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Toward Complete Utilization of Miscanthus in a Hot-Water Extraction-Based Biorefinery

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Abstract: *Miscanthus* (*Miscanthus* sp. Family: *Poaceae*) was hot-water extracted (two h, at 160 °C) at three scales: laboratory (Parr reactor, 300 cm³), intermediate (M/K digester, 4000 cm³), and pilot (65 ft³-digester, 1.841 × 10⁶ cm³). Hot-water extracted miscanthus, hydrolyzate, and lignin recovered from hydrolyzate were characterized and evaluated for potential uses aiming at complete utilization of miscanthus. Effects of scale-up on digester yield, removal of hemicelluloses, deashing, delignification degree, lignin recovery and purity, and cellulose retention were studied. The scale-dependent results demonstrated that before implementation, hot-water extraction (HWE) should be evaluated on a scale larger than a laboratory scale. The production of energy-enriched fuel pellets from hot-water extracted miscanthus, especially in combination with recovered lignin is recommended, as energy of combustion increased gradually from native to hot-water extracted miscanthus to recovered lignin. The native and pilot-scale hot-water extracted miscanthus samples were also subjected to enzymatic hydrolysis using a cellulase-hemicellulase cocktail, to produce fermentable sugars. Hot-water extracted biomass released higher amount of glucose and xylose verifying benefits of HWE as an effective pretreatment for xylan-rich lignocellulosics. The recovered lignin was used to prepare a formaldehyde-free alternative to phenol-formaldehyde resins and as an antioxidant. Promising results were obtained for these lignin valorization pathways.

Keywords: miscanthus; hot-water extraction; scale-up; lignin as an antioxidant; lignin-based formaldehyde-free resins; enzymatic hydrolysis

1. Introduction

The reserves of non-renewable energy resources have been declining exponentially over the past few decades. They continue to irreversibly pollute the planet's atmosphere, and contribute to an increase in the average air temperatures via emission of greenhouse gases. In 2015 the United States consumed 7.08 billion barrels of petroleum, averaging 19.4 million barrels per day [1]. The demand for use of renewable energy has steadily increased and significant research has been done on plant-based fuels as an alternative to fossil fuels. However, the market share of these environmentally friendly fuels is relatively small, with only 10% of the total energy consumption in the U.S. for the year 2015 coming from renewable resources [1]. Most of the early research in renewable energy has been focused on ethanol produced from maize grain, leading to the food/feed vs. fuel dilemma. Furthermore, maize is an annual crop, which consumes a large portion of energy and financial resources, resulting in only a small net positive carbon balance [2]. In contrast, perennial crops such as miscanthus (*Miscanthus* sp.), are attractive alternatives as dedicated energy crops. Comparatively, they require lower amount of

resource input in terms of fertilizers and pesticides, and thus have an improved carbon balance [2]. Miscanthus is a tall, perennial, non-wood rhizomatous C4 grass, consisting of 17 species within the genus, native to Asia. The most commonly studied variant is *Miscanthus × giganteus*, which is a sterile hybrid of *Miscanthus sinensis* and *Miscanthus sacchariflorus*, and has a greater adaptive range and productivity than the wild types. Notable research conducted in the European Union has shown that in comparison to other model grasses, miscanthus yields a higher ratio of biomass to area. Moreover, the yield is consistent regardless of rainfall, nitrogen fertilizer or growing degree days [2]. Miscanthus has also gained attention among U.S. researchers over the past few years. The models obtained from European research have been employed to conduct field trials of miscanthus in Illinois, USA with promising results of biomass yields up to 44 t ha⁻¹ [2,3].

Miscanthus is mainly composed of cellulose (40–60%), hemicelluloses (20–40%), and lignin (10–30%), with arabinoxylans (AXs) as the predominant hemicellulose [3]. AXs are esterified to a certain degree with ferulic acid at the C5 carbon of the arabinose and xylans are sporadically joined through diferulate bridges. Grasses such as miscanthus are characterized by HSG (*p*-hydroxyphenyl-syringyl-guaiacyl) lignin with presence of *p*-coumaroylated and feruloylated units [4–7]. Grass lignin can also be linked to hydroxycinnamic acids (*p*-coumaric and ferulic acids) via ether linkages [5]. Therefore, as other grasses, miscanthus is characterized by a lignin-ferulate-xylan complex which contributes to the additional recalcitrance of grasses [8]. Recalcitrance is a common feature of not only grasses but in general all lignocellulosic biomass [9]. Different pretreatments have been suggested to overcome this issue [10,11]. One important group is that of hydrothermal pretreatments, due to their environmentally friendlier nature and their ability to generate hemicellulosic sugars as an additional revenue stream [12]. Hot-water extraction (autohydrolysis) is an example of such pretreatment, which is a subcritical hydrothermal pretreatment [13]. Supercritical pretreatments have also been applied [13,14].

Keeping an eye on future energy demands, researchers at SUNY-ESF have proposed a lignocellulosic-based biorefinery, to efficiently isolate and recover the various biomass components, viz. cellulose, hemicelluloses, and lignin, which can then be utilized to produce high-volume low-value (biofuels) and low-volume high-value products (bioplastics, biochemicals) [15]. The hot-water extraction process (HWE; water treatment at 160 °C for 2 h) is proposed to be an integral part of the biorefinery with the goal of removing hemicelluloses through autohydrolysis. HWE is commonly applied on Angiosperms rich in xylans and can remove up to 80% of the original xylans [16]. During this process, some lignin also becomes soluble; for example, in M/K digester experiments conducted at SUNY-ESF, 9.4% and 15.7% of the total lignin was removed from sugar maple (*Acer saccharum*) and foxglove-tree (*Paulownia tomentosa*), respectively [17]. Cellulose was found to be largely intact at the end of HWE [15]. Therefore, HWE may be recommended for use in biorefineries to relatively selectively remove most of the xylans from different lignocellulosics, including hardwoods, agriculture residues, and grasses. The process is based on autohydrolysis as it is conducted in water at high temperatures. Thus, it eliminates regeneration costs associated with the use of organic solvents and harsh chemicals and reduces pollution concerns. The potential uses of extracted xylan-based carbohydrates have been explored through fermentation or chemical conversion routes to a great extent in the past. It has been well established by now that an array of products can be produced, including ethanol, furfural, lactic acid, and polyhydroxyalkanoates [15,18,19]. In this report, we explore some possibilities in using other two product streams of HWE; extracted biomass and lignin recovered from hot-water extracts. Our goal is to suggest products which can add value to a biorefinery based on xylan-rich biomass, such as miscanthus. We demonstrate the effects of scaling the process up from a laboratory-sized Parr reactor (300 cm³) to a mid-range M/K digester (4000 cm³) to a full-sized pilot reactor (65 ft³; 1.841 × 10⁶ cm³), on the removal of xylans, inorganics, and lignin (delignification degree expressed as the amount of lignin removed based on the total lignin originally present in percentages), and the retention of cellulose. The native and hot-water extracted miscanthus (MS and EMS, respectively), hot-water extracts, and lignin recovered from hot-water extracts (RecL) by acid precipitation were

characterized. The samples of MS and EMS were hydrolyzed to produce fermentable sugars and evaluate the benefits of HWE in regard to hydrolyzability of miscanthus. Comparative experiments of enzymatic hydrolysis were also conducted in the presence of surfactants. Considering uses of extracted biomass, we also focused on the potential production of fuel pellets and benefits which can be expected if HWE would be performed prior to pelletizing. With this goal in mind, a higher heating value of MS and EMS, and in addition, of RecL as a potential fuel-pellet additive, was measured. RecL was also studied as a raw material for the production of formaldehyde-free adhesives and as an antioxidizing agent. The lignin-based adhesives may be proposed as possible environmentally friendly and safe alternatives to phenol-formaldehyde resins [20–24]. Also, as furfural is a known crosslinking agent for lignin [25–27], lignin and furfural may be considered to replace phenol and formaldehyde, respectively, in the lignin-furfural resin systems [28]. An important advantage of using furfural is its renewable nature, underscored by its selection into the list of top ten designed products of biorefineries [29]. Moreover, furfural and lignin may be produced at the same site [15]. In the present work, the effect of furfural content is studied on the mechanical properties of lignin based resins. Due to a polyphenolic nature, lignin can also be used as an antioxidant [30,31]. Antioxidants are additives used predominantly in pharmaceutical and cosmetic preparations, foods, dyestuffs and synthetic polymers. They are also useful in reduction of oxidative stress in living systems. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are two of the most common, commercially used synthetic antioxidants. Their manufacturing costs, relatively poor efficiencies and non-renewable nature have led to a search for natural antioxidants, which are relatively cheaper and safer than the synthetic ones [32]. Lignin itself has been reported to be useful as an antioxidant in cellulose pulps [33] and in synthetic polymers [34]. Radical scavenging activity of lignin has been also reported on cancer cell lines [35]. Therefore, antioxidation potential of lignin recovered from miscanthus hot-water extracts was analyzed through its radical quenching ability [36] and compared with that of synthetic antioxidants.

These studies have a goal to promote use of miscanthus as a perennial fast growing species as an alternative to fossil fuels for the production of a broad range of products including carbohydrate- and aromatic-based products and hence, to suggest potential directions to increase economics of miscanthus-based biorefineries.

2. Results and Discussion

2.1. HWE Yield and End pH

The yield of unextracted biomass (digester yield, % based on the starting O.D. mass) increased with the scale, from ~60% to ~67% (Table 1).

Table 1. Biomass yield and hydrolysate pH after HWE of miscanthus.

Variable	Digester		
	EMS _{Parr}	EMS _{M/K}	EMS _{Pilot}
Scale-up factor	1	44.4	31,020
W/B	40	10	8
Digester Yield (% OD MS)	59.9 ± 0.34	64.7	66.95 ± 0.21
Hydrolysate pH	3.8 ± 0.04	3.67	3.595 ± 0.007

Determined digester yields were comparatively lower than observed for different hardwoods (such as *Paulownia* sp. and sugar maple) studied earlier under the same conditions [17]. This result suggests that autohydrolysis reactions taking place during HWE lead to extraction of a higher amount of miscanthus constituents than of hardwood constituents. The observed ready availability of miscanthus to HWE may provide an opportunity for valorization of a larger amount of extracted material and also for more effective enzymatic hydrolysis of the remaining cellulose, largely free of hemicelluloses and some lignin. Yield was noticeably affected by the scale-up factor, which can be

explained by the differences in the particle size and water-to-biomass (W/B) ratio (Table 1). Parr reactor experiments resulted in the lowest yields of hot-water extracted biomass, i.e., the highest extraction yields. This can be attributed to a higher accessibility and more ready diffusion of miscanthus constituents, due to the small miscanthus particle size and higher concentration gradients characteristic of small-scale laboratory experiments. The digester yields increased as the particle size increased and the W/B ratio decreased, with the yield obtained in the pilot scale digester being ~17% higher than in the laboratory Parr scale reactor. Contrary to expected inverse correlation between the extraction yields and pH, the extraction yield decreased with the decrease of the end pH, i.e., with the scale-up factor. The lowest pH and lowest extraction yields obtained for the largest scale demonstrate that even though important, the pH is not the only HWE (autohydrolysis)-governing factor. Additional factors such as particle size, W/B ratio, operating scale, and most importantly, character of lignocellulosic biomass should be taken into account in evaluation of the effectiveness of HWE process, before being industrially implemented.

2.2. Chemical Composition of Native Miscanthus (MS), Hot-Water Extracted Miscanthus (EMS) and Recovered Lignin (RecL)

Miscanthus was found to be more susceptible for extraction using ethanol/toluene (ET) than dichloromethane (DCM) as expected in general [37] (Table 2). DCM extracts are rich in waxes, fats, resins, sterols, and non-volatile hydrocarbons, while in addition, ET extracts contain polyphenols, low-molecular weight carbohydrates and water-soluble compounds [37,38]. In accordance with previous studies [39], the extractive content increased after HWE, most likely due to an increased porosity of hot-water extracted biomass [40]. Organic solvents used in the determination of content of extractives and pre-extraction of biomass have been also found to cause delignification of hot-water extracted biomass, resulting in a decrease in the determined lignin content. Furthermore, previous studies have demonstrated a relatively high tendency of ET to extract lignin from the hot-water extracted biomass [39]. Therefore, while ET was used in pre-extraction of native miscanthus, DCM was used as a pre-extraction solvent in experiments with hot-water extracted miscanthus.

Table 2. Chemical composition of native and hot-water extracted miscanthus (% O.D.).

Component	MS	EMS _{Parr}	EMS _{M/K}	EMS _{Pilot}
ET (1:2) extractives	5.57	-	-	8.18
DCM extractives	1.02	4.76	3.03	3.52
Seifert cellulose ¹	39.78 ± 0.32	60.19 ± 0.61	58.05 ± 0.44	57.2 ± 0.51
Cellulose removal ²	-	9.86	5.58	3.8
Klason lignin	20.27 ± 0.26	20.05 ± 0.04	22.02 ± 0.12	21.44 ± 0.49
Acid soluble lignin	1.9 ± 0.006	1.24 ± 0.07	1.12 ± 0.03	1.07 ± 0.05
Total lignin	22.17 ± 0.29	21.3 ± 0.57	23.13 ± 0.14	22.51 ± 0.45
Lignin removal ²	-	42.76	32.47	32.07
Ash	4.24 ± 0.07	1.32 ± 0.47	3.89 ± 0.1	4.97 ± 0.02
Ash removal ²	-	81.45	40.64	21.58
PhOH (mmol/g lignin)	0.63	-	1.47 ± 0.07	1.38 ± 0.18

¹ The Seifert cellulose was corrected for residual lignin (acetyl bromide method). ² Component removal: $100 \times [(\text{component content (MS)} - (\text{component content (EMS)} \times \text{digester Yield})) / \text{component content (MS)}]$, % of original component content in MS.

Detailed carbohydrate analysis revealed xylan as the main hemicellulose component of native miscanthus (~20% O.D., Table 3). The relatively high ratio of xylan to arabinose (indicating linearity of hemicelluloses) and low acetylation degree corroborate reported traits of carbohydrates in grasses [41]. The expected deacetylation taking place during HWE is evident by the decreased acetyl content in hot-water extracted miscanthus (Table 3). Removal of xylan ranging between ~57% (pilot scale) and ~66% (M/K scale) was lower than that reported for hardwoods [15,16]. The lower xylan hydrolyzability may be attributed to relatively low contents of acetyl and arabinose groups [42] and to developed

cross-linked structure characteristically present in miscanthus cell walls (diferulate bridges between xylans and ferulate bridges between xylan and lignin [8,43]. Taking into account a reduced xylan removal and higher extraction yields, it seems that HWE is less selective in the removal of xylans from miscanthus than from hardwoods. Even though removal of xylans varied in experiments of different scales, no trends were observed.

Table 3. Carbohydrate composition of miscanthus; results include furfural as xylose degradation product and acetate resulting from deacetylation (% O.D.).

Component	MS	EMS _{Parr}	EMS _{M/K}	EMS _{Pilot}
Glucose ¹ (Glucan)	41.54 (37.39)	58.02 (52.22)	59.93 (53.94)	56.00 (50.40)
Glucose loss ²	-	16.33	6.66	9.81
Xylose ¹ (Xylan)	20.75 (18.26)	13.31 (11.71)	10.89 (9.58)	13.30 (11.7)
Xylose loss ²	-	61.77	66.04	57.11
Arabinose (Arabinan)	1.45	0.26	0.13	0.26
Furfural	0.31	0.18	0.2	0.3
[Acetate]	0.11	0.05	0.04	0.05

¹ The conversion factor for glucose and xylose to glucan and xylan are 0.9 and 0.88, respectively. ² Loss: $100 \times [(\text{content (MS)} - (\text{content (EMS)} \times \text{digester Yield})) / \text{content (MS)}]$, % of original content in MS.

Cellulose determined by the Seifert method has been found to correlate well with the glucan content of eucalyptus (*Eucalyptus nitens*) acquired with acid hydrolysis followed by HPLC [44]. Seifert cellulose content was determined in these studies and was corrected for the lignin content measured by acetyl bromide method [45]. Although the glucan content of miscanthus (measured by acid hydrolysis followed by ¹H-NMR) may be expected to be higher than the content of Seifert cellulose as in addition to cellulose, miscanthus contains xyloglucan and the mixed linkage (1→3, 1→4)-β-D-glucan [46,47], our experiments showed opposite results (Tables 2 and 3). The discrepancy was especially noticeable in the case of EMS and this effect might be attributed to a higher accessibility of the more porous HW-extracted biomass. These puzzling results highlight a need for the development of more comprehensive methods of carbohydrate analysis to complement growing biorefineries. Seifert cellulose content increased after HWE, with the maximum cellulose content obtained for the smallest Parr reactor scale (which also resulted in the lowest yield of EMS). Although these experiments revealed susceptibility of cellulose to hydrolysis during HWE of miscanthus, cellulose losses decreased with increasing scale-up factor and in regard to cellulose retention pilot experiments showed the best results with less than 4% of the total Seifert cellulose removed.

Total lignin content of native and hot-water extracted miscanthus was fairly similar (Table 2). The lignin removal (synonymous with degree of delignification, fraction of the total lignin dissolved, expressed in percent) decreased for larger scales, i.e., Parr experiments revealed occurrence of a greater delignification than M/K and pilot experiments. Nevertheless, miscanthus lignin was found to be relatively susceptible to hydrolysis as delignification of >30% was observed at all scales. In contrast, hardwoods have exhibited delignification of <20% under the same conditions of HWE in our previous experiments [17,39,48]. The relatively low molecular weight of lignin in miscanthus and/or abundance of lignin and lignin-carbohydrate acid-labile bonds may contribute to its relatively higher hydrolyzability in comparison to lignin in hardwoods. The content of free phenolic hydroxyl groups (PhOH) was determined for MS and EMS (Table 2). The PhOH group content determined for miscanthus milled wood lignin (MWL) by [38] is somewhat higher (0.84 mmol/g lignin) than that found in our studies for MS (0.63 mmol/g lignin) but the small discrepancy may be caused by ball milling (required step in MWL isolation), which is known to cause chemical changes in the functions groups of lignin [49]. The difference may also be attributed to the periodate oxidation method of PhOH content determination used in our studies as this method fails to include any PhOH groups not adjacent to methoxyl group, as in H-units and *p*-coumarate units [50], which are commonly present in HSG lignins in grasses [4–7]. HWE approximately doubled the content of PhOH groups in

EMS. This result suggests an extensive cleavage of acid-labile bonds; aryl ether bonds (α -O-4, β -O-4) and/or phenylglycosidic bonds leading to a ready release of lignin fragments and a relatively higher degree of delignification of miscanthus compared to that of hardwoods (based on MK studies DD 30% found for MS vs 10% for *Acer saccharum* (sugar maple) and 15% for *Paulownia tomentosa* [17]. The increase in PhOH content of miscanthus after HWE was still less than that observed in sugar maple (0.4 mmol/g lignin before HWE to 1.8 mmol/g lignin after HWE, more than four times increase [39]. Based on these results it can be concluded that along with the cleavage of acid-labile bonds other factors contribute to the lignin dissolution during HWE of Miscanthus such as its size and hydrophilicity; notably, *p*-coumarylated and feruloylated end lignin units naturally present in miscanthus [43] carry hydrophilic PhOH groups and may enhance lignin solubility.

Recovered lignin from pilot studies was found to contain some carbohydrates, which remained in lignin even after washing with acidic water at pH 5 (Tables 2 and 3). It appears that lignin recovered from hot-water extracts encompasses lignin bound to carbohydrates in a form of lignin-carbohydrate complex (LCC), which is soluble in water to a certain extent [51].

The inorganic constituents of miscanthus were removed at a notable degree in Parr reactor experiments (>80% of the total inorganics were removed) but deashing decreased with scale-up factor and only ~20% of inorganics were removed during pilot plant experiments. This result may be attributed to some extent to use of water of different quality in different experiments (DI water and tap water in Parr reactor and pilot plant experiments, respectively).

2.3. Near Infrared Spectroscopy (NIR)

Solid state NIR analysis of MS and EMS was performed. Quantitative analysis was not possible due to limited number and variability of MS and EMS sample. Qualitative analysis was conducted on raw and second derivative spectra. Key wavelengths in spectra are labeled according to Table 4.

Table 4. Wavelengths of interest for NIR analysis.

Number	Wavelength (nm)	Bond Vibration	Assignment	Reference
1	1426	1st OT O–H stretch	Amorphous Cellulose	[52]
2	1721	1st OT C–H stretch	Polysaccharides	[53]
4	1909	2nd OT C=O stretch	Hemicelluloses	[54]
3	1916–1942	O–H stretch	Absorbed water	[54]

EMS spectra showed reduction in absorption at associated wavelengths for all constituents. Thus second derivative spectra proved more suitable for interpretation of chemical changes following HWE (Figure 1). Absorption at 1428 nm is associated with amorphous cellulose. There is a reduction in absorption at this wavelength for EMS, which is in agreement with a noticed removal of Seifert cellulose of ~6.4% across all extraction scales (Table 2), originating most probably from amorphous regions of cellulose. Similar results after hot water extraction have been observed previously [53,55]. A decrease in absorption bands at 1728 nm also confirmed results of carbohydrate analysis (Table 3) indicating a higher absorption for MS at this hemicellulose-associated wavelength compared to EMS [56]. The difference in absorption from 1916 nm through 1942 nm is most likely a result of bound water in the MS material; notably, this peak is reduced in EMS compared to MS. Hot water extraction results in decreased hygroscopicity, which explains the decreased absorption band intensity for EMS. This is also in agreement with moisture contents of 8.2% and 7.6% determined for MS and EMS, respectively at 60% relative humidity. The reduction in hygroscopicity is related to the removal of hemicelluloses, which represent the most hydrophilic constituent of lignocellulosics, as seen in reduction in absorption at 1909 nm. Total xylose reduction averaged 61.64% across all scales, while total glucose reduction averaged 10.93% across all scales. This agrees with reduction in absorption intensity at 1909 nm and 1721 nm wavelengths.

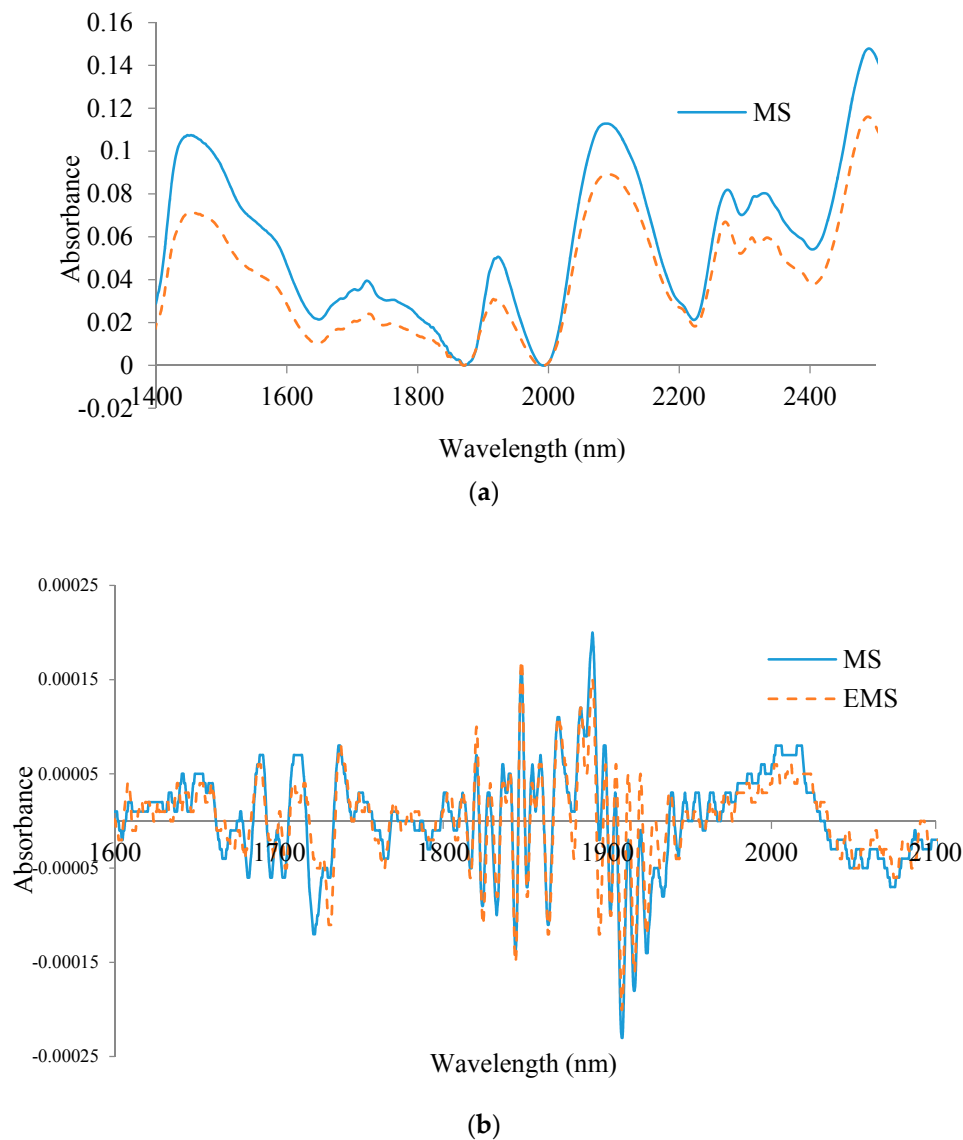


Figure 1. Raw (a) and 2nd derivative (b) NIR spectra of miscanthus MS (solid line) and hot water extracted miscanthus EMS (dashed line).

Degree of crystallinity of cellulose was calculated for MS and EMS using NIR spectra and Equation (1) [53]:

$$CR = \frac{C_I + C_{II}}{C_I + C_{II} + A_m} \quad (1)$$

wherein C_I and C_{II} represent the integral intensities of OH bands corresponding to crystalline regions in cellulose (1550 ± 10 nm and 1590 ± 10 nm respectively), and A_m represents the integral intensity of OH bands corresponding to amorphous regions in cellulose (<1428 nm). These results indicate an increase in degree of crystallinity following HWE from 48.9 to 56.4%. The MS crystallinity index is in agreement with published value of 46.4% determined through X-ray diffraction method [57]. A reduction in amorphous cellulose associated wavelengths, removal of Seifert cellulose, and presence of glucose in hydrolyzate corroborate this observation.

2.4. Lignin Characterization

Lignin recovery yields (1.9% (RecL_{MK}) and 1.7% (RecL_{Pilot}) based on OD miscanthus) accounted for ~25% of the total dissolved lignin, which is higher than that for apricot shells (5%, [55]) but lower than that for sugar maple (47%, [39]). This indicates an extensive cleavage of lignin to hydrophilic, low molecular weight phenolic compounds, which remain soluble even after acidification of the extract. (RecLs) showed similar characteristics independent of the production scale (Table 5). In comparison to miscanthus lignins studied by [38], the PhOH group content (1.96 mmol/g lignin) of RecLs was almost equal to those of Acetosolv and Formosolv lignins (2.19 and 1.92 mmol/g lignin, respectively). These processed lignins contain a greater content of PhOH groups than MWL (0.84 mmol/g lignin) demonstrating cleavage of the aryl-ether bonds in lignin in acid conditions originated from either autohydrolysis reactions (HWE) or added acid (acetic acid/HCl and formic acid/HCl in Acetosolv and Formosolv delignification, respectively). RecLs were characterized by relatively low molecular weights along with moderate polydispersities. These features are similar to those found for Acetosolv and Formosolv miscanthus lignins corroborating their relatively high content of PhOH groups (Table 5; [38]). The carbohydrate analysis showed that xylose was the main sugar present in recovered lignin, followed by arabinose and glucose (Table 6).

Table 5. Characterization of lignin recovered from hot-water extracts of miscanthus produced in MK and Pilot Plant experiments.

Variable	RecL	
	MK	Pilot
Yield (% O.D. MS)	1.9	1.7
Yield (% lignin dissolved)	25.3	23.6
Klason (% O.D. RecL)	77.91	82.5
Acid soluble lignin	3.88	4.5
Total lignin (% O.D.)	81.79	87
Ash (% OD)	-	0.4
PhOH (mmol/g lignin)	1.96	1.96
Mn (Da)	2425	2570
Mw (Da)	9884	9805
Polydispersity	4.1	3.8

Table 6. Carbohydrate analysis of RecL_{Pilot}.

Carbohydrate ¹	% O.D. RecL _{Pilot}
Arabinan	0.48
Glucan	0.60
Xylan	3.85
Total Carbohydrates	4.93

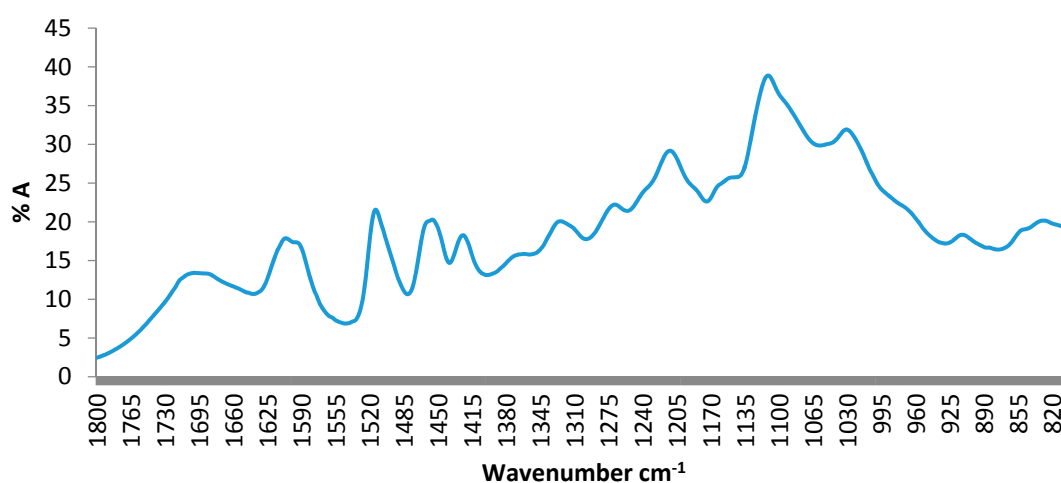
¹ Analysis was done at the USDA-Forest Products Laboratory (FPL) [58].

2.4.1. FT-IR Analysis of RecL_{Pilot}

The IR spectrum showed absorption bands typical of grasses [38,59]. The FT-IR spectrum showed typical lignin bands at 1604 cm⁻¹, 1516 cm⁻¹, and 1429 cm⁻¹ originating from aromatic ring vibrations in addition to C–H deformation vibrations around 1464 cm⁻¹ [6] (Table 7, Figure 2). Lignin bands below 1430 cm⁻¹ are of complex nature, originating from a combination of various vibration modes, and hence are difficult to assign to a single specific structural feature [6].

Table 7. Band assignments for FT-IR spectrum of RecL_{Pilot}.

Band at cm ⁻¹ , % A	Band Origin	Reference
836	C-H out of plane deformation of S units in positions 2 and 6, and in all positions of H units	[59]
1039	Aromatic in-plane bending of G units > S units	[6]
1117	Aromatic in-plane bending of S units	[6]
1218	C–C plus C–O plus C=O stretch	[6]
1278	Aromatic ring breathing of G rings	[6]
1331	Aromatic ring breathing of S and G rings	[6]
1517	Aromatic skeletal vibrations, G units > S units	[6,59]
1711	C=O stretching vibrations of unconjugated ketones and carbonyls or ester groups; or conjugated aldehydes and carboxylic acids	[6]

**Figure 2.** FT-IR spectrum of RecL_{Pilot} miscanthus lignin recovered from HW Extract in pilot scale experiments.

2.4.2. 2D HSQC NMR Analysis of RecL_{Pilot}

The HSQC spectrum showed a dominant presence of characteristic lignin correlations, along with correlations indicating the presence of carbohydrates. The oxygenated aliphatic region of the spectrum showed signals from side-chain linkages of β -O-4, β - β and β -5 (range $\delta C/\delta H = 50$ – $70/3$ – 6 , Figure 3, Table 8). The aromatic region (range $\delta C/\delta H = 110$ – $130/6$ – 7.5) showed presence of S, G rings and H rings as well as ferulate and *p*-coumarate end units, similar to the MWL of miscanthus reported in another study [38]. The aldehyde region (range $\delta C/\delta H = 191$ – $194/9.5$ – 9.7) also indicated the presence of benzaldehyde and cinnamaldehyde residues. Carbohydrates were also seen, notably by the presence of anomeric C₁/H₁ correlations (range $\delta C/\delta H = 88$ – $104/4.1$ – 5.4). The correlations were derived by comparison with literature values [60,61].

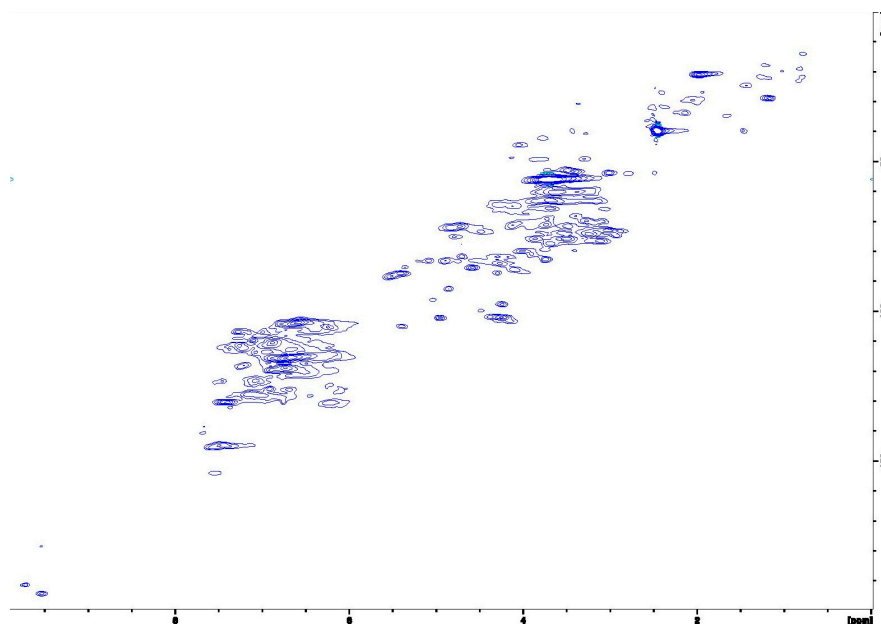


Figure 3. HSQC spectrum of RecL_{pilot} miscanthus lignin recovered from HW Extract in pilot scale experiments, DMSO-d₆.

Table 8. Main assignments of ¹³C-¹H cross-signals in HSQC spectrum of RecL_{Pilot}.

Signal (δC/δH, ppm)	Signal Origin [60,61]
194.0/9.5	C _γ /H _γ , Cinnamaldehyde units
191.1/9.7	C _α /H _α , benzaldehyde units
145.2/7.6	C _α /H _α , ferulate units
144.6/7.5	C _α /H _α , Coumarate units
130.3/7.4	C _{2,6} /H _{2,6} Coumarate units
127.4/7.2	C _{2,6} /H _{2,6} p-Hydroxyphenyl (H) units
128.0/7.0	C _{2,6} /H _{2,6} p-Hydroxyphenyl (H) units
123.3/7.1	C ₆ /H ₆ Ferulate units
116.0/6.7	C _{3,5} /H _{3,5} Coumarate units
113.6/6.9	C _{3,5} /H _{3,5} ferulate units
113.5/7.1	C ₂ /H ₂ Guaiacyl (G) units
111.4/7.3	C ₂ /H ₂ , ferulate units
109.6/7.1	C ₂ /H ₂ Guaiacyl (G) units
106.7/7.3	C _{2,6} /H _{2,6} Syringyl (S) units
104.9/5.4	C ₁ /H ₁ α-L-Arabinofuranose
103.8/6.6	C _{2,6} /H _{2,6} Syringyl (S) units
103.4/4.1	C ₁ /H ₁ β-D-Glucopyranose
102.0/4.2	C ₁ /H ₁ β-D-Xylopyranose
97.7/4.2	C ₁ /H ₁ β-D-Xylopyranose
92.4/4.8	C ₁ /H ₁ α-D-Xylopyranose
88/5.5	C _α /H _α , β-5 linkage
85/4.6	C _α /H _α , β-β linkage
84/4.3	C _β /H _β , β-O-4 linkage
72/4.8	C _α /H _α , β-O-4 linkage
71.1/4.1	C _γ /H _γ , β-β linkage
62.2/3.7	C _γ /H _γ , β-5 linkage
53.4/3.4	C _β /H _β , β-5 linkage
53.2/3	C _β /H _β , β-β linkage
55.9/3.7	C-H in methoxyl (OCH ₃) groups
39.7/2.4	Solvent (DMSO-d ₆)

2.4.3. Higher Heating Value (HHV) of MS, EMS and RecL_{Pilot}

To evaluate the HWE effect on use of miscanthus for energy production/fuel pellets, the higher heating value (HHV) of MS and EMS was measured and the results are presented in Table 9.

Table 9. Higher heating value of MS, EMS and RecL_{Pilot}.

Sample	Higher Heating Value (MJ/kg)
MS	18.60 ± 0.37
EMS _{Parr}	20.11 ± 0.06
EMS _{MK}	19.51 ± 0.08
EMS _{Pilot}	19.80 ± 0.13
RecL _{Pilot}	23.67 ± 0.12

After HWE, the HHV of miscanthus produced at three different scales increased $6.49 \pm 1.6\%$ in average, as expected due to a relative increase in cellulose and lignin contents and decrease in content of hemicellulose/xylan (energy of combustion lignin (~21 MJ/kg) > cellulose (~17 MJ/kg) > hemicelluloses (~16.63 MJ/kg)) [62]. Moreover, increase in HHV observed for miscanthus was higher than reported earlier for sugar maple (2.9%), wheat straw (3.0%) and apricot pit shell (4.1%) after HWE (Parr reactor; [55,63]). As expected, the higher heating value of RecL_{Pilot} is higher than that of EMS_{Pilot} and is comparable to that of coal (23.67 MJ/kg vs. 23–28 MJ/kg, [64]). These results demonstrate utility of HWE in making enhanced fuel pellets either from EMS or from EMS with addition of RecL, which would increase energy of combustion and serve also as a binder for an increased durability [65].

2.5. Enzymatic Hydrolysis of Native and Hot-Water Extracted Miscanthus

Extracted biomass was more accessible to the enzymatic hydrolysis, releasing higher amount of sugars, compared to the native biomass (Figure 4).

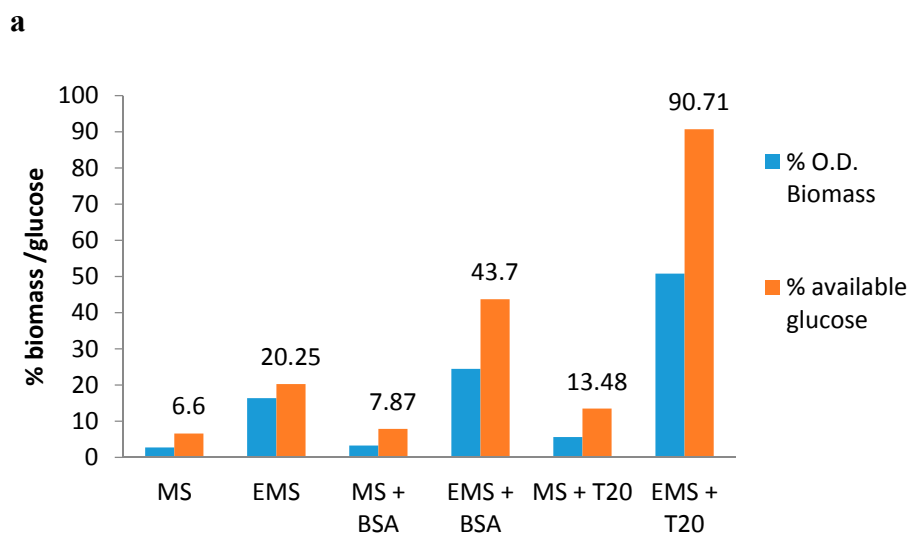


Figure 4. Cont.

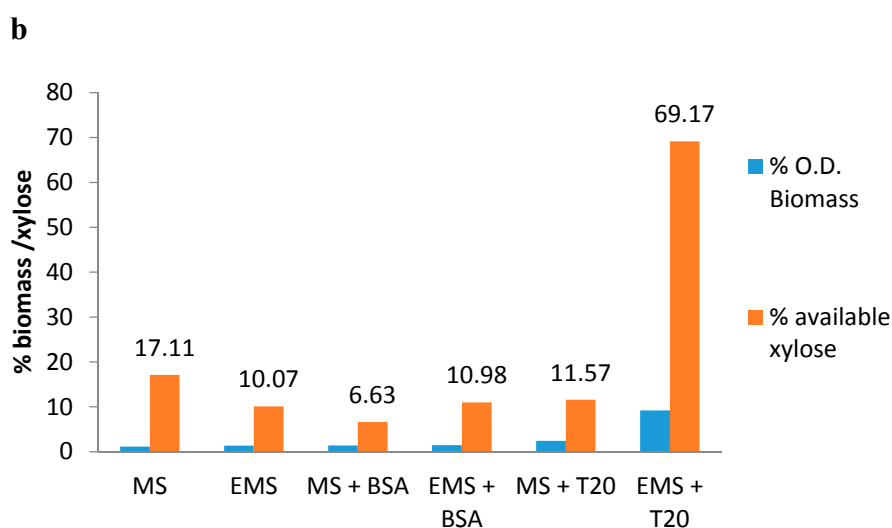


Figure 4. Yields of glucose (a) and xylose (b) after enzymatic hydrolysis of miscanthus (native and hot-water extracted), with BSA and Tween[®] 20 (T20) as enhancing agents.

Higher release of fermentable sugars from extracted biomass may be attributed to increased porosity of the biomass, resulting in increased accessibility and rapid diffusion and also to removal of hemicelluloses, which may exert inhibitory effect on the enzymes when present in higher concentrations [66]. Addition of enhancing agents such as BSA and Tween[®] 20 may prevent unproductive adsorption of the enzymes on biomass, thus preserving enzyme activity and resulting in higher yields [67]. The results provide evidence that enzymatic hydrolysis in presence of surfactants leads to release of ~90% available glucose and ~70% available xylose from EMS while under the same conditions only ~13.5% of available glucose and ~12% of available xylose may be produced from MS. Available glucose and available xylose are the total glucose and xylose contents of the biomass respectively, which is the highest amount of sugars that can be released from the biomass.

2.6. Production of Resin from Recovered Lignin

Given the similar characteristics of RecL_{M/K} and RecL_{Pilot}, the selection of lignin for making adhesive blends was simply based on the available amount, and RecL_{Pilot} was selected (RecL). The miscanthus RecL based adhesives produced in this study were characterized by their mechanical properties—tensile strength and Young's modulus. The SMAH_F0 formulation (SMAH lignin with 0% furfural at pH 0.65) from our previous work [28] was used as a reference because it was similar in strength to commercial phenol-formaldehyde resin and demonstrated the highest strength among all investigated lignin-based formulations in that study. SMAH lignin was isolated from hot water extract of sugar maple wood chips after several steps including filtration and acid hydrolysis. Our previous study also investigated the effect of furfural content (up to 16% on lignin content) on the mechanical properties of the adhesive blends [28]. In this study, furfural content was further increased to 50% and 100%.

Furfural (50% and 100% on lignin content) was reacted with RecL_{Pilot} to produce RecL_F50 and RecL_F100 formulations respectively. 50% furfural was also reacted with SMAH lignin (SMAH_F50). As seen in Figure 5, RecL_F50 demonstrates highest strength (20% and 9.5% higher than RecL_F0 and SMAH_F0, respectively). However, increasing the furfural content to 100% (RecL_100) showed lower strength when compared to RecL_F50 and SMAH_F0 (6% and 2.9% lower, respectively).

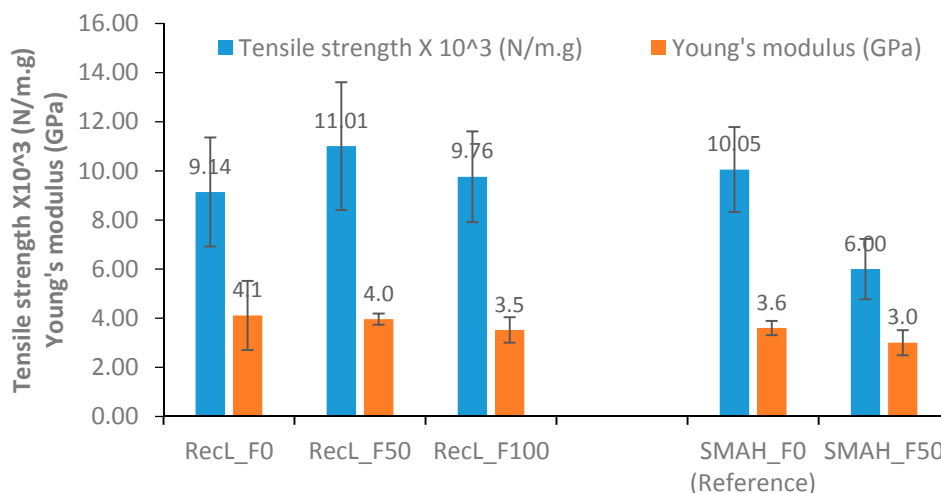


Figure 5. Effect of furfural content on mechanical properties (tensile strength and elastic modulus) of adhesive blends at curing temperature and pressure of 180 °C and 1.9 MPa. Tensile strength per gram (N/mg) was determined using Equation (3).

Adding more furfural to SMAH did not have the same effect (Figure 5). At 50% furfural on SMAH lignin (SMAH_F50), the strength decreased by 40% when compared to the reference (SMAH_F0). Experiments of 100% furfural on SMAH lignin were not performed as it was hypothesized that the strength will further decrease.

It is well understood that lignin undergoes competing polymerization and depolymerization reactions under acidic conditions [68–72]. It was seen from our previous study that extensive cross-linking compromised tensile strength in lignin based adhesive blends [28].

Our previous work also suggested that SMAH lignin is more condensed and has undergone cleaving of aryl ether β -O-4 bonds due to the acid hydrolysis step performed during isolation (characterization via HSQC), when compared to the RecL fraction of sugar maple [28]. Adding 50% furfural (a potential cross-linking agent for lignin) to the already condensed SMAH lignin further promotes cross-linking, reducing flexibility of the polymer and decreasing strength (SMAH_F50). Adding 50% furfural to RecL_{Pilot} promotes cross-linking but simultaneously cleaves ether linkages (lignin-lignin and/or lignin-ferulate) under acidic conditions causing depolymerization. This may result in a polymer that has higher tensile strength (RecL_F50). However, adding 100% furfural could promote extensive cross-linking resulting in decreased strength because of reduced flexibility (RecL_F100).

It was also observed that the Young's modulus for the formulations varied from 3 GPa to 4.1 GPa. The different furfural contents did not affect the Young's modulus as much as tensile strength (Figure 5).

These experiments demonstrated that in regard to the tensile strength of resulting formulations adding furfural is more beneficial to lignin recovered from hot-water extract of miscanthus (RecL_{Pilot}) than to lignin recovered from acid hydrolysate of hot water extract of sugar maple (SMAH). Further investigation of the formulations to define reactivity of lignin units, including end groups such as benzaldehyde, cinnamaldehyde, ferulate and coumarate (all present in miscanthus RecL) should be conducted to understand the chemical structure of the formulation and polymerization/depolymerization reactions taking place.

2.7. Use of Recovered RecL_{Pilot} as an Antioxidant

RecL_{Pilot} was found to have comparable antioxidant activity with vitamin C and poplar organosolv lignins found in literature [31] (Figure 6). This indicates that RecL_{Pilot} may find a potential use as a preservative in commercial preparations.

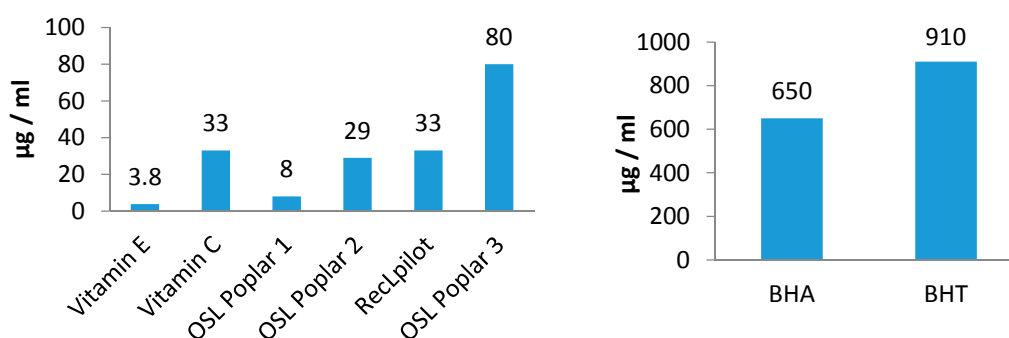


Figure 6. Comparison of antioxidant activity of MS RecL with natural antioxidants (Vitamin E, vitamin C); poplar organosolv lignins (OSL Poplar 1,2,3 [31]); and with synthetic antioxidants (Butylated hydroxyanisole—BHA, Butylated hydroxytoluene—BHT).

3. Experimental Materials and Methods

3.1. Biomass

Miscanthus (MS, *Miscanthus* sp.) biomass was harvested from Bono (AK, USA) in January 2015. The harvested biomass was stored covered in a barn, before removal of roots and processed by a bale breaker and a collision mill, to obtain a sample of $\frac{3}{4}$ screen size, by MESA Reduction Energy and Processing Inc. (Auburn, NY, USA). The processed biomass (the moisture content ranged between 5.05% and 15.21%) was then transferred in 15 separate supersacks (200 lb or 90.7 kg each, 3000 lb or 1360.7 kg total) to SUNY-ESF, Department of Paper and Bioprocess Engineering, where our experiments were performed.

3.2. Hot Water Extraction (HWE) of the Biomass

HWE was performed at three different scales, using a Parr (300 cm³ 4560 Mini bench top reactor, Parr Instrument Company, (Moline, IL, USA; triplicate experiments), M/K (4 L, M/K Systems Inc. (Peabody, MA, USA; one experiment), and Pilot digester (Struthers-Well 65 ft³ stainless lined batch digester (Santa Fe Springs, CA, USA; duplicate experiments) at 160 °C for 2 h, each varying in sample amount (oven dry mass of miscanthus (OD_MS), particle size and water-to-biomass (W/B) ratio (Table 10). Although the heating and cooling periods were different for the different scales, the total effect of combined time and temperature on the hydrolysis of xylans as identified by the P-factor [73] was in a relatively narrow range from 558 (M/K) to 625 (Pilot) with an average value of 585 (st. dev. 35.2). The comparative studies conducted in different scales inevitably include these differences.

Table 10. Hot-water extraction conditions.

Variable	Digester		
	Parr (300 cm ³)	MK (4 L)	Pilot (65 ft ³)
Particle Size (mm)	<0.595	0.420–19.0	~19.0
OD_MS (kg)	0.005	0.222	155.1
Scale up factor	1	44.4	31,020
W/B	40	10	8
Ramped temperature increase to 160 °C, min	60	25	55

Depending on the type of digester used for extraction, the extracted samples were washed twice with 200 mL distilled water (EMS_{Parr}), flowing tap water (EMS_{M/K}), or with tap water equal to the hydrolysate volume (EMS_{Pilot}) at 80 °C, for 15 min. The biomass and the hydrolysate were separated.

The resultant biomass was air-dried and ground to #30 mesh size, for further characterization and was subsequently used as a substrate, along with native miscanthus, for enzymatic hydrolysis. After recording the initial pH value, the hydrolysate was acidified to pH 2 using 20% sulfuric acid to precipitate the dissolved lignin. The acidified hydrolysate was stored in a cold room overnight to allow the lignin to settle, which was then separated by centrifugation as recovered lignin (RecL_{Parr,M/K}). After precipitation, RecL_{Pilot} was collected from the bottom of the hydrolysate storage tank, by washing with a water jet. RecLs were washed with acidic water (pH 5) for further purification and characterized. RecL_{Pilot} was used for the preparation of adhesive blends.

3.3. Characterization of Miscanthus before and after Hot Water Extraction

Chemical Composition

Carbohydrates: The carbohydrate content of native biomass (MS) and hot-water extracted biomass (EMS) was determined by a modified acid hydrolysis procedure [55]. 150 mg of extractive-free biomass was sonicated with 4.8 mL of 72% sulfuric acid for one h at room temperature, after which 6.3 mL deionized water was added to dilute the acid to 4%. The mixture was then placed in a water bath at 80 °C for one h with intermittent mixing and was filtered. The filtrates analyzed by ¹H-NMR [74,75]. All spectra were acquired at 30 °C with an AVANCE III 600 spectrometer (600 MHz ¹H frequency, Bruker, Billerica, MA, USA) equipped with a 5 mm Cryo Prodigy BBO z-gradient probe. Data were acquired and processed in TOPSPIN v3.2 from Bruker BioSpin. Glucosamine was used as the internal standard. The carbohydrates were determined for MS and EMS. The reported results are an average of two experiments. Carbohydrates in lignin recovered from hot water extract hydrolysate (RecL_{Pilot}) were analyzed from acid-hydrolysate resulting from Klason lignin determination at the USDA-FPL [58].

Ash: Ash content was determined in accordance with TAPPI Standard T211 om-02: Ash in wood, pulp, paper, and paperboard: combustion at 900 °C for MS, EMS, and RecL. The reported results are an average of three experiments.

Extractives: Prior to further analyses, the native and hot-water extracted samples were pre-extracted by the Soxhlet method with ethanol/toluene (1:2) and dichloromethane (DCM), respectively, in accordance with TAPPI Standard T 204 cm-97. This organic-solvent pre-extracted biomass was used for lignin and cellulose measurement. [39].

Cellulose: Cellulose content in MS and EMS was determined on pre-extracted samples by Seifert's method [44,76]. The amount of residual lignin in Seifert cellulose was determined by acetyl bromide assay [45]. The reported results are an average of two experiments.

Lignin: Contents of acid-insoluble (Klason lignin) and acid-soluble lignin for MS, EMS, and RecL were determined following the modified Klason lignin method [48,77], with one h sonication in 72% sulfuric acid for 1 h, at room temperature (Branson 3510). The presented results are an average of three experiments.

3.4. Near Infrared Spectroscopy (NIR) and IR

A Bruker FT-NIR Multi-Purpose Analyzer spectrophotometer was used. Biomass (MS, EMS) was vacuum-dried at 40 °C and scanned at 8 cm⁻¹ resolution combining sixteen or thirty two scans per spectra, depending on the method.

3.5. Higher Heating Value (HHV)

The higher heating value was determined by a Parr Calorimeter 6200 in accordance with ASTM method D5865-13: Standard test method for gross calorific value of coal and coke for MS, EMS, and RecL_{Pilot}. The presented results are an average of three experiments. Determination of HHV requires use of oven-dried samples or moisture content (MC) corrections. To simulate exterior conditions

samples of MS and EMS were equilibrated at 60% relative humidity until mass stabilized. These samples were then dried at 105 °C to constant mass for determination of dry basis MC. MS samples averaged 8.2% and EMS averaged 7.6%. Thus the differences in LHV (HHV not corrected for MC) would be greater for MS and EMS. MC was calculated using Equation (2):

$$MC = \frac{m_g - m_d}{m_d} * 100 \quad (2)$$

3.6. Molecular Weight Distribution

Size exclusion chromatography (SEC) was used to determine molecular weight distribution of lignin; Styragel HR0.5, HR3 and HR4E columns (Waters, Milford, MA, USA) with a UV detector at 0.8 mL/min flowing rate. Polystyrene standards (MW: 2×10^6 –466 Da) were used for calibration [28]. Recovered lignin samples (RecL) were acetylated [78] and then dissolved in tetrahydrofuran with 1 mg/mL concentration. A third order polynomial equation was used for quantification.

3.7. Phenolic Hydroxyl Groups (PhOH)

The PhOH group content was measured for MS, EMS, and RecL by periodate oxidation method [79]. Analysis was performed on 400 mg in case of biomass, and on 50 mg in case of isolated lignins. The quantification of resulting methanol was performed by ^1H -NMR run under the same conditions as for sugar analysis. Duplicates were performed.

3.8. 2-D NMR (Heteronuclear Single Quantum Coherence Spectroscopy-HSQC)

Fifty mg of the sample was vacuum dried at 40 °C, for 48 h and was dissolved in DMSO- d_6 . The HSQC correlation spectra were acquired from 9.0 to 0 ppm in F2 (^1H) with 900 data points (acquisition time 200 ms), and from 150 to 0 ppm in F1 (^{13}C) with 512 increments, 128 scans, and a 1-s interscan delay for a total acquisition time of 11 h 12 min. The d_{24} delay was set to 1.72 ms ($J = 145$ Hz).

3.9. Synthesis and Testing of Adhesive Blends

3.9.1. Synthesis of Adhesive Blends

Adhesive blends were made in a slightly modified manner in accordance with Johansson's method [80] where 15–34% sulfite liquor is reacted at pH = 0.3–0.6 with sulfuric or hydrochloric acid at temperatures of 90–160 °C. The resultant products are then applied to wood chips, formed into sheets and cured at temperatures of 150–180 °C and pressures of 0.8 and 2 MPa. In this study, duplicates of 25 g of miscanthus lignin (produced in pilot-scale experiments, RecL_{Pilot}) was subjected to acid hydrolysis at pH = 1 (20% formic acid) and pH = 0.65. Based on the results from a previous study by [28], the reaction was carried out at 90 °C for 1 h without furfural at pH 0.65 and with 50% (based on the amount of lignin used) furfural at pH 1. To obtain pH = 0.65, concentrated hydrochloric acid was added to 20% formic acid until desired pH was reached. After, the product was allowed to cool to room temperature and the pH was adjusted to 2 using 2 M NaOH solution. The adhesive product was then filtered via a Büchner funnel and washed with distilled water several times to remove any water-soluble materials and unreacted furfural and then air-dried. The prepared adhesive blends were then dissolved in acetone:water (9:1) at 20% consistency for treatment of glass fibers (Pall Corporation, Port Washington, NY, USA).

A lignin-based resin formulation (SMAH_F0) from a previous study [28] was used as a reference for comparison. It is important to note that the lignin (SMAH) used to make the reference formulation (SMAH_F0) was isolated from hot water extract of sugar maple in the following manner. After hot water extraction of sugar maple wood chips, the extract undergoes ultrafiltration via a membrane (Hilco HM634-01, ceramic filter with pore size of 0.01 μm). The permeate from the ultrafiltration process further undergoes nanofiltration. The permeate of the nanofiltration process is acid hydrolyzed

with concentrated sulfuric acid (1.5% by mass of extract) at 130 °C for 45 min. The precipitate (SMAH) formed is recovered with dissolution in acetone-water (1:1) mixture and steam stripped to remove the solvents. The recovered precipitate (SMAH) was used as received as the raw material for making the reference formulation without furfural addition (SMAH_F0).

3.9.2. Preparation and Mechanical Testing of Adhesives Reinforced by Glass Fibers

The method used for testing tensile strength was according to [81] and Tappi method T 494-om-01 with modifications. Glass filter fibers were cut into 4" × 1" strips, oven dried at 105 °C and weighed. The strips were then immersed in the prepared adhesive blends for 5 min, removed and left to dry overnight. The dried strips were then pressed in an electric hydraulic press at 180 °C and 1.9 MPa. The pressed glass fibers were oven dried at 105 °C and weighed once again to determine the amount of adhesive absorbed by the glass fiber. The glass fiber strips were conditioned at 23 °C and 50% humidity and tested in MTS 1/S Sintech tensile equipment for tensile properties. Tensile strength defined as the force (N) required to break a strip of a certain width (m) per gram of adhesive, TS (N/m·g) was determined using Equation (3):

$$TS \text{ (N/m g)} = \frac{\text{Tensile strength of reinforced fiber (N/m)} - \text{Tensile strength of the blank glass fiber (N/m)}}{\text{Mass of adhesive absorbed by the glass fiber (g)}} \quad (3)$$

The inert glass fibers do not swell in acetone:water (9:1) and any change in mechanical properties can be associated with the resin and not the fiber substrate itself.

3.10. Antioxidant Activity Assay

The antioxidant activity of RecL was measured with the use of DPPH (2,2-diphenyl-1-picrylhydrazyl) as the free radical generator, as per the method described by [31].

3.11. Enzymatic Hydrolysis of the Biomass

Native and hot water-extracted miscanthus (EMS_{Pilot}) was treated with enzymes Cellic[®] CTec and Cellic[®] Htec2 enzymes (Novozymes North America, Inc. Franklinton, NC, USA) to release monomeric sugars. Surfactants Tween[®] 20 (Sigma-Aldrich, St. Louis, MO, USA) and Bovine Serum albumin (BSA, Sigma-Aldrich) were added into the reaction mixture to improve the efficacy of hydrolysis [82]. The effects of the two surfactants on sugar release from MS and EMS_{Pilot} were compared. The procedure was carried out at 50 °C, for 72 h, in a 50 mM citric acid buffer of pH 4.8, under constant agitation in a reciprocating shaking water bath. The concentration of the surfactants was 5 g/L. The concentration of the enzymes was 2 mL/L for CTec (equivalent to 1.14 FPU/g solid biomass; 3.05 FPU/g total glucan for MS, 2.26 FPU/g total glucan for EMS) and 1 mL/L for Htec (equivalent to 1.95 IU/g solid biomass; 10.68 IU/g total xylan for MS, 16.67 IU/g total xylan for EMS). At the end of the reaction period, the reaction was terminated by immersing the reaction tubes in a boiling water bath for 15 min. Composition of the released sugars was measured on a Waters Breeze HPLC system equipped with an RI detector, using Waters IonPak ion exclusion (H⁺ Counter ion) column maintained at 55 °C, with 0.01 N sulfuric acid as eluent. Release profiles of glucose and xylose was followed.

4. Conclusions

These studies investigated the effects of scale-up on the process of hot-water extraction of miscanthus. The results produced at three different scales differ from each other highlighting the need to increase the scale in preliminary studies conducted before the commercialization. HWE of miscanthus resulted in less selective removal of xylans than in the case of HWE of hardwoods; i.e., at the end of HWE less xylans and more lignin were removed from miscanthus than from hardwoods. HWE pretreatment results in a biomass that is more accessible to cellulase and hemicellulase enzymes, and hence results in higher yields of glucose and xylose released by enzymatic hydrolysis. Hence, the extracted biomass can be used as a feedstock for fermentation products. Lignin removed during

the process can be recovered and used for the production of formaldehyde-free adhesives which are competitive in strength to phenol-formaldehyde resins. Lignin also displays antioxidant behavior, and can be developed into a relatively cheaper and greener additive for industries ranging from food and medicine to cosmetics to plastics and packaging. Recovered lignin has higher energy content and lower ash content as compared to the native and hot-water extracted miscanthus. Hence, it can be used as an additive in fuel pellets, where it can serve as a binder to produce stronger pellets, and will also result in a pellet with higher combustion energy.

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Author Contributions: Kuo-Ting Wang and Chengyan Jing: Hot water extraction experiments, biomass and lignin characterization, Christopher Wood: Pilot plant operations. Aditi Nagardeolekar: Lignin and biomass characterization, coordination of manuscript writing. Neil Kohan: NIR analysis of biomass, higher heating value experiments. Prajakta Dongre: Synthesis and testing of lignin-based adhesives. Thomas E. Amidon: Advising and supporting, Biljana M. Bujanovic: Advising, coordination of manuscript writing.

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

Nomenclature

AX	Arabinoxylan
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BSA	Bovine serum albumin
DCM	Dichloromethane
DD	Delignification degree
EMS	Hot water extracted miscanthus biomass
ET	Ethanol/toluene (1:2)
HHV	Higher heating value
HMF	Hydroxymethyl furfural
HSQC	Heteronuclear single quantum coherence
HWE	Hot water extraction
LCC	Lignin carbohydrate complex
MC	Moisture content
MS	Miscanthus biomass
MWL	Milled wood lignin
NIR	Near infra-red
NMR	Nuclear magnetic resonance
OD	Oven dry mass
OSL	Organosolv lignin
PhOH	Phenolic hydroxyl groups
RecL _{MK}	Lignin recovered after hot water extraction of miscanthus biomass in an M/K digester
RecL _{Parr}	Lignin recovered after hot water extraction of miscanthus biomass in a Parr reactor
RecL _{Pilot}	Lignin recovered after hot water extraction of miscanthus biomass in a pilot digester
SMAH lignin	Sugar maple acid hydrolyzed lignin
T20	Tween 20
W/B ratio	Water to biomass ratio

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