agriculture

MDPI

Article

Isolation of Lignin from Anaerobically Digested Unhydrolyzed Solids Produced in a Biorefinery

Xiaoyu Tang [1,*], Kunyang Zhao [2], Chunlin Gao [1], Xionghui Gao [2], Venkatesh Balan [3] and Wenguo Wang [2,*]

[1] Institute of Modern Agricultural Equipment, Xihua University, Chengdu 610039, China
[2] Biogas Institute of Ministry of Agriculture and Rural Affairs, Chengdu 610041, China
[3] Department of Engineering Technology, College of Technology, University of Houston-Sugarland, Houston, TX 77479, USA
* Correspondence: tangxiaoyu@mail.xhu.edu.cn (X.T.); wangwenguo@caas.cn (W.W.)

Abstract: About 30–40% of lignin-rich unhydrolyzed solids (UHS) are left behind after subjecting lignocellulosic biomass to thermochemical pretreated processes followed by enzymatic hydrolysis (EH) to produce sugars that are fermented to fuels and chemicals in a biorefinery. Ammonia Fiber Expansion (AFEX) is one of the leading alkaline pretreatment processes that use volatile ammonia that can be recovered and reused beneficially for the environment. In this work, we used AFEX-EH-UHS which are produced after subjecting corn stover to AFEX followed by EH and contain carbohydrates, ashes, and other impurities that are detrimental to the conversion of lignin to high-value products. In the study, we discovered that ~80% of the carbohydrates present in AFEX-EH-UHS were hydrolyzed and consumed during the AD process. The resulting solids, hereafter called AD-UHS, were subjected to lignin extraction using different combinations of solvents under reflux conditions. The solvent-extracted lignin was subjected to thermogravimetry, nuclear magnetic resonance (NMR) spectroscopy, and molecular weight analysis. Among the solvents, acetic acid could produce 95% pure lignin with some chemical modification, while aqueous ethanol was able to produce 80% pure lignin without any chemical modification.

Keywords: unhydrolyzed solids; lignin; anaerobic digestion; solvent extraction

Citation: Tang, X.; Zhao, K.; Gao, C.; Gao, X.; Balan, V.; Wang, W. Isolation of Lignin from Anaerobically Digested Unhydrolyzed Solids Produced in a Biorefinery. *Agriculture* **2022**, *12*, 1621. https://doi.org/10.3390/agriculture12101621

Academic Editor: Tomasz Płociniczak

Received: 3 August 2022
Accepted: 3 October 2022
Published: 6 October 2022

1. Introduction

Lignin is the second most abundant terrestrial polymer on Earth next to cellulose [1]. Globally, the annual available amount of lignin in the world is about 300 billion tons and is expected to increase by about 20 billion tons every year [2]. The plant cell wall is comprised of cellulose and hemicellulose sugar polymer and aromatic lignin polymer. Lignin is made up of three phenylpropane monomers, p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. The composition of lignin varies depending on the plant source, for example, softwood (25–35%), hardwood (18–25%), and grasses (12–20%). Softwood lignin has mostly coniferyl alcohol monomers, hardwood lignin has coniferyl alcohol and sinapyl alcohol monomers, and grass lignin has three types of monomers (coniferyl, sinapyl, and p-coumaryl alcohol). These monomers are linked through ether bonds (β-O-4 and α-O-4 linkages), biphenyl ether bonds (4-O-5 and 5-O-4), and/or resistant carbon–carbon bonds (β-5, β-β, and 5-5) [3]. In recent years, considerable attention has been paid to lignin because of its potential as an alternative to aromatic resources that are currently obtained from the non-renewable petroleum refining process [4]. The extracted lignin could be used for different applications such as producing composite materials, soil conditioners, filler in rubber products, phenolic resins, or adhesives, providing additional revenue to the biorefinery.

Several methods [5,6], including cracking using catalysts such as rhenium oxides ($MeReO_3$), copper oxides (CuO), lignin-degrading enzyme (peroxidase and laccases) hydrolysis, reduction reactions using hydrogen, and microbial oxidation, have been devel-

oped to depolymerize lignin into aromatic building blocks and monomeric precursors [7,8]. However, most of the research methods have been demonstrated by lab scale and very few commercial processes exist. Native lignin structures (e.g., β-aryl ether linkages) are preferred that can facilitate efficient depolymerization [5]. Different sources of commercial lignin include soda lignin, Kraft lignin, hydrolyzed lignin, organosolv lignin, and lignosulfonates [9]. Extractive Ammonia (EA) pretreatment is another approach that produced crude lignin during pretreatment, which could fractionate pure lignin streams [10,11]. On the other hand, the second-generation biorefinery uses different chemical pretreated lignocellulosic biomasses, which are subjected to separate hydrolysis and fermentation using carbohydrate-degrading enzymes (cellulases, hemicellulose, and pectinase) and native or genetically modified microorganisms such as yeast or bacteria, respectively, to produce biofuel such as ethanol [12]. Lignin-rich unhydrolyzed solids (UHS) (about 30–40% of the feedstock) left behind after enzyme hydrolysis is a sustainable feedstock for producing lignin [13]. Developing new processing methods to isolate lignin from UHS will help to bring additional revenue to biorefinery and reduce the cost of producing biofuels from lignocellulosic biomass.

More importantly, enzymatic hydrolysis is carried out at mild processing conditions (pH 5, 50 °C for 3 to 5 days), which does not make significant structural changes to lignin [14]. However, the UHS contains impurities such as carbohydrates, proteins, and ashes [15], that will inevitably influence its downstream applications. Most of the lignin-upgrading methods also prefer a source of lignin that is enriched and free from impurities. Therefore, the development of cost-effective technologies that can isolate lignin from UHS without extensively affecting the lignin structure is necessary for valorization.

At present, dilute acid pretreatment is a widely used method that results in the production of condensed lignin forming new C-C bonds [16–18]. The highly condensed lignin is hard to break and will consume much higher energy to convert them into valuable products. Anaerobic digestion (AD) is a mature technology for organic waste treatment and has been widely used for biogas production using native and pretreated cellulosic raw materials such as straws, wood chips, energy plants, and municipal solid waste in recent years [19]. The enzymes produced by anaerobic microorganisms can especially break down the lignin–carbohydrate complex (LCC), particularly from recalcitrant biomass [20], making the carbohydrate available to be metabolized. However, the lignin is hardly modified during the AD process [21,22]. Ammonia Fiber Expansion (AFEX) is a mild alkaline pretreatment that uses volatile ammonia which could be recovered and re-used benefiting the environment. This process does not significantly modify the lignin structure according to the previous study [23]. The carbohydrates bound to AFEX pretreated enzymatically hydrolyzed UHS (AFEX-EH-UHS) are highly recalcitrant and cannot be cleaved by commercial fungal enzyme cocktails [24]. Our previous research demonstrated that the carbohydrates bound to UHS can be effectively decomposed by microbes during the AD process and converted into biogas under optimized conditions [25].

The presence of inorganic minerals has been considered a barrier to valorize the lignin present in AFEX-EH-UHS to fuels and chemicals because of its interference with the catalytic process [14]. The isolation of lignin from these impurities is necessary to obtain lignin with desirable quality. In this work, we extracted lignin from AD-UHS using different combinations of organic solvents (acetic acid, aqueous acetic acid, and aqueous ethanol). The extracted lignin was subjected to thermogravimetric, NMR, and gel permeation chromatography analysis to understand the physical and chemical properties and to provide insights into their potential use in various applications.

2. Materials and Methods

2.1. Biomass

The Corn stover (CS) was harvested at Wisconsin (USA) in the year 2010 and oven dried to <6% (dry weight basis). The CS sample was milled to 4-mesh size and stored in zip-lock bags at room temperature. The composition of this untreated CS was 31% glucan,

19% xylan, 3% arabinan, 14% lignin, 13% ash, and 20% extractives. AFEX pretreatment was performed at GLBRC (Biomass Conversion Research Laboratory, Michigan State University, Lansing, MI, USA). Pretreatment was carried out using the procedure reported before [26]. The pretreatment conditions were 1:1 ammonia to biomass ratio (dry weight), 60% moisture loading, and 140 °C for 15 min total residence time. The pretreated sample was kept in the hood overnight to evaporate residual ammonia and the moisture was reduced to <8%. The AFEX-CS was then sealed in zip bags and stored in a 4 °C refrigerator prior to further usage.

2.2. Preparation of AFEX-EH-UHS

The AFEX-CS was then enzymatically hydrolyzed with a commercial enzyme mixture as previously reported [27]. The enzyme mixture was comprised of Ctec2 (28.5 mL/kg CS), Htec2 (16.0 mL/kg CS), and Pectinase (38.2 mL/kg CS) which were generously donated by Novozymes. The glucan loading used for the hydrolysis was 6% by weight, which was equivalent to about 18% solids loading. The enzymatic hydrolysis was conducted in a 5 L bioreactor at 50 °C, pH 4.8, and 250 rpm for 96 h. The AFEX-EH-UHS solids were harvested by centrifugation at 8000 rpm for 30 min to remove the liquid hydrolysate and washed at least twice with deionized water (100 mL for 10 g) to remove absorbed sugar and proteins. Each time the solids were vortex-agitated and centrifuged at 8000 rpm for 30 min. The AFEX-EH-UHS was oven dried at 50 °C until the moisture content was <8% and then milled through to an 80-mesh sieve for further use. The composition of the obtained AFEX-EH-UHS was shown in Table 1.

Table 1. Composition of the inoculum, AFEX-EH-UHS, and AD-UHS.

Sample	Composition (wt.%)			
	Glucan	Xylan	AIL	Ash
Inoculum	2.95 ± 0.04	2.13 ± 0.04	23.04 ± 0.43	31.16 ± 0.16
AFEX-EH-UHS	8.44 ± 0.08	4.18 ± 0.09	64.68 ± 0.44	4.44 ± 0.01
AFEX-EH-UHS + INC	4.46 ± 0.23	2.48 ± 0.15	34.79 ± 0.73	20.95 ± 0.10
AD-UHS-day20	2.83 ± 0.02	1.79 ± 0.33	39.58 ± 1.55	22.27 ± 0.13

INC–Inoculum.

2.3. AD of UHS

The inoculum used in the experiment was taken from a 20 L-AD bioreactor of kitchen waste located in the Biogas Institute of the Ministry of Agriculture and Rural Affairs (Chengdu, China). Effluent from the digester was first passed through an 80-mesh sieve to reduce the particle size and anaerobically incubated at 35 °C until there was no biogas produced. The sludge was then used as inoculum for the AD process. The batch anaerobic experiment was conducted using optimized conditions described before [28]: fermentation temperature of 35 °C, total solids loading (TS) of 2%, inoculum to substrate ratio ($R_{I/S}$, based on volatile solids) of 2:1. The AD was performed in 610 mL serum bottle with a working volume of 250 mL (Figure 1). After mixing UHS with inoculum in the bottle, tap water was added to match the desired TS. The initial pH was adjusted to 7.0 with either 1 M HCl or 1 M NaOH. To maintain an anaerobic atmosphere, each bottle was flushed with nitrogen gas for 3 min. and then sealed with the rubber stopper and aluminum cap. AD experiment was initiated by placing the bottles in an incubator under mesophilic conditions (35 °C). All fermentation experiments were carried out in triplicate and the bottles filled with inoculum only served as control.

While performing AD of AFEX-EH-UHS, biogas production and methane content were analyzed every two days. After sampling, the stopper was pierced with a needle to release the pressure inside the serum bottle. At the end of the AD process, the left-behind solids (AD-UHS) were harvested by centrifugation at 12,000 rpm for 15 min. The obtained solids were washed at least twice with deionized water (100 mL for 10 g). Each time the slurry was vortex-agitated and centrifuged at 12,000 rpm for 15 min. Then, the AD-UHS was oven

dried at 80 °C for 24 h and milled through an 80-mesh sieve for further use. The biogas yield was measured using a pressure meter and the methane content was analyzed by Gas Chromatography (GC). The GC analysis was performed on a GC 122 chromatography system equipped with a carbon molecular sieve column (TDX-01, 2 m × 2 mm) and a thermal conductivity detector. The column temperature, injector temperature, and detector temperature were set at 120 °C, 120 °C, and 150 °C, respectively. Hydrogen was used as carrier gas and the gas pressure was maintained at 0.38 Mpa. The injection volume was 500 µL [29].

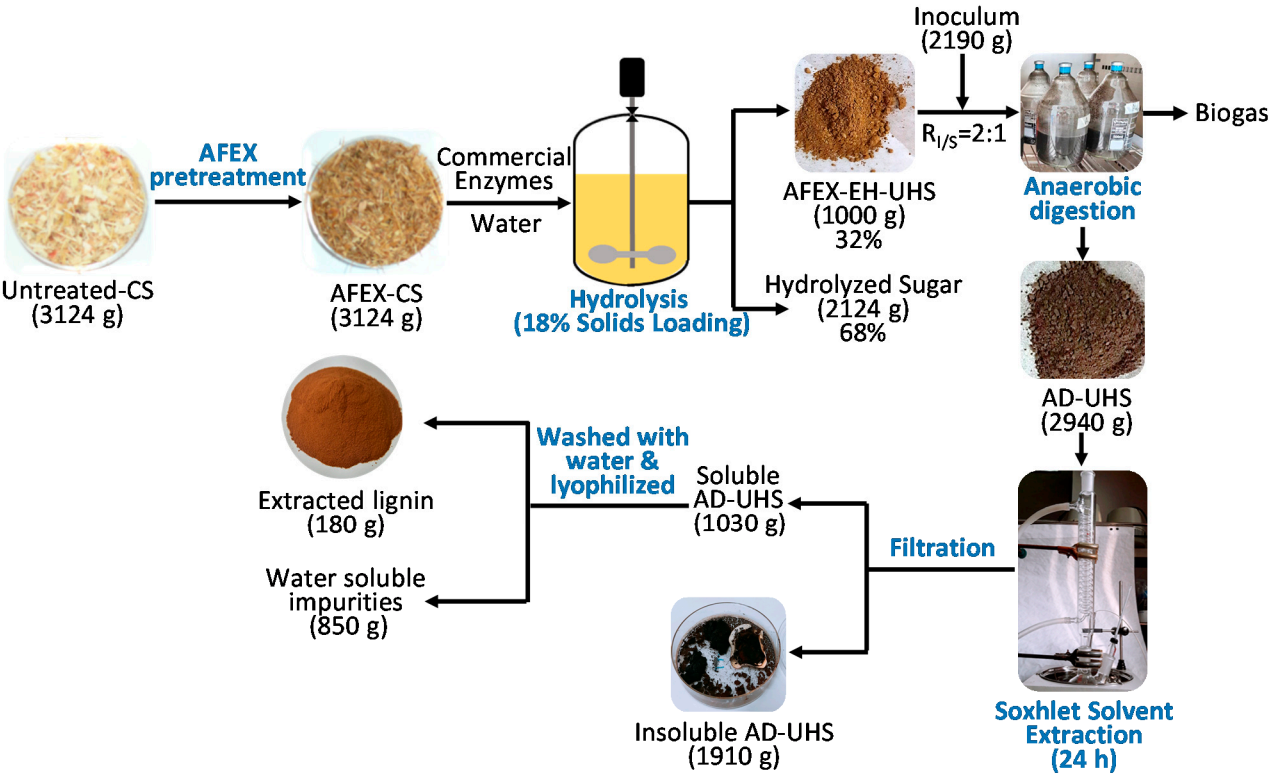

Figure 1. Schematic diagram of the process showing the extraction of lignin from AD-UHS using AcOH solvent.

2.4. Isolation of Extracted Materials from ADUHS

AD-UHS was extracted by different solvents according to a slightly modified published procedure [30]. The experimental scheme was shown in Figure 1, and the solvents used during Soxhlet extraction and the ratio of organic solvent to water ratio were given in Table 2. AD-UHS (10 g) and solvent (200 mL) were refluxed for 24 h in a Soxhlet apparatus. After refluxing, the mixture was centrifugated at 10,000 rpm for 10 min. The obtained solids were washed twice with deionized water (200 mL for 10 g) and then oven dried. The liquid stream containing the extractives was concentrated to 50 mL under vacuum in a rotary evaporator at 60 °C. The same volume of deionized water was added to precipitate lignin and the mixture was centrifugated at 12,000 rpm for 15 min. The centrifuged solids were immersed in liquid nitrogen and subjected to lyophilization.

Table 2. The mass yields extracted and extraction conditions of AD-UHS using three solvents.

Solvent	Refluxing Temp (°C)	Time (h)	Mass Yield (%)
AcOH	105	24	35.20
AcOH: H_2O ratio (2:1)	105	24	37.37
EtOH: H_2O ratio (2:1)	85	24	21.57

2.5. Isolation of Standard Lignin

The standard lignin (employed as a control) was prepared according to the reported procedure [30–32]. The AFEX-EH-UHS and AD-UHS were extracted using 200 mL of dioxane: H_2O (96:4) by mixing in a shake flask for 24 h in the dark (100 rpm, 27 °C). This extraction was repeated three times and 20 mL of solvent (per gram of solid) was used each time. All the dioxane: H_2O extracts were combined, and the solvent was removed under vacuum in a rotary evaporator. The solid lignin was then solubilized in 90% acetic acid and precipitated in deionized water (20 times the volume of acetic acid). The precipitated liquid was centrifugated (12,000 rpm, 15 min) and then lyophilized. The freeze-dried solid was dissolved in 1,2-dichloroethane: ethanol (2:1, *v*/*v*) and precipitated in hexane, then washed with cold hexane. The solid sample was oven dried at 40 °C overnight.

2.6. Analytical Methods

2.6.1. Compositional Analysis of Solids Sample

The carbohydrates, acid-insoluble lignin (AIL) and acid-insoluble ash contents of AFEX-EH-UHS, AD-UHS, extracted materials and solids remaining after extraction were determined using the NREL protocols [33]. The monosaccharides after two-stage acid hydrolysis were analyzed by an HPLC (LC 1200 series, Agilent) equipped with an HPX-87H Aminex column (Bio-rad, Hercules, CA, USA) and a Refractive Index Detector (RID). The mobile phase was comprised of 5 mM H_2SO_4 solution at a flow rate of 0.6 mL/min and the column temperature was maintained at 35 °C. The AIL content was obtained by subtracting the mass of residual acid insoluble ashes from the mass of acid insoluble solids. The ash content in acid insoluble solids was measured based on the weight loss that occurred after subjecting to a muffle furnace at 575 °C for 12 h.

2.6.2. Gel permeation Chromatography (GPC) Analysis

Before the gel permeation chromatography analysis, the sample was acetylated according to the previously reported procedure [10] with minor modifications. About 15 mg of samples were dissolved in a 2 mL mixture of acetic anhydride/pyridine (1:1, *v*/*v*). The solution was stirred at room temperature overnight. Then, 5 mL of anhydrous ethanol was added, and after 30 min stirring, the solvent was removed by rotary evaporation under reduced pressure. The above procedure was repeated several times until both acetic acid and pyridine were completely removed. The acetylated sample was then dissolved in the minimum amount of chloroform and precipitated by adding diethyl ether (20 times the volume of chloroform) dropwise. The precipitate was separated by centrifugation and washed with diethyl ether. The washing and separation steps were repeated at least three times to ensure the complete removal of chloroform. The obtained solid was then dried under a vacuum at 40 °C overnight.

The acetylated samples were dissolved in tetrahydrofuran (THF, 0.5 mg/mL) and then filtered through a nylon membrane (0.45 μm). The GPC analysis was performed on a Waters 1515 chromatography system equipped with two MIXED-C columns (7.5 × 300 mm) and a Refractive Index (RI) detector. THF was used as the eluent at 35 °C with a flow rate of 1.0 mL/min. A calibration curve was established based on six narrow polystyrene standards with molecular weight ranging from 1.5×10^3 to 3.6×10^6 g/mol.

2.6.3. Thermogravimetric Analysis (TGA)

Thermal gravimetric analysis data were obtained using a TA-Thermogravimetric Analyzer (TGAr Q500). Samples were put into a ceramic crucible with a lid. The temperature ramp rate was 20 °C/min from 25 to 800 °C. Nitrogen was used as the flushing gas set at a flow rate of 2.0 mL/min.

2.6.4. NMR Analysis

Standard lignin of AFEX-EH-UHS and AD-UHS, materials extracted from AD-UHS with various solvents, were analyzed by NMR spectroscopy [30]. For ^{1}H NMR and ^{13}C

NMR, 50 mg samples were dissolved in 600 µL DMSO-d6. For 2D HSQC NMR, 80 mg samples were dissolved in 500 µL DMSO-d6. Both ^{1}H NMR and ^{13}C NMR and 2D HSQC NMR spectra were obtained using a Bruker Avance 600 MHz spectrometer at the temperature of 25 °C. The HSQC analysis was performed using a hsqcetgpsisp 2.2 pulse sequence with a 90° pulse, a 2 s cycle delay, a JC-H of 145 Hz, and 6 scans (16 black scans). The acquisition of 128 data points and 0.002 s time were used for F1, and the acquisition of 1024 data points and 0.05 s time were used for F2.

3. Results and Discussion

3.1. The Effect of AD on UHS Composition

Previous studies showed that the carbohydrates present in UHS were highly recalcitrant and unable to hydrolyze by commercial fungal enzymes [24]. However, our previous work demonstrated that those recalcitrant carbohydrates in AFEX-EH-UHS can be efficiently degraded during the AD process [34]. In this study, AD was used to hydrolyze recalcitrant carbohydrates present in AFEX-EH-UHS to facilitate lignin extraction.

The following process conditions were used while carrying out the AD process: inoculum-to-substrate ratio of 2:1, the temperature of 35 °C, total solid loading (TS) of 2%, and 20 days [25]. Under these conditions, the carbohydrates in AFEX-EH-UHS can be converted into biogas with a cumulative methane yield of 103.2 mL/g·VS. The composition of insoluble solids (AD-UHS-day20) obtained after the AD process for 20 days was shown in Table 1. From the results, the contents of carbohydrates (both glucan and xylan) and lignin present in the inoculum were lower compared to AFEX-EH-UHS with inoculum. Thus, the addition of inoculum inevitably led to the decrease in carbohydrates and lignin contents of AFEX-EH-UHS. In particular, the lignin content was decreased to 35%. After subjecting AFEX-EHS-UHS to the AD process for 20 days, the glucan and xylan were further reduced to 2.83% and 1.79% from 4.46% and 2.48%, respectively, while the lignin was enriched to about 40%. On the other hand, the ash content of AFEX-EH-UHS with inoculum was about 21%, which was much higher than 4% in AFEX-EH-UHS. Elemental analysis of inoculum using ICP analysis showed the presence of Ca, Fe, K, Mg, Mn, Zn, and Al. Interestingly, Si was not detected in the inoculum which would contaminate the lignin sample and could negatively impact the catalytic process [10].

When we doubled the amount of inoculum to the substrate, we saw a significant decrease in lignin content and the introduction of more ashes. One possible way to reduce the ash in the inoculum is by recycling part of digested sludge resulting from the first AD cycle. In fact, this strategy should be desirable, as the anaerobic microbial community could be acclimated to the substrate of AFEX-EH-UHS during the recycling process.

Compositional analysis and mass balance (Figure 1) showed that about 80% of carbohydrates present in AFEX-EH-UHS were digested during the AD process, as the amount of carbohydrates in the inoculum remained stable (data not shown). We found that methane productivity was dependent on the amount of carbohydrates in AFEX-EH-UHS when similar AD conditions were used. Our previous work also demonstrated that UHS derived from different pretreatment and/or enzymatic hydrolysis conditions gave different methane yields, due to varying amounts of carbohydrates [34]. On the other hand, the amount of total AIL (in inoculum and AD-UHS) was almost constant during AD, which demonstrated that the lignin could not be degraded by mesophilic anaerobic microbes during the AD process. In other words, we found that there was no mass loss of lignin during the AD process. This result was also in accordance with other observations that lignin was non-degradable in the absence of oxygen [35].

3.2. Mass Yield from Solvent Extraction of ADUHS

To extract lignin from AD-UHS, different solvents, such as AcOH, aqueous AcOH (AcOH: H_2O = 2:1), and aqueous EtOH (EtOH: H_2O = 2:1), were used. We chose these solvents because they gave higher mass yields when extracting UHS according to the previous literature report [30]. Both ethanol and acetic acid were commonly used solvents in

the organosolv pulping process [4]. The mild solvent extraction conditions were employed to avoid any chemical modification of lignin. The solvents were mixed with AD-UHS and refluxed for 24 h at a set temperature. Table 3 showed the % of extracted AIL from AD-UHS when using different solvents. The results showed that AcOH extracted more AIL from AD-UHS compared to using aqueous AcOH and EtOH. The results agreed with previously reported observations [36]. The lignin extraction using organic solvent relies on the lignin solubility, which would be determined by the interaction between the solvent and lignin due to their hydrogen bonding. The relatively lower yield of EtOH was partially attributed to the better solvency of AcOH to lignin fragments of AD-UHS, as the aqueous acid system would contain higher hydroxonium ion concentration and it can dissolve lignin by catalyzing the cleavage of ether linkages in lignin macromolecule [36]. It was important to note that the mass loss from extracting AD-UHS was more when compared to extracting AFEX-EH-UHS. This was probably due to the presence of soluble extractives from microorganisms, enzymes, and ashes after the AD process.

Table 3. Compositional analysis of the extractive when using three solvents.

Solvent	Glucan (wt.%)	Xylan (wt.%)	AIL (wt.%)	Ash (wt.%)
AcOH	0.76 ± 0.01	1.52 ± 0.02	94.59 ± 0.01	2.44 ± 0.30
AcOH: H_2O = (2:1)	0.95 ± 0.15	0.90 ± 0.01	60.92 ± 0.55	5.43 ± 0.59
EtOH: H_2O = (2:1)	0.79 ± 0.00	1.83 ± 0.01	79.41 ± 1.46	3.36 ± 0.20

3.3. Compositional Analysis of Extracts and Solid Residues

The compositional analysis of the materials extracted from AD-UHS and the solids remaining after extraction was carried out to determine the relative proportions of carbohydrates, AIL, and ash (shown in Tables 3 and 4). The contents of arabinan in both extracts and remaining solids were less than 0.5 wt%; thus, it was not shown in the table. Although most of the carbohydrates were degraded during the AD process, there were still a few residual carbohydrates and a significant amount of ashes in leftover solids.

Table 4. Compositional analysis of the left-over solids after extraction with three solvents.

	Glucan (wt.%)	Xylan (wt.%)	AIL (wt.%)	Ash (wt.%)
AcOH	2.26 ± 0.46	1.47 ± 0.31	54.88 ± 2.00	18.78 ± 0.14
AcOH: H_2O = (2:1)	2.56 ± 0.07	1.75 ± 0.14	58.14 ± 0.59	19.87 ± 0.85
EtOH: H_2O = (2:1)	3.25 ± 0.37	2.35 ± 0.26	34.03 ± 0.65	24.11 ± 0.50
Unextracted AD-UHS	2.83 ± 0.02	1.79 ± 0.33	39.58 ± 1.55	22.27 ± 0.13

As shown in Table 3, the material extracted with AcOH (95%) had the most lignin, followed by extraction with EtOH: H_2O (79%) and AcOH: H_2O (61%). All the materials extracted from AD-UHS contained a small proportion of carbohydrates (<3%) and the ratios of carbohydrates to AIL for extracted solids in this study were lower than those materials directly extracted from AFEX-EH-UHS without the AD process [30]. The material extracted with AcOH (2.4%) had the lowest ash content when compared with the materials extracted with EtOH: H_2O (3.4%) and AcOH: H_2O (5.4%).

Since there was a large proportion of solids left behind after extraction, the composition analysis was carried out (Table 4). The proportions of carbohydrates and ashes in solids remaining after extraction with AcOH and AcOH: H_2O were lower than EtOH: H_2O. The results suggested that aqueous organic acid can extract both carbohydrates and ashes in AD-UHS. The AcOH/AcOH: H_2O solvent is capable of acetylating carbohydrates [37] and producing water-soluble saccharides. The material extracted with AcOH and AcOH: H_2O contained small amounts of carbohydrates and ashes because both solubilized ashes and saccharides were mostly removed in the water-washing step. However, the solids remaining after extraction with the AcOH and AcOH: H_2O solvent system had relatively higher lignin compared to unextracted AD-UHS.

3.4. Molecular Weight Analysis

To investigate the influence of AD and solvent extraction on the molecular weight of lignin, gel permeation chromatography (GPC) was conducted (Table 5). The AD-UHS, AFEX-EH-UHS, and extracted lignin using three solvents were analyzed. All the samples were acetylated to facilitate the dissolution. The molecular weights for standard lignin decreased after AD, as demonstrated by the weight-average molecular weight (Mw) values dropping from 6408 (AFEX-EH-UHS) to 4429 (AD-UHS), while the number-average molecular weight (Mn) had no significant difference. Since the composition of AD-UHS was complicated, it was hard to tell the exact reason for the decrease. There may be residual carbohydrates attached to the lignin, leading to a higher Mn value for the standard lignin of AFEX-EH-UHS as reported earlier [10]. The AD process may introduce a fraction of lignin with lower molecular weight, or cause chemical modification on the lignin, resulting in a lower Mw value of AD-UHS lignin. The polydispersity of standard lignin of AFEX-EH-UHS was 2.77, which was close to the values of dioxane-extracted lignin [38]. After subjecting AFEX-EH-UHS to the AD process, the standard lignin exhibited relatively narrower molecular weight distributions compared to AFEX-EH-UHS lignin. We can also see that the materials extracted with solvents had even lower Mw and Mn values than that of AD-UHS lignin. It was noted that the materials extracted with AcOH, AcOH: H_2O, and EtOH: H_2O showed no significant difference in their weight-average molecular weight, which ranged Mw from 2679 to 2964 g/mol. These results suggested that solvent extraction had no substantial influence on the Mw of materials extracted from AD-UHS. Moreover, the materials extracted with three solvents exhibited similar polydispersity, ranging between 1.51 and 1.56.

Table 5. Number-average molecular weight (Mn), Weight-average molecular weight (Mw), and polydispersity (Mw/Mn) of materials extracted from AD-UHS with three solvents, and standard lignin of AFEX-EH-UHS and AD-UHS.

	Mn (g/mol)	Mw (g/mol)	Mw/Mn
AFEX-EH-UHS	2315	6408	2.77
AD-UHS	2260	4429	1.95
AcOH	1860	2808	1.51
AcOH: H_2O = (2:1)	1740	2679	1.54
EtOH: H_2O = (2:1)	1900	2964	1.56

3.5. Thermogravimetric Analysis (TGA)

The thermal stability of lignin is an important physical property for its potential applications and was determined by thermogravimetric analysis (TGA). The TGA curves of all samples were shown in Figure 2. This figure showed similar profiles associated with the thermal decomposition of materials extracted with AcOH and AcOH: H_2O as well as the standard lignin. A substantial mass loss was observed for all samples at the temperature range between 100 and 550 °C, after which no major decomposition was recorded.

The materials extracted from AD-UHS with AcOH, AcOH: H_2O, and EtOH: H_2O experienced less than a 10% weight loss at temperatures below 180 °C. The initial thermal stability can be explained by the low carbohydrate content and low amounts of volatile organic compounds [10]. The onset temperatures were in the range of 180 and 250 °C, followed by a rapid decrease in mass (~50%) up to 450 °C. Then, there was a slow decrease in mass until 800 °C. The residual weights of materials extracted with AcOH, AcOH: H_2O, the standard lignin of AFEX-EH-UHS, and AD-UHS were 40%, 36%, 38%, and 33%, respectively. Similar TGA curves and corresponding residue mass percentages were observed in other work [39], in which the isolated lignin also contained little amounts of carbohydrates. The results confirmed that the presence of recalcitrant carbohydrates with lignin would change the required temperature for decomposition, and thus, affect the amount of produced biochar. The results also suggested that neither AD nor solvent reflux process would significantly affect the thermal decomposition of lignin.

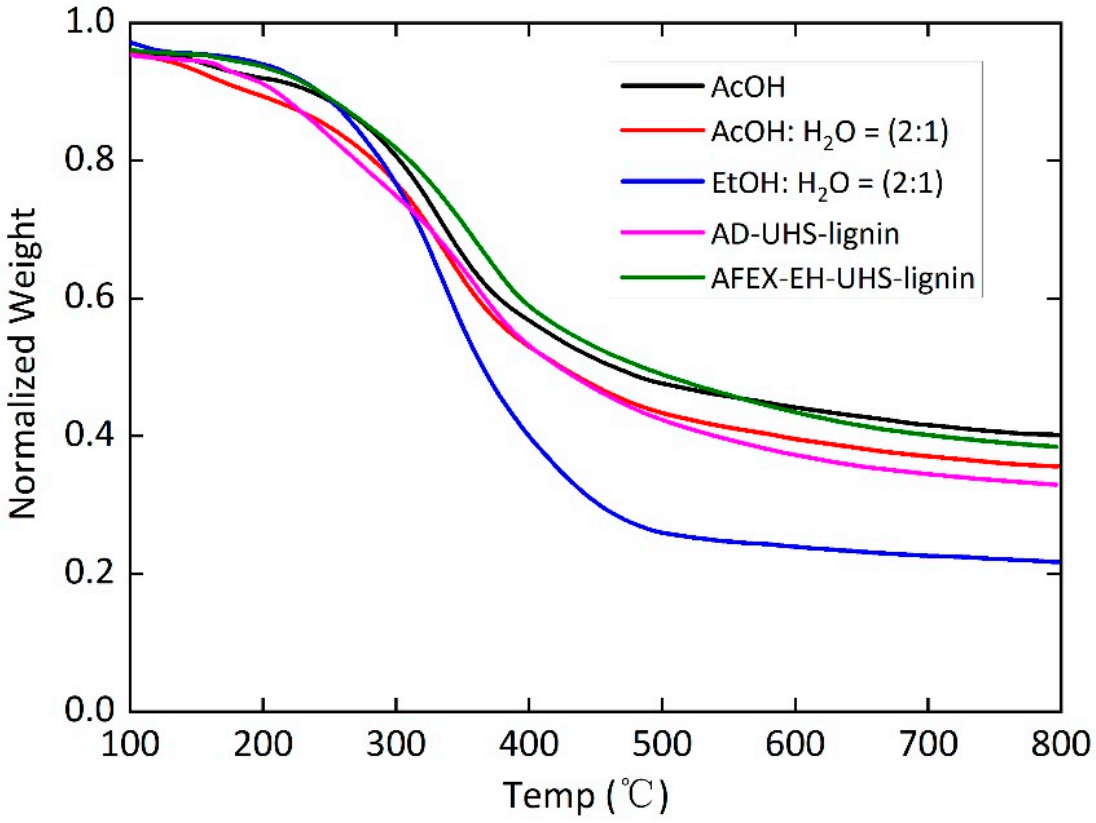

Figure 2. Thermogravimetric curves for material extracted from AD-UHS with three solvents, and standard lignin of AFEX-EH-UHS and AD-UHS.

In contrast to the above samples, the material extracted with EtOH: H_2O had the same onset temperature as standard lignin of AFEX-EH-UHS; however, it behaved differently at temperatures ranging from 250 to 450 °C. The rapid thermal decomposition resulted in a mass loss of approximately 70%. A smaller amount of residues (~22%) was also left behind compared with the other samples at 800 °C. Its profile was like the previous work [10], in which the samples were also extracted with EtOH: H_2O and contained more than 92% of lignin. The results from this study showed that materials extracted with EtOH: H_2O pyrolyzed at lower temperatures, leaving behind relatively low amounts of biochar compared with other samples. It suggested that material extracted from AD-UHS with EtOH: H_2O may be more suitable candidates for thermochemical conversion to liquid fuel by pyrolysis compared to extracted with AcOH.

3.6. Nuclear Magnetic Resonance Analysis (NMR)

Lignin chemistry is dependent on its structure and functionalities, which are usually determined by NMR. 2D HSQC NMR is the preferred method for lignin-related sub-structure analysis due to its superior resolution and the ability to separate overlapping cross-peaks in the ^{13}C NMR spectrum. To understand the effect of AD and the solvent extraction system on the chemical and molecular structure of lignin, the standard lignin from AFEX-EH-UHS and AD-UHS, as well as the materials extracted from AD-UHS using AcOH and EtOH: H_2O, were subjected to whole-cell-wall gel 2D HSQC NMR analysis. The material obtained after AcOH: H_2O extraction was not used because of its low solubility in the DMSQ-d6 solvent. Figures 3 and 4 showed the NMR spectra for all the lignin samples, while the aromatic region (δC/δH: 90–150 ppm/5.5–8.3 ppm) and the aliphatic region (δc/δH: 50–100 ppm/2.0–6.1 ppm) were displayed in Figures 3 and 4, respectively. The corresponding peak assignments were listed in Table 6. As expected, the NMR spectrum for standard lignin of AFEX-EH-UHS showed the presence of various lignin sub-structural units that represented native lignin. The aromatic monomers detected

included guaiacyl (G), oxidized guaiacyl (G′), oxidized syringyl (S′), syringyl (S), ferulate (FA), p-coumarate (pCA), and 4-hydroxybenzyl (H) units, among which the guaiacyl unit was the most abundant, followed by syringyl and p-coumarate unit. While the linkages between aromatic monomers that were detected in the aliphatic region included β-aryl ether (A), phenylcoumaran (C) and resinol (B) linkages as well as the methoxyl group, and the β-aryl ether linkages were the most prevalent.

Table 6. The assignments of major components in the HSQC NMR spectra.

Name	δC/δH	Assignments
pCA_7	144.5/7.54	C_α-H_α in p-coumarate (pCA)
$pCA_{2,6}$	129.6/7.48	$C_{2,6}$-$H_{2,6}$ in p-coumarate (pCA)
$H_{2,6}$	127.8/7.25	$C_{2,6}$-$H_{2,6}$ in 4-hydroxycinnamyl units (H)
FA_6	123.1/7.17	C_6-H_6 in ferulate (FA)
G'_6	122.8/7.12	C_6-H_6 in oxidized (C=O) guaiacyl units (G′)
G_6	118.8/6.75	C_6-H_6 in guaiacyl units (G)
G_5	115.2/6.75	C_5-H_5 in guaiacyl units (G)
pCA_8	114.0/6.28	C_β-H_β in p-coumarate (PCA)
FA_2	110.8/6.25	C_2-H_2 in ferulate (FA)
G_2	110.1/6.92	C_2-H_2 in guaiacyl units (G)
$S'_{2,6}$	106.1/7.28	$C_{2,6}$-$H_{2,6}$ in oxidized syringyl units(S′)
$S_{2,6}$	103.7/6.69	$C_{2,6}$-$H_{2,6}$ in syringyl units (S)
C_α	87.6/5.50	C_α-H_α in phenylcoumaran ©
$A_{\beta(S)}$	85.8/4.10	C_β-H_β in β-O-4 linked to S(A)
$A_{\beta(G)}$	83.4/4.42	C_β-H_β in β-O-4 linked to G (A)
A_α	72.0/4.85	C_α-H_α in β-O-4 unit (A)
B_γ	71.8/3.81	C_γ-H_γ in β-β resinol (B)
A'_γ	63.4/4.16	C_γ-H_γ in γ-acylated β-O-4(A′)
A_γ	62.53/4.40	C_γ-H_γ in β-O-4 substructures (A)
OCH_3	55.9/3.72	C-H in methoxyl group (OMe)

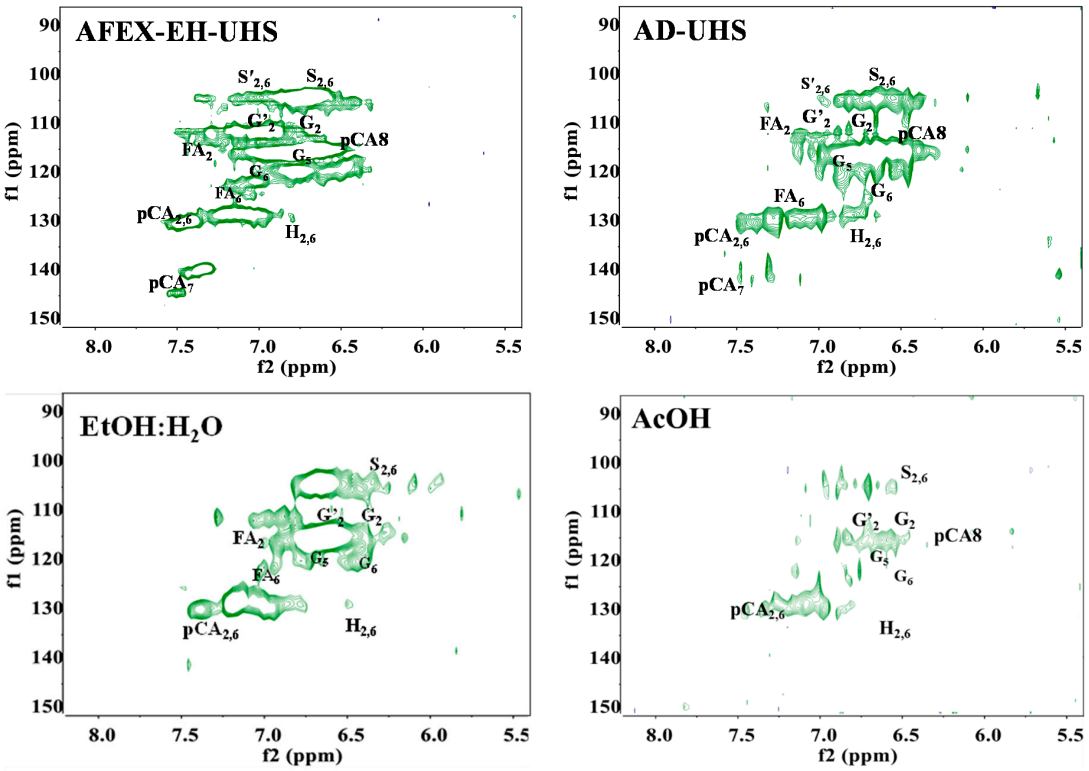

Figure 3. Aromatic region of the 2D-HSQC NMR spectra of material extracted from AD-UHS with different solvents, and standard lignin of AFEX-EH-UHS and AD-UHS.

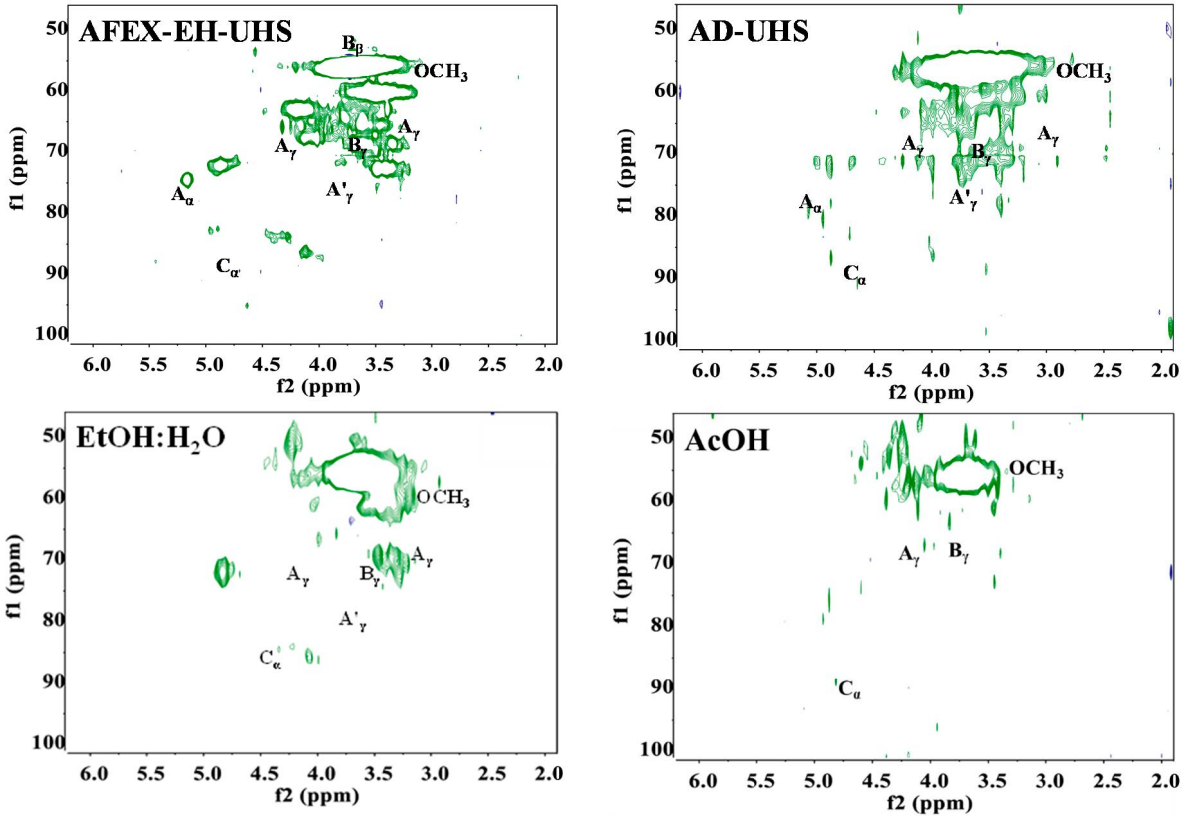

Figure 4. Aliphatic region of the 2D-HSQC NMR spectra of material extracted from AD-UHS with different solvents, and standard lignin of AFEX-EH-UHS and AD-UHS.

The HSQC NMR spectrums for the standard lignin of AFEX-EH-UHS and AD-UHS in both aromatic and aliphatic regions were nearly identical. As seen in Figures 3 and 4, the HSQC NMR spectrum of standard lignin of AD-UHS contained unaltered cross-peaks involving all the monomer units and inter-monomer linkages compared to standard lignin of AFEX-EH-UHS, though the former exhibited slightly weaker signals in Cα and Cγ in β-aryl ether structures and methoxyl substituents that were shown in ^{13}C NMR spectra (data not shown). The results indicated that the AD process did not significantly influence the lignin structure that was present in AFEX-EH-UHS.

The HSQC NMR spectrum of the materials extracted from AD-UHS with EtOH: H_2O was also like that of standard lignin of AFEX-EH-UHS and AD-UHS, except for the disappearance of S′ unit and some sub-structural units susceptible to degradation such as pCA. It was notable that the most sensitive functionalities, the β-aryl ether linkages, were well preserved during solvent extraction by EtOH: H_2O. These types of functionalities are the selected target for many catalytic processes that depolymerize lignin followed by the conversion to value-added products [5,6,40]. Our results suggested that extracting AD-UHS using EtOH: H_2O was well suited for upgrading lignin via the catalytic process. On the other hand, both the aromatic and aliphatic regions of the HSQC NMR spectra of material extracted from AD-UHS using AcOH demonstrated that there was degradation or chemical alteration to lignin and disappearance of FA units. This could be explained by the acetylation reaction during delignification, in which the aliphatic hydroxyl group/phenolic hydroxyl group would react with AcOH, leading to the production of condensed lignin similar to dilute acid pretreatment.

4. Conclusions

In this work, the AFEX-EH-UHS was subjected to the AD process followed by solvent extraction to produce lignin. The batch AD performed under mesophilic conditions for 20 days resulted in 80% biodegradation of recalcitrant carbohydrates in AFEX-EH-UHS and

gained a cumulative methane yield of 103.2 mL/g·VS. However, there was no significant loss of lignin during the AD process. The characterization of the lignin present in AFEX-EH-UHS and AD-UHS suggested that both lignins had a similar structure and thermochemical properties, suggesting the AD process did not significantly alter the lignin structure. When AD-UHS was extracted using AcOH and EtOH: H_2O solvents, we found low levels of ashes and carbohydrates. The ashes extracted by AcOH solvent were removed during the washing step resulting in 95% pure lignin. However, we found some degradation of lignin in the AcOH extracted lignin. On the other hand, the EtOH: H_2O solvent exhibited higher selectivity for lignin extraction from AD-UHS and could preserve much of lignin's functionality such as β-aryl ether linkages, which showed potential for lignin valorization.

Author Contributions: Conceptualization, X.T.; methodology, X.T and K.Z.; formal analysis, X.T. and K.Z.; investigation, K.Z. and X.G.; resources, V.B. and W.W.; data curation, C.G. and K.Z.; writing—original draft preparation, X.T. and K.Z.; writing—review and editing, V.B. and W.W.; funding acquisition, X.T. and V.B. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the National Science Foundation of China (31600013), the Sichuan Science and Technology Program (2019YFH0036), Sichuan Province Engineering Technology Research Center of Modern Agriculture Equipment Program (XDNY2021-002) and Talents Introduction Program of Xihua University (Z212011). VB thanks University of Houston and State of Texas for his startup funds.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: We thank the constructive comments by anonymous reviewers, which improved the presentation of this paper. We thank Biomass Conversion Research Laboratory of Michigan State University for providing AFEX-EH-UHS. We also thank Leonardo da Costa Sousa and Bruce Dale from Michigan State University for the help with this research and Yan Jiang from Chengdu Institute of Biology, Chinese Academy of Sciences for performing NMR structure characterization. We thank Novozymes for providing commercial enzymes Ctec2, Htec2 and pectinase for this project. We also thank GLBRC for providing corn stover.

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

References

1. Becker, J.; Wittmann, C. A field of dreams: Lignin valorization into chemicals, materials, fuels, and health-care products. *Biotechnol. Adv.* **2019**, *37*, 107360. [CrossRef] [PubMed]
2. Fernández-Rodríguez, J.; Erdocia, X.; Sánchez, C.; Alriols, M.G.; Labidi, J. Lignin depolymerization for phenolic monomers production by sustainable processes. *J. Energy Chem.* **2017**, *26*, 622–631. [CrossRef]
3. Parsell, T.; Yohe, S.; Degenstein, J.; Jarrell, T.; Klein, I.; Gencer, E.; Hewetson, B.; Hurt, M.; Kim, J.I.; Choudhari, H.; et al. A synergistic biorefinery based on catalytic conversion of lignin prior to cellulose starting from lignocellulosic biomass. *Green Chem.* **2015**, *17*, 1492–1499. [CrossRef]
4. Gillet, S.; Aguedo, M.; Petitjean, L.; Morais, A.; Lopes, A.; Lukasik, R.M.; Anastas, P.T. Lignin transformations for high value applications: Towards targeted modifications using green chemistry. *Green Chem.* **2017**, *19*, 4200–4233. [CrossRef]
5. Zakzeski, J.; Bruijnincx, P.; Jongerius, A.L.; Weckhuysen, B.M. The catalytic valorization of lignin for the production of renewable chemicals. *Chem. Rev.* **2010**, *110*, 3552–3599. [CrossRef]
6. Rinaldi, R.; Jastrzebski, R.; Clough, M.T.; Ralph, J.; Kennema, M.; Bruijnincx, P.C.A.; Weckhuysen, B.M. Paving the way for lignin valorisation: Recent advances in bioengineering, biorefining and catalysis. *Angew. Chem.* **2016**, *128*, 8296–8354. [CrossRef]
7. Bourbiaux, D.; Pu, J.; Rataboul, F.; Djakovitch, L.; Geantet, C.; Laurenti, D. Reductive or oxidative catalytic lignin depolymerization: An overview of recent advances. *Catal. Today* **2021**, *373*, 24–37. [CrossRef]
8. Crestini, C.; Crucianelli, M.; Orlandi, M.; Saladino, R. Oxidative strategies in lignin chemistry: A new environmental friendly approach for the functionalisation of lignin and lignocellulosic fibers. *Catal. Today* **2010**, *156*, 8–22. [CrossRef]
9. Lignin and Its Derivaties. Available online: http://polymerdatabase.com/polymer%20classes/Lignin%20type.html#:~{}:text=COMMERCIAL%20Lignin%20Products&text=The%20most%20important%20types%20of,Weyerhaeuser%20among%20several%20other%20companies (accessed on 2 August 2022).

10. Da Costa Sousa, L.; Foston, M.; Bokade, V.; Azarpira, A.; Lu, F.; Ragauskas, A.J.; Ralph, J.; Dale, B.; Balan, V. Isolation and characterization of new lignin streams derived from extractive-ammonia (EA) pretreatment. *Green Chem.* **2016**, *18*, 4205–4215. [CrossRef]
11. Da Costa Sousa, L.; Jin, M.; Chundawat, S.P.S.; Bokade, V.; Tang, X.; Azarpira, A.; Lu, F.; Avci, U.; Humpula, J.; Uppugundla, N.; et al. Next-generation ammonia pretreatment enhances cellulosic biofuel production. *Energy Environ. Sci.* **2016**, *9*, 1215–1223. [CrossRef]
12. Robak, K.; Balcerek, M. Review of second generation bioethanol production from residual biomass. *Food Technol. Biotechnol.* **2018**, *56*, 174–187. [CrossRef] [PubMed]
13. Xu, C.; Arancon, R.A.D.; Labidi, J.; Luque, R. Lignin depolymerisation strategies: Towards valuable chemicals and fuels. *Chem. Soc. Rev.* **2014**, *43*, 7475–7485. [CrossRef] [PubMed]
14. Li, C.; Zhao, X.; Wang, A.; Huber, G.W.; Zhang, T. Catalytic transformation of lignin for the production of chemicals and fuels. *Chem. Rev.* **2015**, *115*, 11559–11624. [CrossRef]
15. Holtman, K.M.; Chang, H.; Kadla, J.F. Solution-state nuclear magnetic resonance study of the similarities between milled wood lignin and cellulolytic enzyme lignin. *J. Agric. Food Chem.* **2004**, *52*, 720–726. [CrossRef] [PubMed]
16. Trajano, H.L.; Engle, N.L.; Foston, M.; Ragauskas, A.J.; Tschaplinski, T.J.; Wyman, C.E. The fate of lignin during hydrothermal pretreatment. *Biotechnol. Biofuels* **2013**, *6*, 110. [CrossRef] [PubMed]
17. Van Aelst, K.; Van Sinay, E.; Vangeel, T.; Cooreman, E.; Van den Bossche, G.; Renders, T.; Van Aelst, J.; Van den Bosch, S.; Sels, B.F. Reductive catalytic fractionation of pine wood: Elucidating and quantifying the molecular structures in the lignin oil. *Chem. Sci.* **2020**, *11*, 11498–11508. [CrossRef] [PubMed]
18. Yang, H.; Zhang, Y.; Yoo, C.G.; Meng, X.; Chen, X.; Ragauskas, A.J.; Yao, L. Physico-chemical properties of lignin fractions from acid pretreated corn stover and their effects on enzymatic hydrolysis of microcrystalline cellulose. *BioResources* **2020**, *15*, 4898–4911.
19. Yadav, M.; Balan, V.; Varjani, S.; Tyagi, V.K.; Chaudhary, G.; Pareek, N.; Vivekanand, V. Multidisciplinary pretreatment approaches to improve the bio-methane production from lignocellulosic biomass. *BioEnergy Res.* **2022**. [CrossRef]
20. Chirania, P.; Holwerda, E.K.; Giannone, R.J.; Liang, X.; Poudel, S.; Ellis, J.C.; Bomble, Y.J.; Hettich, R.L.; Lynd, L.R. Metaproteomics reveals enzymatic strategies deployed by anaerobic microbiomes to maintain lignocellulose deconstruction at high solids. *Nat. Commun.* **2022**, *13*, 3870. [CrossRef]
21. Chandra, R.; Takeuchi, H.; Hasegawa, T. Methane production from lignocellulosic agricultural crop wastes: A review in context to second generation of biofuel production. *Renew. Sustain. Energy Rev.* **2012**, *16*, 1462–1476. [CrossRef]
22. Kirk, T.K.; Farrell, R.L. Enzymatic "combustion": The microbial degradation of lignin. *Annu. Rev. Microbiol.* **1987**, *41*, 465–505. [CrossRef] [PubMed]
23. Singh, S.; Cheng, G.; Sathitsuksanoh, N.; Wu, D.; Varanasi, P.; George, A.; Balan, V.; Gao, X.; Kumar, R.; Dale, B.E.; et al. Comparison of different biomass pretreatment techniques and their impact on chemistry and structure. *Front. Energy Res.* **2015**, *2*, 1–12. [CrossRef]
24. Gunawan, C.; Xue, S.; Pattathil, S.; Da Costa Sousa, L.; Dale, B.E.; Balan, V. Comprehensive characterization of non-cellulosic recalcitrant cell wall carbohydrates in unhydrolyzed solids from AFEX-pretreated corn stover. *Biotechnol. Biofuels* **2017**, *10*, 82. [CrossRef] [PubMed]
25. Chen, S.; Tang, X.; Xiao, Z.; Wang, W.; Yin, X.; Balan, V.; Wu, B.; Hu, Q. Biogas production of unhydrolyzed solid from corn stover hydrolysate by anaerobic digestion. *J. Agro-Environ. Sci.* **2016**, *35*, 584–589.
26. Balan, V.; Bals, B.; Chundawat, S.P.S.; Marshall, D.; Dale, B.E. Lignocellulosic biomass pretreatment using AFEX. In *Biofuels: Methods and Protocols*; Mielenz, J.R., Ed.; Humana Press: Totowa, NJ, USA, 2009; pp. 61–77.
27. Uppugundla, N.; Da Costa Sousa, L.; Chundawat, S.P.; Yu, X.; Simmons, B.; Singh, S.; Gao, X.; Kumar, R.; Wyman, C.E.; Dale, B.E.; et al. A comparative study of ethanol production using dilute acid, ionic liquid and AFEX™ pretreated corn stover. *Biotechnol. Biofuels* **2014**, *7*, 72. [CrossRef] [PubMed]
28. Zhao, K.; Gao, X.; Zhu, Q.; Tang, X. Effect of anaerobic digestion on lignin derived from unhydrolyzed solid of corn stover. *J. Agro-Environ. Sci.* **2020**, *39*, 2661–2667.
29. Gao, X.; Tang, X.; Zhao, K.; Balan, V.; Zhu, Q. Biogas production from anaerobic co-digestion of spent mushroom substrate with different livestock manure. *Energies* **2021**, *14*, 570. [CrossRef]
30. Meyer, J.R.; Waghmode, S.B.; He, J.; Gao, Y.; Hoole, D.; Da Costa Sousa, L.; Balan, V.; Foston, M.B. Isolation of lignin from ammonia fiber expansion (AFEX) pretreated biorefinery waste. *Biomass Bioenergy* **2018**, *119*, 446–455. [CrossRef]
31. Guerra, A.; Mendonça, R.; Ferraz, A.; Lu, F.; Ralph, J. Structural characterization of lignin during *Pinus taeda* wood treatment with *Ceriporiopsis subvermispora*. *Appl. Environ. Microbiol.* **2004**, *70*, 4073–4078. [CrossRef]
32. Holtman, K.M.; Chang, H.M.; Jameel, H.; Kadla, J.F. Quantitative ^{13}C NMR characterization of milled wood lignins isolated by different milling techniques. *J. Wood Chem. Technol.* **2006**, *26*, 21–34. [CrossRef]
33. Biomass Compositional Analysis Laboratory Procedures. Available online: https://www.nrel.gov/bioenergy/biomass-compositional-analysis.html (accessed on 17 July 2022).
34. Zhu, T.; Zhu, Q.; Li, Q.; Tang, X.; Zhao, K.; Wu, B. Anaerobic degradation of recalcitrant carbohydrates in lignocellulose hydrolysis residues. *China Biogas* **2018**, *36*, 43–47.

35. Khan, M.U.; Ahring, B.K. Lignin degradation under anaerobic digestion: Influence of lignin modifications—A review. *Biomass Bioenergy* **2019**, *128*, 105325. [CrossRef]
36. Xu, F.; Sun, J.; Sun, R.; Fowler, P.; Baird, M.S. Comparative study of organosolv lignins from wheat straw. *Ind. Crop. Prod.* **2006**, *23*, 180–193. [CrossRef]
37. Borrero-López, A.M.; Valencia, C.; Ibarra, D.; Ballesteros, I.; Franco, J.M. Lignin-enriched residues from bioethanol production: Chemical characterization, isocyanate functionalization and oil structuring properties. *Int. J. Biol. Macromol.* **2022**, *195*, 412–423. [CrossRef] [PubMed]
38. Sun, X.F.; Wang, H.H.; Zhang, G.C.; Fowler, P.; Rajaratnam, M. Extraction and characterization of lignins from maize stem and sugarcane bagasse. *J. Appl. Polym. Sci.* **2011**, *120*, 3587–3595. [CrossRef]
39. Xu, Y.; Zeng, P.; Li, M.; Bian, J.; Peng, F. γ-valerolactone/water system for lignin fractionation to enhance antibacterial and antioxidant capacities. *Sep. Purif. Technol.* **2021**, *279*, 119780. [CrossRef]
40. Macala, G.S.; Matson, T.D.; Johnson, C.L.; Lewis, R.S.; Iretskii, A.V.; Ford, P.C. Hydrogen transfer from supercritical methanol over a solid base catalyst: A model for lignin depolymerization. *ChemSusChem* **2009**, *2*, 215–217. [CrossRef]