.78 and 71.2/4.17	C_γ-H_γ in β-β resinol substructures	[55]
Bγ	71.2/4.86	C_γ-H_γ in β–O–4 substructures	[57]
X_2	72.4/3.06	C_2-H_2 in xylan	[57]
X_3	73.7/3.27	C_3-H_3 in xylan	[57]
X_4	74.7/3.53	C_4-H_4 in xylan	[57]
Cα	84.9/4.62	C_α-H_α in β-β resinol substructures	[55]
X_1	101.4/4.29	C_1-H_1 in xylan	[57]
$S_{2,6}$	103.8/6.68	$C_{2,6}$-$H_{2,6}$ in syringyl units	[56]
$S'_{2,6}$	106.9/7.21	$C_{2,6}$-$H_{2,6}$ in oxidized syringyl units	[56]
G_5/G_6	115.1/6.71	$C_{2,6}$-$H_{2,6}$ in guaiacyl units	[56]

There are three main regions in the spectra of lignin samples: non-oxygenated aliphatic, oxygenated aliphatic region or literal chain, and aromatic region [57]. The non-oxygenated aliphatic region does not provide structural information about lignin. From the side chain region, it is possible to note different bonds between terminal units and lignin structures along with carbohydrate traces. The most prominent signal found is the C-H of the methoxy group (O-CH_3). Some signals refer to the presence of xylans X_1, X_2, X_3, X_4, and X_5. C_α-H_α, C_β-H_β, and C_γ-H_γ in β-β resinol substructures were also found at 84.9/4.62, 53.5/3.07, and 70.7/3.78 ppm and 71.2/4.17 ppm, respectively. Signals assigned to C_γ-H_γ in β-O-4 substructures and C_α-H_α in β-O-4 substructures attached to S units were found at 71.2/4.86 and 59.3/3.23 ppm, respectively [55,57]. In the aromatic region, signals indicating the presence of S and G units in lignin were found. It was also possible to obtain information about the oxidation of S and G unit side chains [56]. The characteristics of the NMR spectrum of recovered lignin presented similarities with the Kraft lignin obtained from *Eucalyptus globulus*, reported in another work [57].

3.6. Total Phenolic Content and Antioxidant Assays

The TPC presented by lignin from eucalyptus sawdust was 290.8 ± 4.55 mgGAE/g lignin. The observed value was significantly higher than other results from *Miscanthus sinensis* lignin (220 mg GAE/g lignin) isolated by autohydrolysis [50], Kraft lignin (181 mg GAE/g) [58], and pine residual sawdust lignin (226–270 mgGAE/g) [21]. Cavali and co-workers reported the influence of temperature on the TPC of lignin from pine residual sawdust, showing that 150 °C presented higher values than 130 and 170 °C [21].

The phenolic content of a substance is directly related to its antioxidant activity [21,59]. The scavenging potential of LIRES on DPPH radicals is shown in Figure 6 (left).

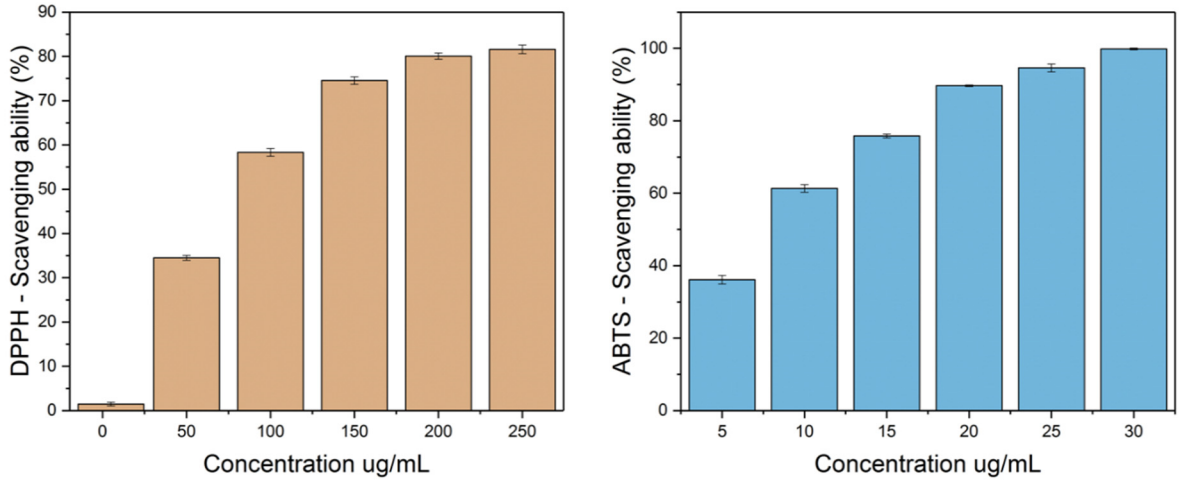

Figure 6. Antioxidant activity of lignin from residual sawdust of eucalyptus according to DPPH (**left**) and ABTS (**right**) assays.

The inhibition capacity of lignin for DPPH radicals increased proportionally to its concentration. However, at higher concentrations, there was no significant increase in the antioxidant capacity since the maximum capacity was already reached. The minimum concentration of LIRES required to inhibit 50% of the DPPH radicals (IC_{50}) was 60 µg/mL (R^2 = 0.9592). That value was 2.7 times higher than the exhibited by Trolox (21.92 µg/mL) (R^2 = 0.9981). Trolox is a water-soluble analog of vitamin E, and it is used as a control antioxidant standard. The IC_{50} of the LIRES was lower than those obtained in other works, such as lignin from oil palm empty fruit bunches (84.43 µg/mL), Acacia nilotica lignin (79.89 to 149.96 µg/mL), and pine residual sawdust (92.72 to 109.91 µg/mL) [21,59,60].

The results regarding ABTS radical inhibition by LIRES are presented in Figure 6 (right). The IC_{50} of the LIRES was 7.39 µg/mL (R^2 > 0.99), while the IC_{50} of Trolox was 3.33 µg/mL (R^2 > 0.97). A concentration of 30 µg/mL of lignin inhibited 99.86% of ABTS. This concentration is 2.5 times higher than the obtained using Trolox (12 µg/mL). However, higher concentrations of LIRES could attain the same antiradical activity as some efficient and well-known commercial antioxidants, such as Trolox [61]. To inhibit 100% of the ABTS radical, 80 µg/mL of lignin isolated from the black liquor of rice straw was required [33]. The antioxidant activity of lignin is directly related to its structure. Thus, the structural modifications of lignin inevitably cause changes in the antioxidant assay [33]. Non-etherified phenolic –OH groups, ortho-methoxy groups, hydroxyl groups, and the double bond between the outermost carbon atoms in the side-chain contribute to the radical scavenging ability of lignin [62]. Thence, this biopolymer has been presented as an excellent source of antioxidants for different products and materials, such as sunscreens [63], polyurethane [64], and biodiesel [65], in addition to many other applications of high-added value [48].

4. Conclusions

Sequential acid-alkaline treatment of the residual sawdust of eucalyptus was efficient to recover 69.38% of lignin while removing 68.15% of hemicellulosic carbohydrates and yielding a cellulose-rich fraction. This fractionation is the first step toward a biorefinery of eucalyptus residual sawdust. Regarding the lignin obtained from residual sawdust of eucalyptus (LIRES), TGA, FTIR, and NMR analyses provided its characteristics, which will be useful when defining subsequent applications for this biopolymer. LIRES also presented a good performance in antioxidant assays, obtaining an IC_{50} of 60 µg/mL and 7.39 µg/mL for DPPH and ABTS assays, respectively. Other works might study future applications of lignin from residual sawdust of eucalyptus to improve its antioxidant properties. Accordingly, residual sawdust of eucalyptus has the potential to be better used as raw material to obtain high value-added products, utilizing its three main lignocellulosic components: cellulose, hemicellulose, and lignin.

Author Contributions: D.T.: conceptualization, methodology, investigation, writing—original draft, data curation, formal analysis. M.C.: conceptualization, methodology, investigation, writing—review and editing, data curation, formal analysis. V.d.O.A.T.: methodology, investigation, writing—review and editing, data curation, formal analysis. L.A.Z.T.: methodology, investigation, writing—review and editing, data curation, formal analysis. A.S.R.: methodology, investigation, writing—review and editing, data curation, formal analysis. A.Z.F.: supervision, project administration, writing—review and editing. C.R.S.: supervision, project administration, writing—review and editing. A.L.W.: supervision, project administration, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data are available from the corresponding author.

Acknowledgments: The authors thank the support of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), a Brazilian federal government agency.

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

References

1. Arevalo-Gallegos, A.; Ahmad, Z.; Asgher, M.; Parra-Saldivar, R.; Iqbal, H.M. Lignocellulose: A sustainable material to produce value-added products with a zero waste approach—A review. *Int. J. Biol. Macromol.* **2017**, *99*, 308–318. [CrossRef]
2. Medina, J.D.C.; Woiciechowski, A.; Filho, A.Z.; Noseda, M.D.; Kaur, B.S.; Soccol, C.R. Lignin preparation from oil palm empty fruit bunches by sequential acid/alkaline treatment—A biorefinery approach. *Bioresour. Technol.* **2015**, *194*, 172–178. [CrossRef] [PubMed]
3. Bohn, L.R.; Dresch, A.P.; Cavali, M.; Vargas, A.C.G.; Führ, J.F.; Tironi, S.P.; Fogolari, O.; Mibielli, G.M.; Alves, S.L., Jr.; Bender, J.P. Alkaline pretreatment and enzymatic hydrolysis of corn stover for bioethanol production. *Res. Soc. Dev.* **2021**, *10*, e149101118914. [CrossRef]
4. Letti, L.A.J.; Woiciechowski, A.L.; Medeiros, A.B.P.; Rodrigues, C.; de Carvalho, J.C.; Vandenberghe, L.P.D.; Karp, S.G.; Torres, L.A.Z.; Guarnizo, A.F.C.; Colonia, B.S.O.; et al. Valorization of solid and liquid wastes from palm oil industry. In *Waste Biorefinery: Value Addition through Resource Utilization*; Bhaskar, T., Pandey, A., Varjani, S., Rene, E.R., Eds.; Elsevier: Amsterdam, The Netherlands, 2021; pp. 235–265. [CrossRef]
5. Vallejos, M.E.; Felissia, F.E.; Area, M.C.; Ehman, N.V.; Tarrés, Q.; Mutjé, P. Nanofibrillated cellulose (CNF) from eucalyptus sawdust as a dry strength agent of unrefined eucalyptus handsheets. *Carbohydr. Polym.* **2016**, *139*, 99–105. [CrossRef] [PubMed]
6. European Organization of the Sawmill Industry. *Annual Report of the European Sawmill Industry 2020–2021*; European Organization of the Sawmill Industry: Brussels, Belgium, 2021.
7. Schneider, V.E.; Peresin, D.; Trentin, A.C.; Bortolin, T.A.; Sambuichi, R.H.R. *Diagnóstico dos Resíduos Orgânicos do Setor Agrossilvopastoril e Agroindústrias Associadas*; Instituto de Pesquisa Econômica Aplicada (Ipea): Brasília, Brasil, 2012; 134p.
8. Brazilian Tree Industry. *Annual Report—2020*; Brazilian Tree Industry: São Paulo, Brasil, 2020.
9. Myburg, A.A.; Grattapaglia, D.; Tuskan, G.A.; Hellsten, U.; Hayes, R.D.; Grimwood, J.; Jenkins, J.; Lindquist, E.; Tice, H.; Bauer, D.; et al. The genome of *Eucalyptus grandis*. *Nature* **2014**, *510*, 356–362. [CrossRef]
10. Rominiyi, O.L.; Adaramola, B.A.; Ikumapayi, O.M.; Oginni, O.T.; Akinola, S.A. Potential Utilization of Sawdust in Energy, Manufacturing and Agricultural Industry; Waste to Wealth. *World J. Eng. Technol.* **2017**, *5*, 526–539. [CrossRef]
11. Gujjula, P.; Kumar, N.; Lynam, J.G. Pretreatment of Loblolly Pine Tree Needles Using Deep Eutectic Solvents. *Biomass* **2021**, *1*, 1–10. [CrossRef]

12. Celiktas, M.S.; Alptekin, F.M. Biorefinery Concept: Current Status and Future Prospects. *Int. Conf. Eng. Technol.* **2017**, *7*, 9.
13. Okolie, J.A.; Nanda, S.; Dalai, A.K.; Kozinski, J.A. Chemistry and Specialty Industrial Applications of Lignocellulosic Biomass. *Waste Biomass-Valoriz.* **2020**, *12*, 2145–2169. [CrossRef]
14. Gupta, A.; Verma, J.P. Sustainable bio-ethanol production from agro-residues: A review. *Renew. Sustain. Energy Rev.* **2015**, *41*, 550–567. [CrossRef]
15. Ragauskas, A.J.; Beckham, G.T.; Biddy, M.J.; Chandra, R.; Chen, F.; Davis, M.F.; Davison, B.H.; Dixon, R.A.; Gilna, P.; Keller, M.; et al. Lignin Valorization: Improving Lignin Processing in the Biorefinery. *Science* **2014**, *344*, 1246843. [CrossRef] [PubMed]
16. Figueiredo, P.; Lintinen, K.; Hirvonen, J.T.; Kostiainen, M.A.; Santos, H.A. Properties and chemical modifications of lignin: Towards lignin-based nanomaterials for biomedical applications. *Prog. Mater. Sci.* **2018**, *93*, 233–269. [CrossRef]
17. Xu, G.; Shi, Z.; Zhao, Y.; Deng, J.; Dong, M.; Liu, C.; Murugadoss, V.; Mai, X.; Guo, Z. Structural characterization of lignin and its carbohydrate complexes isolated from bamboo (*Dendrocalamus sinicus*). *Int. J. Biol. Macromol.* **2018**, *126*, 376–384. [CrossRef] [PubMed]
18. Vanholme, R.; Demedts, B.; Morreel, K.; Ralph, J.; Boerjan, W. Lignin Biosynthesis and Structure. *Plant Physiol.* **2010**, *153*, 895–905. [CrossRef]
19. Liu, Q.; Luo, L.; Zheng, L.Q. Lignins: Biosynthesis and Biological Functions in Plants. *Int. J. Mol. Sci.* **2018**, *19*, 335. [CrossRef]
20. Roy, R.; Rahman, S.; Amit, T.A.; Jadhav, B. Recent Advances in Lignin Depolymerization Techniques: A Comparative Overview of Traditional and Greener Approaches. *Biomass* **2022**, *2*, 130–154. [CrossRef]
21. Cavali, M.; Soccol, C.R.; Tavares, D.; Torres, L.A.Z.; Tanobe, V.O.D.A.; Filho, A.Z.; Woiciechowski, A.L. Valorization of lignin from pine (*Pinus* spp.) residual sawdust: Antioxidant activity and application in the green synthesis of silver nanoparticles for antibacterial purpose. *Biomass Convers. Biorefinery* **2021**, *11*, 1–13. [CrossRef]
22. Barapatre, A.; Meena, A.S.; Mekala, S.; Das, A.; Jha, H. *In vitro* evaluation of antioxidant and cytotoxic activities of lignin fractions extracted from *Acacia nilotica*. *Int. J. Biol. Macromol.* **2016**, *86*, 443–453. [CrossRef]
23. de Carvalho, J.C.; Cardoso, L.C.; Ghiggi, V.; Woiciechowski, A.L.; de Souza Vandenberghe, L.P.; Soccol, C.R. *Biotransformation of Waste Biomass into High Value Biochemicals*; Springer: New York, NY, USA, 2014.
24. Grossman, A.; Vermerris, W. Lignin-based polymers and nanomaterials. *Curr. Opin. Biotechnol.* **2019**, *56*, 112–120. [CrossRef]
25. Li, T.; Lyu, G.; Liu, Y.; Lou, R.; Lucia, L.A.; Yang, G.; Chen, J.; Saeed, H.A.M. Deep Eutectic Solvents (DESs) for the Isolation of Willow Lignin (*Salix matsudana cv. Zhuliu*). *Int. J. Mol. Sci.* **2017**, *18*, 2266. [CrossRef]
26. Liu, L.-Y.; Patankar, S.; Chandra, R.P.; Sathitsuksanoh, N.; Saddler, J.; Renneckar, S. Valorization of Bark Using Ethanol–Water Organosolv Treatment: Isolation and Characterization of Crude Lignin. *ACS Sustain. Chem. Eng.* **2020**, *8*, 4745–4754. [CrossRef]
27. Zikeli, F.; Vinciguerra, V.; Taddei, A.R.; D'Annibale, A.; Romagnoli, M.; Mugnozza, G.S. Isolation and characterization of lignin from beech wood and chestnut sawdust for the preparation of lignin nanoparticles (LNPs) from wood industry side-streams. *Holzforschung* **2018**, *72*, 961–972. [CrossRef]
28. Zhang, X.; Zhu, J.; Sun, L.; Yuan, Q.; Cheng, G.; Argyropoulos, D.S. Extraction and characterization of lignin from corncob residue after acid-catalyzed steam explosion pretreatment. *Ind. Crop. Prod.* **2019**, *133*, 241–249. [CrossRef]
29. Torres, L.A.Z.; Woiciechowski, A.L.; Tanobe, V.O.D.A.; Filho, A.Z.; de Freitas, R.A.; Noseda, M.D.; Szameitat, E.S.; Faulds, C.; Coutinho, P.; Bertrand, E.; et al. Lignin from oil palm empty fruit bunches: Characterization, biological activities and application in green synthesis of silver nanoparticles. *Int. J. Biol. Macromol.* **2020**, *167*, 1499–1507. [CrossRef]
30. Cavali, M.; Soccol, C.R.; Tavares, D.; Torres, L.A.Z.; Tanobe, V.O.D.A.; Filho, A.Z.; Woiciechowski, A.L. Effect of sequential acid-alkaline treatment on physical and chemical characteristics of lignin and cellulose from pine (*Pinus* spp.) residual sawdust. *Bioresour. Technol.* **2020**, *316*, 123884. [CrossRef] [PubMed]
31. National Renewable Energy Laboratory. *Biomass Compositional Analysis*; National Renewable Energy Laboratory: Golden, CO, USA, 2020.
32. Jaramillo, D.T.; Vélez, S.P.M.; Díaz, J.C.Q. Evaluación de pretratamientos químicos sobre materiales lignocelulósicos. *Ingeniare Rev. Chil. Ing.* **2017**, *25*, 733–743. [CrossRef]
33. Jiang, B.; Zhang, Y.; Gu, L.; Wu, W.; Zhao, H.; Jin, Y. Structural elucidation and antioxidant activity of lignin isolated from rice straw and alkali-oxygen black liquor. *Int. J. Biol. Macromol.* **2018**, *116*, 513–519. [CrossRef]
34. Guigou, M.; Cabrera, M.N.; Vique, M.; Bariani, M.; Guarino, J.; Ferrari, M.D.; Lareo, C. Combined pretreatments of eucalyptus sawdust for ethanol production within a biorefinery approach. *Biomass Convers. Biorefinery* **2018**, *9*, 293–304. [CrossRef]
35. Wang, H.-M.; Wang, B.; Wen, J.-L.; Yuan, T.-Q.; Sun, R.-C. Structural Characteristics of Lignin Macromolecules from Different *Eucalyptus* Species. *ACS Sustain. Chem. Eng.* **2017**, *5*, 11618–11627. [CrossRef]
36. Gallina, G.; Cabeza, Á.; Biasi, P.; García-Serna, J. Optimal conditions for hemicelluloses extraction from *Eucalyptus globulus* wood: Hydrothermal treatment in a semi-continuous reactor. *Fuel Process. Technol.* **2016**, *148*, 350–360. [CrossRef]
37. Rangel, J.; Hornus, M.; Felissia, F.E.; Area, M.C. Hydrothermal treatment of eucalyptus sawdust for a forest biorefinery. *Cellul. Chem. Technol.* **2016**, *50*, 521–528.
38. Mankar, A.R.; Pandey, A.; Modak, A.; Pant, K. Pretreatment of lignocellulosic biomass: A review on recent advances. *Bioresour. Technol.* **2021**, *334*, 125235. [CrossRef] [PubMed]
39. Woiciechowski, A.L.; Neto, C.J.D.; de Souza Vandenberghe, L.P.; de Carvalho Neto, D.P.; Sydney, A.C.N.; Letti, L.A.J.; Karp, S.G.; Torres, L.A.Z.; Soccol, C.R. Lignocellulosic biomass: Acid and alkaline pretreatments and their effects on biomass recalcitrance—Conventional processing and recent advances. *Bioresour. Technol.* **2020**, *304*, 122848. [CrossRef]

40. Anwar, Z.; Gulfraz, M.; Irshad, M. Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: A brief review. *J. Radiat. Res. Appl. Sci.* **2014**, *7*, 163–173. [CrossRef]
41. Yáñez-S, M.; Matsuhiro, B.; Nuñez, C.; Pan, S.; Hubbell, C.A.; Sannigrahi, P.; Ragauskas, A.J. Physicochemical characterization of ethanol organosolv lignin (EOL) from *Eucalyptus globulus*: Effect of extraction conditions on the molecular structure. *Polym. Degrad. Stab.* **2014**, *110*, 184–194. [CrossRef]
42. Kim, J.S.; Lee, Y.Y.; Kim, T.H. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. *Bioresour. Technol.* **2016**, *199*, 42–48. [CrossRef]
43. dos Santos, P.S.B.; de Cademartori, P.H.G.; Prado, R.; Gatto, D.A.; Labidi, J. Composition and structure of organosolv lignins from four eucalypt species. *Wood Sci. Technol.* **2014**, *48*, 873–885. [CrossRef]
44. Chen, X.; Zhang, K.; Xiao, L.-P.; Sun, R.-C.; Song, G. Total utilization of lignin and carbohydrates in *Eucalyptus grandis*: An integrated biorefinery strategy towards phenolics, levulinic acid, and furfural. *Biotechnol. Biofuels* **2020**, *13*, 2. [CrossRef]
45. Sharma, A.; Thakur, M.; Bhattacharya, M.; Mandal, T.; Goswami, S. Commercial application of cellulose nano-composites—A review. *Biotechnol. Rep.* **2019**, *21*, e00316. [CrossRef]
46. Brienzo, M.; Fikizolo, S.; Benjamin, Y.; Tyhoda, L.; Görgens, J. Influence of pretreatment severity on structural changes, lignin content and enzymatic hydrolysis of sugarcane bagasse samples. *Renew. Energy* **2017**, *104*, 271–280. [CrossRef]
47. Sarkar, N.; Ghosh, S.K.; Bannerjee, S.; Aikat, K. Bioethanol production from agricultural wastes: An overview. *Renew. Energy* **2012**, *37*, 19–27. [CrossRef]
48. Torres, L.A.Z.; Woiciechowski, A.L.; Tanobe, V.O.D.A.; Karp, S.G.; Lorenci, L.C.G.; Faulds, C.; Soccol, C.R. Lignin as a potential source of high-added value compounds: A review. *J. Clean. Prod.* **2020**, *263*, 121499. [CrossRef]
49. Canevarolo, S.V., Jr. *Técnicas de Caracterização de Polímeros*; Artliber: São Carlos, Brazil, 2017.
50. García, A.; Toledano, A.; Andrés, M.; Labidi, J. Study of the antioxidant capacity of *Miscanthus sinensis* lignins. *Process Biochem.* **2010**, *45*, 935–940. [CrossRef]
51. Zhao, J.; Xiuwen, W.; Hu, J.; Liu, Q.; Shen, D.; Xiao, R. Thermal degradation of softwood lignin and hardwood lignin by TG-FTIR and Py-GC/MS. *Polym. Degrad. Stab.* **2014**, *108*, 133–138. [CrossRef]
52. Lopes, M.S. Obtenção e Caracterização de Ligninas e Nanopartículas de Lignina Klason e Kraft. Master's Thesis, Universidade Federal do Paraná, Curitiba, Brazil, 2018.
53. Romaní, A.; Ruiz, H.A.; Teixeira, J.; Domingues, L. Valorization of Eucalyptus wood by glycerol-organosolv pretreatment within the biorefinery concept: An integrated and intensified approach. *Renew. Energy* **2016**, *95*, 1–9. [CrossRef]
54. Sunthornvarabhas, J.; Liengprayoon, S.; Suwonsichon, T. Antimicrobial kinetic activities of lignin from sugarcane bagasse for textile product. *Ind. Crop. Prod.* **2017**, *109*, 857–861. [CrossRef]
55. Wen, J.-L.; Sun, S.-L.; Yuan, T.-Q.; Sun, R.-C. Structural elucidation of whole lignin from *Eucalyptus* based on preswelling and enzymatic hydrolysis. *Green Chem.* **2015**, *17*, 1589–1596. [CrossRef]
56. Klamrassamee, T.; Tana, T.; Laosiripojana, N.; Moghaddam, L.; Zhang, Z.; Rencoret, J.; Gutierrez, A.; del Rio, J.C.; Doherty, W.O.S. Effects of an alkali-acid purification process on the characteristics of eucalyptus lignin fractionated from a MIBK-based organosolv process. *RSC Adv.* **2016**, *6*, 92638–92647. [CrossRef]
57. Fernández-Costas, C.; Gouveia, S.; Sanromán, M.A.; Moldes, D. Structural characterization of Kraft lignins from different spent cooking liquors by 1D and 2D Nuclear Magnetic Resonance spectroscopy. *Biomass Bioenergy* **2014**, *63*, 156–166. [CrossRef]
58. Faustino, H.; Gil, N.; Baptista, C.; Duarte, A.P. Antioxidant Activity of Lignin Phenolic Compounds Extracted from Kraft and Sulphite Black Liquors. *Molecules* **2010**, *15*, 9308–9322. [CrossRef]
59. Medina, J.D.C.; Woiciechowski, A.L.; Filho, A.Z.; Bissoqui, L.; Noseda, M.; Vandenberghe, L.P.D.S.; Zawadzki, S.F.; Soccol, C.R. Biological activities and thermal behavior of lignin from oil palm empty fruit bunches as potential source of chemicals of added value. *Ind. Crop. Prod.* **2016**, *94*, 630–637. [CrossRef]
60. Aadil, K.R.; Barapatre, A.; Sahu, S.; Jha, H.; Tiwary, B.N. Free radical scavenging activity and reducing power of *Acacia nilotica* wood lignin. *Int. J. Biol. Macromol.* **2014**, *67*, 220–227. [CrossRef] [PubMed]
61. Alzagameem, A.; El Khaldi-Hansen, B.; Büchner, D.; Larkins, M.; Kamm, B.; Witzleben, S.; Schulze, M. Lignocellulosic biomass as source for lignin-based Environmentally Benign Lignin-based Antioxidants isolated from Lignocellulose Feedstock. *Molecules* **2018**, *23*, 2664. [CrossRef] [PubMed]
62. Dizhbite, T.; Telysheva, G.; Jurkjane, V.; Viesturs, U. Characterization of the radical scavenging activity of lignins—Natural antioxidants. *Bioresour. Technol.* **2004**, *95*, 309–317. [CrossRef] [PubMed]
63. Qian, Y.; Qiu, X.; Zhu, S. Lignin: A nature-inspired sun blocker for broad-spectrum sunscreens. *Green Chem.* **2015**, *17*, 320–324. [CrossRef]
64. Ponomarenko, J.; Tatiana, D.; Lauberts, M.; Viksna, A.; Dobele, G.; Bikovens, O.; Telysheva, G. Characterization of Softwood and Hardwood LignoBoost Kraft Lignins with Emphasis on their Antioxidant Activity. *Bioresources* **2014**, *9*, 2051–2068. [CrossRef]
65. Harahap, A.F.P.; Gozan, M. Utilization of lignin as natural antioxidant for biodiesel Utilization of lignin as natural antioxidant for biodiesel. *J. Clin. Immunol.* **2018**, *2024*, 020024. [CrossRef]