

Characterization of Fermentation Residues from the Production of Bio-Ethanol from Lignocellulosic Feedstocks

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Solid residues from the conversion of four lignocellulosic biomass feedstocks, Miscanthus, Switchgrass, hybrid poplar and red maple, to bioethanol in a pilot plant have been characterized for their lignin and carbohydrate content, heating value, ash content and inorganic elements profile. These residues have also been analyzed with solid-state ^{13}C NMR spectroscopy to understand their bulk chemical composition. The hemicellulose contents of residues from all four feedstocks are significantly lower than the untreated biomass, indicating utilization or degradation during the steam autohydrolysis pretreatment and bioconversion. Residues from red maple show the highest lignin content and heating value. All the residues have high ash content which includes very high proportions of Ca, P, K and S. The presence of these elements in these biofuel residues may challenge their use for combustion and will need to be addressed.

Keywords: Fermentation Residue, Pilot-Plant, ^{13}C NMR.

1. INTRODUCTION

Ethanol produced by the bioconversion of a variety of biomass sources is widely recognized as a transportation fuel with strong economic, environmental and socio-political advantages. Second-generation biofuels can be produced from a range of lignocellulosic feedstock including agricultural and forestry residues, wood, municipal waste and dedicated energy crops. The production of biofuels from lignocellulosic biomass results in higher greenhouse gas reductions compared to first-generation biofuels from starch-based sources.¹ The three main components of lignocellulosic biomass, cellulose, hemicellulose and lignin are present in various proportions in biomass from different sources. Due to the close association of the three components, some form of chemical pretreatment is employed to render the cellulose amenable to enzymatic deconstruction.^{2,3} Most pretreatments depolymerize lignin and/or hemicellulose which are then extracted in to the liquid stream and produce a cellulose-rich solid phase that is more susceptible to enzymatic hydrolysis. Steam explosion and acid treatment are currently the most frequently used pretreatments and both result in the solubilization of the hemicellulose fraction, release of acetic

acid and degradation to products such as furfural.³ These compounds, together with hydroxymethyl furfural, which is formed from glucose degradation, and lignin fragments, are known to be inhibitory to fermentation.⁴

Regardless of whether the pretreated biomass is processed by simultaneous or separate saccharification and fermentation, the residues from the production of bioethanol from lignocellulosic feedstocks are primarily composed of lignin, unconverted sugars, unrecovered enzymes, metals/ash and should be effectively utilized to ensure waste minimization and increase revenue.⁵ This material is frequently proposed to be utilized as an energy resource for power generation, in part, because there are few efficient chemical conversion processes available that can convert lignin into transportation fuels or higher value chemical substrates. Although a fraction (~40%) of the dried lignin-rich solid stream after ethanol production is necessary to meet the thermal requirements of bioethanol production which includes pretreatment and ethanol distillation,⁶ modern biological cellulosic processing plants will have ~60% excess of lignin that could be utilized as a feedstock for biogasoline/green diesel and/or green chemicals, thus offering a significant opportunity for enhancing the operation of a lignocellulosic biorefinery. Most techno-economic evaluations of bioethanol production currently available utilize the residual lignin stream as a green energy source for the process and do

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not take in to account the value added by converting the lignin to liquid fuels or biomaterial.^{7,8} Recent studies by Shabtai et al.⁹ have described a lignin refining process which involves an initial aqueous alkaline-catalyzed depolymerization of lignin present in biomass or industrial lignin-rich process streams, followed by hydroprocessing of the depolymerized lignin to C7-10 alkylbenzenes, which were proposed to be used for improving the octane level of petroleum-based fuels. Chen and Koch¹⁰ reported a method that combines alkaline fragmentation with metal catalyzed hydrogenation to initially yield phenolics followed by their conversion to substituted cyclohexanes, which are also deoxygenated.

Despite the potential of fermentation residues as a source of energy and as a precursor for lignin-based fuels and biomaterials, very limited information is available on the composition of this material. Most studies on the conversion of biomass to ethanol focus on enzymatic sugar release and ethanol yields and overlook the solid residues after bioconversion. With increasing commercial bio-ethanol production, these residues will be available in greater quantities and be of interest as energy sources or as precursors for the production of bio-based chemicals and material. Compositional characteristics such as heating value, ash content and composition and elemental C, H, N, O content are key to determining whether the bio-conversion residues are indeed suitable as an energy source. Based on these characteristics, conventional combustors may require modifications for optimal performance. A few studies have focused on the combustion related compositional properties of fermentation residues,^{11,12} while pelletization of residues before being used as an energy source has also been explored as an option.¹³ In a novel approach, Bjorklund et al.¹⁴ studied the use of the lignin-rich residue from acid hydrolysis of spruce as a hydrophobic agent for detoxification of the hydrolyzate prior to fermentation. Treatment with lignin was shown to be most effective for the removal of furfural, followed by hydroxymethyl furfural.

In this paper, we present results from the compositional analysis of the fermentation residues from the pilot-plant scale production of ethanol from four common biomass feedstocks Miscanthus, Switchgrass, hybrid poplar and red maple. The compositional characteristics of the fermentation residues have been compared to those from untreated feedstock from values presented in the literature.

2. MATERIAL AND METHODS

Fermentation residues from a commercial pilot plant scale bioconversion of Switchgrass, Miscanthus, hybrid poplar and red maple were provided by a commercial bioethanol company in the US. The feedstock source and details of the bioconversion process are proprietary to the company and cannot be disclosed here. All four biomass feedstocks

were subjected to steam autohydrolysis pretreatment at severities ranging from 4.0 to 4.2. For autohydrolysis pretreatments severity is calculated as:

$$R_0 = t \exp[(T - T_{\text{ref}})/14.7]$$

where, t = pretreatment time (min); T = pretreatment temperature ($^{\circ}\text{C}$); $T_{\text{ref}} = 100^{\circ}\text{C}$.

These severity values are in the range of those reported in the literature.¹⁵ The pretreatment conditions employed were optimized for each biomass. Following pretreatment, the material was fermented in batch fermentors using commercially available cellulase enzymes and *Saccharomyces cerevisiae* (Baker's yeast). The fermentation residues were rinsed with water and dried overnight at 60°C . The samples were received in the form of a dry powder and were stored in a freezer prior to analysis.

2.1. Ash Content and Composition

Ash content of the fermentation residues was measured by ramped heating to 525°C in a programmable muffle furnace. The samples were first dried at 105°C overnight prior to ashing. The contents of different inorganic elements in the ash were determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) after digestion with nitric acid. The total halogen content (Cl + Br + I) was determined using a micro-coulometric method by Huffman Laboratories, Golden, CO.

2.2. Higher Heating Value (HHV) and Elemental Analysis

Heating value is the net enthalpy released when a material is reacted with oxygen under isothermic conditions. If water vapor formed during the reaction condenses at the end of the process, the latent enthalpy of condensation contributes to what is called the *higher heating value (HHV)*. These measurements are carried out in an Isoperibol bomb calorimeter (Model 1261, Parr Instruments, Moline, IL). The weight percent C, H, N and O of the dry fermentation residues were measured by elemental microanalysis. The nitrogen contents were used to estimate the protein content of the samples using the relation: Weight % protein = Weight % Nitrogen * 6.25. It is noted that the value of 6.25 used here for the nitrogen factor yields a rough estimate of the protein content. More accurate estimates of the nitrogen factor and hence the protein content, require the determination of the amino acid profile of the biomass.¹⁶

2.3. Carbohydrate and Lignin Content

Carbohydrate and acid soluble and insoluble (Klason) lignin contents of the fermentation residues were determined according to NREL standard analytical procedures by hydrolyzing with 72% sulfuric acid.¹⁷ Given the

potentially high ash content of these residues, the Klason lignin contents were also determined on an ash-free basis as given in the NREL standard analytical procedure.¹⁷ This is referred to as the “ash free Klason lignin” and was measured by ashing the filters with Klason lignin in a muffle furnace. The residue after ashing is the ash content of the Klason lignin fraction, which is then subtracted from the total Klason lignin to give the “ash free Klason lignin.” The sugar profiles were measured with a Dionex HPAEC equipped with a pulsed amperometric detector.

2.4. Solid-State CP/MAS ¹³C NMR

Aliquots of the biomass fermentation residues were packed in 4 mm diameter zirconium oxide rotors fitted with Kel-F caps. Solid-state CP/MAS ¹³C NMR experiments were performed on a Bruker Avance-400 spectrometer, operating at a ¹³C frequency of 100.59 MHz. 4096 scans were acquired with a 3 second recycle delay, 1 millisecond contact time and 10 KHz spinning speed. Glycine was used for the Hartman-Hahn matching procedure and as an external standard for the calibration of the chemical shift scale relative to tetramethylsilane (TMS). All the experiments were carried out at ambient temperature using a Bruker 4 mm MAS probe. Spectral processing was performed offline using the MestReNova software package.

3. RESULTS AND DISCUSSION

3.1. Lignin and Carbohydrate Contents

The ash, Klason lignin, ash-free Klason lignin and acid soluble lignin contents of the fermentation residues are presented in Table I. Due to the unavailability of the process mass balance information, the results are reported as % dry weight of the solid material. The results presented in Table I indicate that the residues from Miscanthus and Switchgrass have much higher ash contents than the two hardwood feedstocks, as would be expected from the higher ash contents of these grasses (~6% for Switchgrass and ~3–6% for Miscanthus). Overall the ash contents from these fermentation residues are higher than those previously reported for woody hydrolysis residues.¹¹ The

Table I. Ash, Acid insoluble (Klason) and soluble lignin contents of the fermentation residues from four feedstocks after a pilot-plant scale conversion.

% dry weight	Miscanthus	Red Maple	Switchgrass	Hybrid poplar
Ash	18.9	4.3	11.8	7.1
Klason lignin	74.4	78.2	66.5	50.4
Ash-free Klason lignin	55.4	76.7	58.6	50.3
Acid soluble lignin	3.0	3.2	2.8	2.7
Klason lignin in untreated biomass ^a	25.1	26.0	17.0–21.0	21.5–27.2

^aThe Klason lignin contents of the untreated feedstocks are from the literature.^{19–22}

Table II. Relative proportions of sugars in the carbohydrate fraction of the fermentation residues from four feedstocks after a pilot-plant scale conversion.

% dry weight	Miscanthus	Red Maple	Switchgrass	Hybrid poplar
Arabinan	0.4	0.1	0.8	0.0
Galactan	0.6	0.5	0.9	0.7
Glucan	21.4	15.3	28.7	39.7
Xylan	3.3	1.4	5.2	1.2
Mannan	1.0	1.4	1.2	2.3
Cellulose ^a	44.0	37.7	31–38	42–49
Hemicellulose ^a	18.0	22.1	32.0	16–23

^aThe cellulose and hemicellulose contents of the untreated feedstocks are from the literature.^{19–23}

greatest difference in values for Klason lignin and ash-free Klason lignin are seen in case of the residues from Miscanthus and Switchgrass. For these samples, there is a significant contribution of ash to the Klason lignin values. After taking in to account the corrections for ash content, the residual material after conversion of these four feedstocks to ethanol comprises 53 to 80% total lignin.

According to the results in Table I, the fermentation residues from red maple have higher total lignin (Klason lignin + acid soluble lignin) content than those from hybrid poplar, switchgrass and Miscanthus. The lignin contents of the untreated feedstocks range from 17–21% by dry weight for Switchgrass^{18–20} to 26% by dry weight for red maple.²¹ In general, hybrid poplar and maple have higher lignin contents than the other two feedstocks. The acid soluble lignin contents of all these fermentation residues are high as is usually found in case of hardwood and herbaceous biomass with higher proportions of syringyl lignin.

The relative proportions of different sugars in the residual solids after bioconversion of the four feedstocks are presented in Table II. These values were calculated from the relative amounts of sugars obtained from the HPAEC-PAD results and assuming that the residues are mainly composed of Klason lignin, acid soluble lignin and carbohydrates. The proportions of cellulose and hemicellulose in the untreated feedstocks have been compiled from the literature and are presented for comparison in Table II.^{19–23}

Results from carbohydrate analyses show that the hemicellulose fraction is mostly utilized or degraded during the bioconversion of these feedstocks. Red maple and Miscanthus have the lowest proportion of glucose in the

Table III. Heating value of the fermentation residue from four biomass feedstocks after a pilot-plant scale conversion.

	Hybrid poplar	Red maple	Miscanthus	Switchgrass
Heating value (MJ/kg)	20.92	24.16	19.02	20.78
Wt% C	50.29	56.84	45.56	50.14
Wt% H	5.64	5.73	4.65	5.46
Wt% N	0.85	0.98	1.36	1.54
Wt% O	39.70	34.96	34.07	36.53
Wt% Proteins	5.31	6.13	8.50	9.63

fermentation residue, while hybrid poplar has the highest. The pretreatment and conversion process applied to these feedstocks appears to be most effective for red maple and Miscanthus. Factors that could account for the enhanced digestibility of biomass include lower cellulose crystallinity and higher proportion of more labile syringyl units in the lignin fraction.²⁴

3.2. Heating Value and Elemental Composition

Results of heating value measurements for the fermentation residues from the four biomass feedstocks are presented in Table III. Among the biomass fermentation residues studied here, the residue with the highest lignin content (on an ash-free basis), red maple correspondingly has the highest heating value. The heating values of the lignin-rich residue from the conversion of hardwood (yellow poplar) and softwood have been previously reported as 21.45 and 23.50 MJ/kg respectively,⁹ while that from spruce has been reported as 21.5 MJ/kg.¹³ Both these laboratory pilot-plant studies used a two-step dilute sulfuric acid pretreatment followed by simultaneous saccharification and fermentation (SSF). Heating values are also related to and are sometimes estimated from the elemental composition of biomass and other fuels with higher C contents corresponding to higher heating values. The red maple residues have the highest C content as well as heating value (Table III). The average heating value of biomass feedstocks such as hybrid poplar, switchgrass, pine and wheatstraw is ~18 MJ/kg.²² This is comparable to the heating value of the fermentation residues, indicating that it is of value to use these residues as a fuel source as opposed to using untreated biomass. Cellulose and hemicellulose have higher heating values of 18.6 MJ/kg, whereas lignin has values ranging from 23.2 to 25.6 MJ/kg.²⁵ The extractives fraction of biomass can have even higher heating values than lignin, and are significant in softwoods where resins and terpenes comprise a large fraction of the extractives.¹³ While the high heating values make these residues suitable as a green energy source for the bioconversion process, given the high proportion of lignin, it is of interest to explore their conversion to liquid transportation fuels and to lignin based biomaterials.⁵

The results from elemental analysis indicate that Miscanthus and Switchgrass fermentation residues have higher nitrogen contents than the woody biomass residues. This suggests higher protein contents for these two feedstock residues, which can be attributed to a combination of the higher inherent protein content of grasses and protein from residual enzymes from the bioconversion process. Protein contents of Miscanthus and Switchgrass feedstock have been reported as 3.4% and 7.3% respectively.²⁶ Estimates of the protein contents of woody biomass are not readily available in the literature. When calculated from

the average N contents (using a nitrogen factor of 6.25), the protein content of hybrid poplar ranges from 0.5 to 3.8%. Thus the nitrogen (and protein) contents of fermentation residues from all four feedstocks are higher than the starting material indicating a contribution from the bioconversion process.

3.3. Inorganic Elements Profile

The contents of major inorganic elements in the fermentation residues as determined by ICP-AES are plotted in Figure 1. Among the inorganic elements measured in this study, Ca, K, P and S are present at very high concentrations in the fermentation residues (Fig. 1). Of these elements, potassium and phosphorus could be contributed by the nutrients added for SSF. Gypsum, which

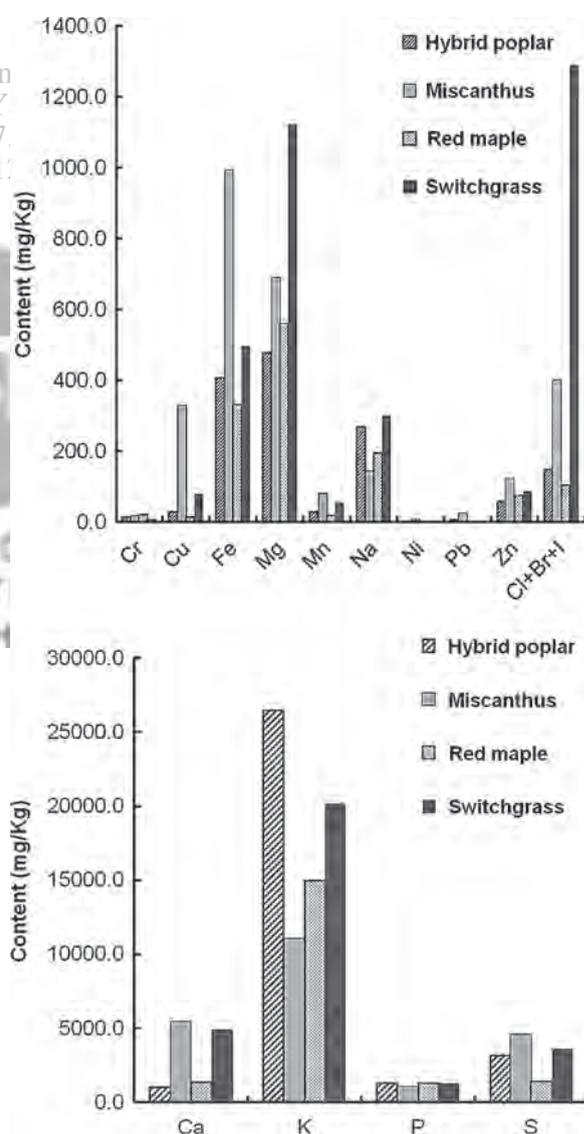


Fig. 1. Major inorganic element contents (mg/Kg) of fermentation residues from the conversion of Miscanthus, Switchgrass, red maple and hybrid poplar to ethanol in a commercial pilot plant.

is sometimes added to detoxify the pretreated slurry prior to SSF,⁴ can also account for the increased Ca and S content of the solid residue. In general, grasses like Switchgrass and Miscanthus have high content of Si and other inorganic elements like K, P, Ca and Cl. Si content of Miscanthus and Switchgrass has been reported to range from 5000 mg/kg to 9900 mg/kg.^{19,27} The total halogen content of the Switchgrass residue is an order of magnitude higher than the other samples. Stems and leaves from Switchgrass have Cl contents on the order of 1600 mg/kg,¹⁹ with some varieties having values as high as 5000 mg/kg.²⁷ The residues from the bioconversion of red maple show the lowest concentration of inorganic elements, which is consistent with the generally lower ash and inorganic content of woody biomass. During combustion, volatile elements such as S and Cl can form sub-micron particles, which upon reaction with K and Si at high temperatures (e.g., in a combustor) can form sticky deposits.²⁷ The presence of oxides of elements such as Ca, Al, Na can also lead to the formation of slag during combustion of the fermentation residues and can be detrimental to their use as an energy source. P can also increase the slagging potential of the residues, while other alkali metals such as Na and Mg can combine with sulfides, chlorides and

carbonates to form lower melting point solids. Thus the inorganic elements profile of the solid residue should be carefully determined prior to deciding upon its use as an energy source. Based on their relatively low metal content, the fermentation residue from red maple appear to be the best suited for combustion among the feedstocks studied here.

3.4. Solid-State CP/MAS ¹³C NMR

Solid-state CP/MAS ¹³C NMR spectra of the fermentation residues are presented in Figure 2. Peaks have been assigned based on the literature,^{28,29} and are shown on the spectra. The peak at δ 21 ppm can be attributed to the acetyl group of hemicellulose and is more prominent in the spectra from Miscanthus and Switchgrass residues. The alkyl peak at δ 35 ppm is associated with compounds present in the extractives fraction. The strong signal at δ 56 ppm corresponds to the aryl methoxyl carbons of lignin and is present in all the biomass fermentation residues. The peaks in the region between 60 and 110 ppm are predominantly due to the different carbons of cellulose. There is usually some spectral overlap with hemicellulose in this region, if they are not removed by chemical treatment or spectral editing. The cellulose signals are least

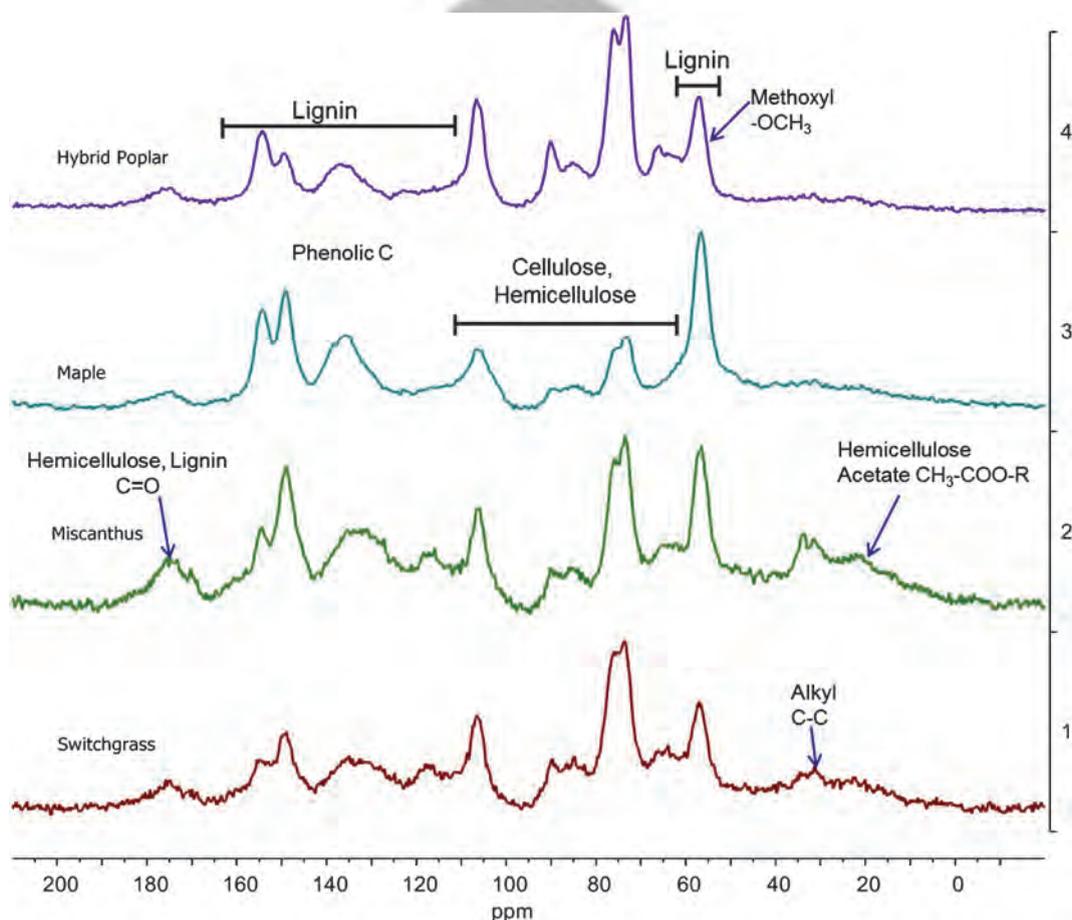


Fig. 2. Solid-state CP/MAS ¹³C NMR spectra of the pilot plant fermentation residues from Miscanthus, Switchgrass, red maple and hybrid poplar.

developed in the red maple fermentation residue, which correlates to its high lignin content and low carbohydrate content (Tables I, II). Signals from the lignin phenolic groups arising from syringyl (S) and guaiacyl (G) type lignin are present from δ 110–160 ppm. In this region, the peaks from 110 to 120 ppm can be assigned to $-\text{CH}-$ carbons of G lignin and are more distinct in the Miscanthus and Switchgrass spectra. The peaks between δ 120–140 ppm correspond to quaternary C in S and G lignin and are seen in all the spectra. Syringyl signals can be present at δ 105 ppm, but there is spectral overlap with cellulose signals in solid-state NMR spectra. The compositional information obtained from lignin and carbohydrate analyses are corroborated by the solid-state ^{13}C CP/MAS NMR results. NMR peaks arising from lignin, cellulose and hemicellulose are seen for all the residues (Fig. 2). The NMR spectra of the red maple and hybrid poplar residues show more prominent lignin signals than the other biomass. The cellulose signals (δ 60–110 ppm) are not very well developed in the red maple fermentation residue, reflecting its low glucose content from carbohydrate analysis. The higher hemicellulose contents of the grass residues are seen in the distinct hemicellulose acetyl peak (δ 21 ppm) in their NMR spectra. Thus, solid-state NMR provides a relatively rapid method to obtain a compositional overview of the fermentation residues.

4. CONCLUSIONS

Solid residues from the bioconversion of four biomass feedstocks to ethanol in a pilot-plant were found to be mainly composed of lignin and ash and to a lesser extent cellulose. The hemicellulose contents of the residues are lower than the untreated feedstock, indicating their effective utilization or degradation. Red maple has the highest proportion of lignin in the residue and also the highest heating value. While these properties make these residues suitable as a green energy source for the bioconversion process, given the high proportion of lignin, it is also of interest to explore their conversion to liquid transportation fuels and lignin-based biomaterials.

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