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Comparative study of organosolv lignin extracted from prairie cordgrass, switchgrass and corn stover

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HIGHLIGHTS

- Organosolv lignin extracted from three feedstocks was analyzed.
- Lignin origin influences its properties.
- Examined lignins were found low in contaminants.
- Examined lignins were found highly phenolic and applicable in vanillin production.

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ABSTRACT

Lignin extracted from prairie cordgrass, switchgrass, and corn stover (using ethyl acetate–ethanol–water organosolv pretreatment) was analyzed and characterized using several methods. These methods included analysis of purity (by determination of Klason lignin, carbohydrate, and ash contents), solubility (with several organic solvents), phenolic group analysis (ultraviolet ionization difference spectra, and nitrobenzene oxidation), and general functional group analysis (by ^{1}H NMR). Results showed that all the examined lignin samples were relatively pure (contained over 50% Klason lignin, less than 5% carbohydrate contamination, and less than 3% ash), but switchgrass-derived lignin was observed to be the purest. All the lignins were found to contain high amounts of phenolic groups, while switchgrass-derived lignin was the most phenolic, according to the ionization difference spectra. Nitrobenzene oxidation revealed that all the lignin samples contained available guaiacyl units in high amounts.

1. Introduction

Lignin represents the most abundant natural phenolic polymer (Lora and Glasser, 2002). It occurs in lignocellulosic plants and plays a major role in maintaining their rigidity and resistance against environmental conditions (Brown, 2003). Lignin is known as an effluent from paper making processes, such as kraft or sulfite pulping (Madakadze et al., 1999).

The monomers which undergo polymerization and contribute to lignin's complex structure include *p*-hydroxycinnamyl (coumaryl), coniferyl and sinapyl alcohols. These correspond to three main structural (phenylpropanoid) units of lignin polymer: *p*-hydroxyphenyl, guaiacyl, and syringyl, respectively (Lin and Dence, 1992). Depending on the material, these structural units occur in different proportions. Softwood lignin tends to contain mostly coniferyl alcohol-derived units, while for herbaceous lignin, coumaryl alcohol-derived units are more typical (Gosselink et al., 2004). Main functional groups observed in lignin structures include hydroxyl (partly phenolic), methoxyl, carbonyl, and carboxyl (Gosselink et al., 2004). Polymerization of coumaryl groups results in formation of aryl-ether bonds (α and β), which among ester and ketal bonds occur most commonly in lignin polymers (Xu et al., 2006).

The organosolv pretreatment originated from an organosolv pulping process, and represents an alternative to traditional pulping methods. The principle is based on lignin solubility in certain organic solvents (e.g. alcohols, organic acids, ketones) (Taherzadeh and Karimi, 2008; Zhao et al., 2009). In the 1990s, the organosolv process was applied specifically as a biomass pretreatment method in ethanol production. Two processes (ALCELL™ and Lignol) were demonstrated on a commercial scale, both using ethanol as a lignin solvent (Arato et al., 2005; Pye and Lora, 1991).

Organosolv pretreatment cleaves mainly α-aryl ether bonds, while the β-aryl ether bonds are cleaved to a lesser extent (Meshgini and Sarkanen, 1989). New phenolic groups are formed

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as a result of ether bond cleavage, producing highly phenolic organosolv lignin (Xu et al., 2006). In addition to being highly phenolic, organosolv lignin is considered relatively pure (i.e. low in carbohydrates, free of sulfur, and low in ash), low molecular weight and highly hydrophobic (Pye and Lora, 1991). Generally, organosolv lignin contains less contaminants than lignin obtained from other processes, such as kraft or sulfite (Lora and Glasser, 2002).

The majority of lignin obtained from pulping processes has been used as fuel to generate energy needed for recovery of other chemicals, due to its high energy content (Kleppe, 1970). However, lignin offers a vast range of potential applications in many industries, beyond that of just a combustion fuel. For example, kraft and sulfite lignin has been used as binders and dispersants (Lora and Glasser, 2002). Organosolv lignin offers additional utilization possibilities, primarily because they are sulfur-free (organosolv processing can eliminate emissions of sulfur compounds during heat processing) and highly phenolic (with a wide range of possible oxidation products) (Lora and Glasser, 2002; Pye and Lora, 1991). One of the potential organosolv lignin applications is production of resins. Using lignin in production of phenolic resins (utilized in plastics manufacture) offers advantages such as limiting the use of toxic formaldehyde, and improved wear behavior. Organosolv lignin has also shown beneficial features when used as a material for oriented strand board binders. Epoxy resins are yet another potential lignin application (e.g. printed circuit board production). Furthermore, organosolv lignin derivatives (modified with alkylene oxides) have been used to produce polyurethane and isocyanurate resins (Lora and Glasser, 2002).

Highly phenolic lignin processing can yield a variety of commercially important compounds, such as vanillin, guaiacol, vanilic acid, acetovanillone, 5-carboxyvanillin, vanillil, vanillovanillone, and syringaldehyde (Pearl, 1967). Vanillin is commonly used as food (e.g. ice cream, bakery products, candy) and beverage additives (as flavoring and scent). It is also a substrate for production of many other chemicals, including papaverine (heart medication), hydrazones (herbicide), trimethoprim (antibacterial agent). Vanillin has been widely produced from guaiacyl units occurring in lignin from kraft and sulfite processes. However, utilizing both kraft lignin and lignin sulfonates resulted in toxic by-products, and was phased out in the late 1980s due to environmental issues and availability of petroleum-based vanillin products (Hocking, 1997; Schulz, 1940). Organosolv lignin is free of sulfur, and thus do not generate hazardous by-products, and could result in an opportunity for sustainable vanillin production (Gogotov et al., 1996).

This work compares lignin extracted from three types of feedstock (prairie cordgrass, switchgrass and corn stover) using organosolv treatment (clean fractionation). The extracted lignin samples were characterized by their original fractional yield, Klason lignin content, solubility in various organic solvents, and contamination by carbohydrates and ash. Spectroscopic methods were also employed to analyze phenolic functional group occurrence and configuration. These included UV–vis ionization difference spectra method and ^{1}H nuclear magnetic resonance (NMR) spectroscopy. Additionally, nitrobenzene oxidation was performed to evaluate guaiacyl unit content, and possible lignin application was examined by measuring vanillin yield. The main purpose of this research was to compare lignins of various origins based on their purity and possible industrial application.

2. Methods

2.1. Materials

Prairie cordgrass (PCG), switchgrass (SG) and corn stover (CS) were used as feedstocks for lignin extraction in this study. All of these materials were obtained locally near Brookings, South Dakota. Prior to processing, all of the samples were ground using a laboratory mill (Model 3375-E15, Thomas Scientific, USA) to pass through a 1 mm screen. Composition of each material, including structural carbohydrates, lignin, ash and moisture content, was measured according to NREL/TP-510-42618 procedure (Sluiter et al., 2008c). All reagents and solvents used in this study were of analytical grade.

2.2. Organosolv pretreatment (clean fractionation)

The clean fractionation process used in this study was based on the National Renewable Energy Laboratory (NREL) optimized process (Black et al., 1998). Ethyl acetate was used as lignin solvent and was mixed with ethanol and water in various ratios. A catalyst (sulfuric acid) was added to the process to initiate ether bond cleavage and improve lignin extraction. The clean fractionation conditions, including temperature, solvent composition and catalyst amount, were optimized for each of the materials using Response Surface Methodology. One of the primary response variables was lignin recovery, which was based on the gravimetric measurement. Optimizations performed for each grass have been presented in different studies (unpublished results). Predetermined optimal conditions of the clean fractionation for each material can be found in Table 2.

The pretreatment was performed in a custom-made experimental set-up, which included an aluminum heating block that held six stainless steel reactor tubes (each of 250 mL capacity). Each reactor tube was equipped with a pressure gauge and a thermocouple. Thermocouples were connected to a temperature controller (Autotune Temperature Controller C9000A Series, Omega, Stamford, CT) monitored by LabView computer software version 8.2 (National Instruments, Austin, TX).

Raw biomass (10 g, dry mass) was loaded into the reactors and treated with the solvent mixture at optimal temperature for 20 min. Liquid-to-solid ratio (%w/w) was 10:1. After the treatment, the slurry was filtered to separate liquid from solid fraction. The cellulose (solid) fraction was washed with approximately 2 L of water to remove solvent residues, and subsequently enzymatically hydrolyzed and fermented to ethanol (Dowe and McMillan, 2008; Selig et al., 2008). The liquid fraction was then separated into two phases: organic (containing lignin dissolved in ethyl acetate and ethanol) and aqueous (containing hemicellulose dissolved in water and ethanol). Lignin was reprecipitated by solvent evaporation, weighed, and stored for analysis.

2.3. Lignin analyses and characterization

2.3.1. Klason lignin, contaminants, and ash analyses

Lignin purity was evaluated by measuring Klason lignin (acid insoluble lignin) content and the amount of contaminants present in the extracted samples. The analysis was carried out by sulfuric acid hydrolysis according to NREL's standard procedure (Sluiter et al., 2008c). Carbohydrate contamination was measured by analyzing the hydrolyzate using High Performance Liquid Chromatography (Agilent HPLC 1200 Series) (Sluiter et al., 2008b). Ash content was also analyzed according to NREL's standard procedure (Sluiter et al., 2008a).

2.3.2. Solubility

Another characteristic of lignin is solubility in various organic solvents. The choice of solvents evaluated in this study was based on literature (Gosselink et al., 2004; Lin and Dence, 1992; Sarkanen and Ludwig, 1971), and included dimethyl sulfoxide (DMSO), dioxane, methanol, ethanol, ethyl acetate, and MIBK. Solutions were prepared in 100 mL volumetric flasks, using 0.05 g of each lignin type. The solutions were kept at 40 °C with constant agitation for

48 h. Subsequently, the solutions were filtered through paper filter (Whatman No. 1), and the solid residue was air dried and weighed. The percent solubility of the lignin sample in a particular solvent was based on the comparison between the initial sample weight and remaining solid residue after the analysis. Solubility tests were performed for both raw and purified (Klason) lignins.

2.3.3. Phenolic hydroxyl groups

The supernatants obtained in the solubility tests with DMSO, dioxane, methanol, and ethanol were used for spectroscopic measurement of phenolic functional groups. The analysis was performed using the ionization difference ultraviolet spectra method as described in literature (El Mansouri and Salvadó, 2007; Lin and Dence, 1992). This method provides a qualitative and quantitative determination of phenolic structures with no need for a reference sample, and is based on a difference in absorbance between neutral and alkalized (ionized) lignin solutions. The difference is observable due to a hyperchromic effect of the ionization process (Lin and Dence, 1992).

Spectra were obtained using a UV–vis spectrophotometer (UV-2450, Shimadzu Scientific Instruments, Columbia, MD, USA). The quantitative analysis of the phenolic groups was possible by comparing the results of ionization absorbance difference to already analyzed model compounds. Four main types of phenolic functional groups have been analyzed and described previously (Lin and Dence, 1992).

Each type of phenolic group absorbs UV light at different wavelengths, and its quantity can be evaluated using the following equation:

$$\text{Phenolic OH(at given wavelength)}\,[\%] = \Delta D/\Delta\varepsilon\,[\text{mol/g}] \times 17\,[\text{g/mol}] \times 100\% \quad (1)$$

where:

$$\Delta D - \text{specific difference absorptivity}\left[\frac{L}{\text{g}\cdot\text{cm}}\right] = D_{\text{ionized}} - D_{\text{neutral}} \quad (2)$$

$$\Delta\varepsilon - \text{molar difference absorptivity}\,\frac{L}{\text{mol}\cdot\text{cm}} \quad (3)$$

Specific difference absorptivity was calculated directly from measured absorbance, using the Beer–Lambert equation (Johnson et al., 1961; Lin and Dence, 1992):

$$D = \frac{A}{c\cdot b} \quad (4)$$

where: D – specific absorptivity $\left[\frac{L}{\text{g}\cdot\text{cm}}\right]$; A – absorbance [unitless]; c – concentration [g/L]; b – light pathway (thickness of the cuvette's wall) [cm].

Molar difference absorptivity was found using an empirical equation based on the absorptivity's linear relationship with the wave number, and therefore wavelength. This relationship is linear for all four phenolic structure types, as previously described by (Lin and Dence, 1992), and has the following form:

$$\Delta\varepsilon = 1.435 \times 10^5 - 4.15 \times v \quad (5)$$

where: v – wave number [cm^{-1}].

2.3.4. Proton nuclear resonance spectroscopy (^{1}H NMR)

Acetylation of the lignin (1 g samples) was performed prior to ^{1}H NMR analysis using 20 mL of mixture of pyridine with acetic anhydride (1:1 w/w). The reaction was carried out under nitrogen, at room temperature, for 24 h. After completion, 25 mL of ice-cooled 100% methanol was added to quench the remaining acetic anhydride. Subsequently, the mixture was evaporated to dryness, suspended in toluene, and evaporated again (repeated 3 times). Finally, methanol was added to the remaining solid and then dried under reduced pressure (Lin and Dence, 1992).

The ^{1}H NMR spectra were obtained using an NMR spectrometer (Bruker 400 MHz, Bruker BioSpin, Billerica, MA, USA). Acetylated lignin samples of 25 mg were prepared for the analysis by dissolving in 1.0 mL of deuterated dimethyl sulfoxide (DMSO-d_6). The analysis was performed in solution state, using 16 scan mode.

2.3.5. Nitrobenzene oxidation

Lignin oxidation to vanillin was carried out to explore its possible industrial application. A number of methods have been developed over the years to utilize lignin for vanillin production. Most of them included nitrobenzene in alkaline solution as an oxidizing agent. There were also trials that used pure oxygen, alkali with copper sulfate/oxide, or silver dioxide (Sarkanen and Ludwig, 1971).

In this work, oxidation was performed as described in (Billa and Monties, 1995). Alkaline solution of nitrobenzene (5 mL 2 M sodium hydroxide and 0.5 mL of nitrobenzene) was used to oxidize 2 mg of lignin sample. The mixture was heated to 160 °C and agitated for 3 h. Then, the mixture was cooled down and diluted with 20 mL of water. Remaining nitrobenzene was extracted with 25 mL of dichloromethane (three times). The organic fraction was discarded, while the aqueous fraction was adjusted to pH 1 with 2 M hydrogen chloride, and then extracted three times with dichloromethane. This time, the aqueous fractions were discarded, while the organic fractions were combined, extracted with 25 mL of water, then dried using anhydrous sodium sulfate (Nadji et al., 2009). The vanillin which was produced was quantitatively analyzed using UV–vis spectroscopy. Percent yield of the vanillin was determined based on the Klason lignin content.

3. Results and discussion

3.1. Klason lignin, contaminant and ash analyses

Results of acid insoluble lignin content and original composition of the feedstocks used for lignin extraction are presented in Table 1. Lignin content in corn stover was found significantly different (>95% confidence) from switchgrass and prairie cordgrass. Switchgrass and prairie cordgrass contained similar amount of lignin in their structure.

Source of lignin influenced the lignin extraction yield as well as its Klason lignin and contaminants content. Prairie cordgrass-originated lignin was found significantly different from two others (Table 3). The highest yield of the extracted organosolv lignin was found when corn stover was used as a feedstock. This lignin was also relatively pure, containing over 70% acid Klason lignin, was low in ash and carbohydrate contamination (all below 5%) (Xu et al., 2006). The lowest yield and purity was obtained in the experiments

Table 1
Composition of the feedstocks used for lignin extraction (means* ± standard deviation).

Material	Prairie cordgrass	Switchgrass	Corn stover
Moisture [%]	$6.02^a \pm 0.02$	$6.47^b \pm 0.00$	$7.94^c \pm 0.23$
Glucose [% DM]	$36.70^a \pm 0.01$	$36.69^a \pm 0.94$	$36.38^a \pm 0.44$
Xylose [% DM]	$13.52^a \pm 2.00$	$20.72^b \pm 0.68$	$16.99^c \pm 0.19$
Arabinose [% DM]	$1.59^a \pm 0.57$	$2.94^b \pm 0.18$	$2.96^b \pm 0.06$
Mannose [% DM]	$0.30^a \pm 0.00$	$0.20^b \pm 0.05$	$0.33^a \pm 0.00$
Galactose [% DM]	$1.40^a \pm 0.50$	$1.30^a \pm 0.01$	$1.20^a \pm 0.03$
Lignin [% DM]	$20.96^a \pm 0.52$	$20.45^a \pm 0.41$	$16.29^b \pm 1.01$
Ash [% DM]	$5.65^a \pm 0.04$	$2.61^b \pm 0.30$	$6.59^a \pm 1.41$

* Mean values followed by the same letters in the superscript for a given dependent variable are not significantly different at $p < 0.05$ (based on a Tukey's comparison of means).

Table 2
Predetermined optimal clean fractionation conditions used to extract lignin from raw materials used in this study.

Material	Prairie cordgrass	Switchgrass	Corn stover
Temperature [°C]	139.71	140.00	139.99
Solvent composition [ethyl acetate:ethanol:water] [% w/w]	36.87:25.49:37.64	36.71:25.00:38.29	36.99:26.31:37.70
Catalyst concentration [% w/w]	0.39	0.46	0.46

Table 3
Lignin purity analysis (means* ± standard deviation).

Material	Prairie cordgrass	Switchgrass	Corn stover
Fractional lignin yield [%]	51.33a ± 6.24	59.03^{ab} ± 7.01	66.77^{b} ± 4.20
Klason lignin content [%]	65.85^{a} ± 1.56	73.49^{b} ± 1.23	72.55^{b} ± 0.44
Carbohydrate contamination [%]:			
Glucose	4.17^{a} ± 0.01	0.33^{b} ± 0.04	0.53^{c} ± 0.10
Xylose	2.75^{a} ± 0.00	0.99^{b} ± 0.43	1.44^{b} ± 0.12
Arabinose	2.12^{a} ± 0.05	1.29^{b} ± 0.12	1.31^{b} ± 0.12
Mannose	–	–	–
Galactose	–	–	–
Ash content [%]	2.93^{a} ± 0.11	0.44^{b} ± 0.01	1.13^{c} ± 0.06

* Mean values followed by the same letters in the superscript for a given dependent variable are not significantly different at $p < 0.05$ [based on Tukey's comparison of means].

with prairie cordgrass. This lignin contained relatively high amounts of carbohydrates and ash. Relatively high amount of carbohydrate residues indicates a strong association of hemicellulose and lignin in this grass species. Lignin extracted from switchgrass presented the highest purity, containing the lowest amounts of carbohydrates (2.61% total sugars) and ash (0.44%), and over 70% Klason lignin. The contamination level was lower than that of Alcell™ lignin (maximum 3.2% sugar residues) analyzed by (Gosselink et al., 2004).

3.2. Solubility

Analysis of solubility of the lignin samples in various solvents (and finding the best solvent for each lignin type) is very practical for any type of spectroscopic analysis, since lignins are insoluble in most of the organic solvents. All lignin types were somewhat soluble in organic solvents examined (Table 4). Generally, the highest solubility was observed in methanol and dioxane. All the lignin samples were non-significantly soluble in ethyl acetate and MIBK at the applied temperature (40 °C). The test was also performed using acid-purified lignin samples (Klason lignin), which showed much lower solubility in the examined solvents than raw lignin. Since raw lignin showed low solubility in ethyl acetate and MIBK, these solvents were not tested in the Klason lignin solubility analysis. Methanol turned out to be the best organic solvent for the acid insoluble lignin (Klason lignin samples). Methanol was also found the most effective in unpurified organosolv lignin's dissolution by other researchers (Cook and Sellers, 1989).

3.3. Phenolic hydroxyl groups

In order to evaluate the potential of the lignins for vanillin production, analysis of phenolic groups has been performed. Quantitative analysis of phenolic hydroxyl groups showed that the only types of phenolic structures present in all the examined lignin samples were type 1 (structures with saturated side chains, such as guaiacyl- and syringyl-propane units) and type 4 (*p*,*p*′ stilbene). These two structures produced two distinct absorptivity peaks in the ionization difference spectrum of each of the lignin samples analyzed, first occurring at 290–300 nm, and second at 360–370 nm (Fig. 1). According to Gaertner et al. (1999), structures absorbing UV light between 350 and 370 nm represent all conjugated phenolic structures, however, 350 nm peaks represent mainly phenolic structures conjugated with carbonyl groups, and 370 nm peaks are mainly associated with stilbene (Gaertner et al., 1999). The total phenolic structures were detected in highest amounts in solutions with the highest lignin concentrations, which were present in methanol solution of switchgrass-originated lignin (5.45% type 1 and 0.47% type 4) and in dioxane solution of corn stover-originated lignin (1.58% type 1 and 0.59% type 4). However, for prairie cordgrass, the highest amount of total phenolic groups (3.41% type 1 and 0.60% type 4) was found in DMSO solution (with only 32.87% lignin solubility). Overall, phenolic structures (type 1 and 4) were found to be detectable in dioxane using ionization difference spectroscopy in all samples (Table 5).

In the case of Klason lignin samples analysis (Table 5), dioxane solutions were found to have the highest concentrations of type 1 and 4 of the phenolic structures, except for switchgrass type 4 structure, which was found to be highest in the DMSO solution. Comparison of the ionization difference spectra of all Klason lignin samples in dioxane is presented in Fig. 2. Peaks assigned to both structures (type 1 at 290–300 nm and type 4 at 360–370 nm) were very distinct in case of all three lignin samples, showing better resolution and intensity than raw lignin samples spectra (Fig. 1). Overall, Klason lignin samples showed higher concentrations of phenolic structures (up to 11.47% type 1 and 0.60% type 4 in switchgrass-originated lignin), which suggests their higher applicability than raw, unpurified lignin. Generally, these results show high content of phenolic groups present in all the organosolv lignin samples examined in this work, and are reflective of other published work (El Mansouri and Salvadó, 2007; Lin and Dence, 1992). All the lignins showed higher contents of the phenolic structures than found by Mansouri and Salvadó (2006) (2.66%), which were analyzed using the same method (Mansouri and Salvadó, 2006). In another study, organosolv lignin extracted from

Table 4
Solubility of lignins in various organic solvents.

	DMSO [% w/w]	Dioxane [% w/w]	Methanol [% w/w]	Ethanol [% w/w]	Ethyl acetate [% w/w]	MIBK [% w/w]
PCG raw lignin	32.87	50.90	76.57	56.42	22.04	27.58
SG raw lignin	17.78	36.74	43.46	20.58	28.37	29.70
CS raw lignin	46.77	57.68	43.98	31.58	29.22	27.34
PCG Klason lignin	17.34	14.23	27.18	31.95	–	–
SG Klason lignin	7.11	11.79	13.97	10.66	–	–
CS Klason lignin	28.35	32.14	49.90	28.34	–	–

PCG is prairie cord grass; CS is corn stover; SG is switchgrass.

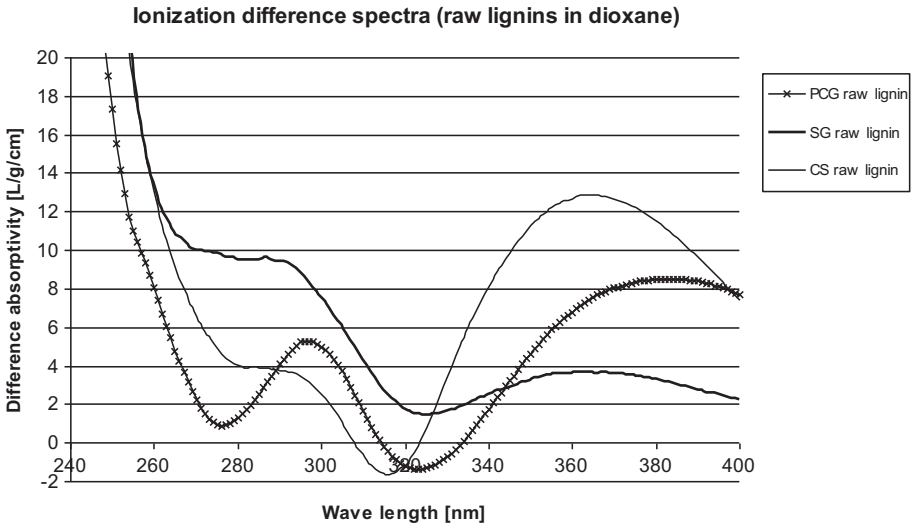

Fig. 1. Ionization difference spectra of raw lignins. PCG is prairie cord grass; SG is switchgrass; CS is corn stover.

Table 5
Phenolic structures in various lignin samples.

	DMSO		Dioxane		Methanol		Ethanol	
	Type 1 [% w/w]	Type 4 [% w/w]	Type 1 [% w/w]	Type 4 [% w/w]	Type 1 [% w/w]	Type 4 [% w/w]	Type 1 [% w/w]	Type 4 [% w/w]
PCG raw lignin	3.41	0.60	2.20	0.39	0.85	0.17	1.06	0.20
SG raw lignin	0.71	0.44	3.66	0.16	5.45	0.47	1.13	0.28
CS raw lignin	0.00	0.54	1.58	0.59	0.00	0.34	0.00	0.44
PCG Klason lignin	2.89	0.45	9.06	0.45	1.63	0.22	1.37	0.18
SG Klason lignin	6.63	0.87	11.47	0.60	1.62	0.32	2.20	0.30
CS Klason lignin	1.96	0.53	4.20	0.55	1.45	0.23	1.95	0.38

PCG is prairie cord grass; CS is corn stover; SG is switchgrass.

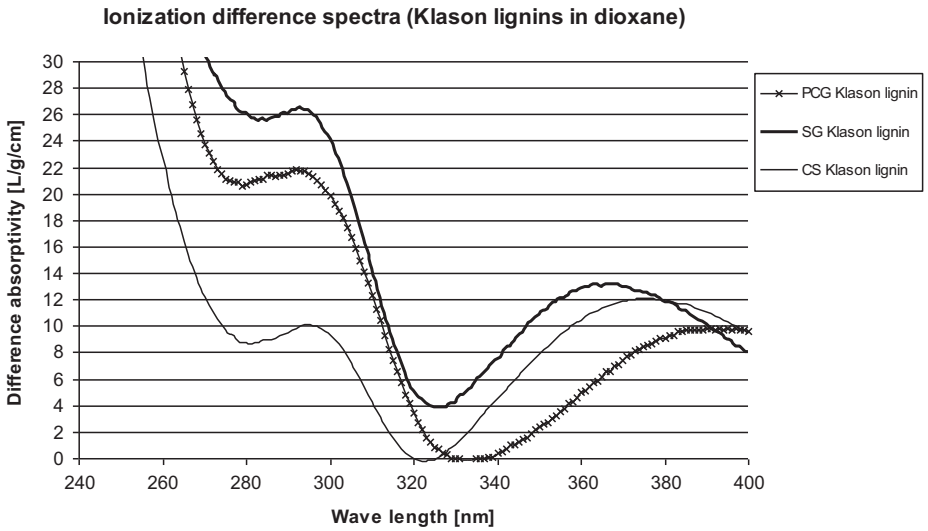

Fig. 2. Ionization difference spectra of Klason lignins. PCG is prairie cord grass; SG is switchgrass; CS is corn stover.

raw miscanthus, analyzed by ^{31}P NMR contained 1.55% of phenolic structures (Obama et al., 2012). Also, detection of the stilbene structures (type 4 structure) proves high extent of ionization of the phenolic groups, since biphenyl structures are often involved in an internal hydrogen bond, which makes them difficult to ionize (Gaertner et al., 1999).

The main purpose of using this technique was to evaluate the content of the phenolic groups and compare it among the analyzed lignins. It gives a rapid, simple and accurate analysis of the conjugated (e.g. stilbene) and unconjugated (e.g. guaiacyl- and syringylpropane) phenolic structures, giving a good characteristic of the lignin potential for oxidation with production of chemicals, such as vanillin or syringylaldehyde (Gaertner et al., 1999; Mansouri and Salvadó, 2006).

Proton NMR analysis was performed to confirm the presence of aromatic (i.e. phenolic) groups in the lignin samples, and to

identify their types, in addition to the ionization difference ultraviolet spectra method. Acetylation of the raw and Klason lignin samples prior to the ^{1}H NMR analysis ensured elimination of the effect of the hydrogen bonds in which phenolic groups are often involved. Hydrogen bonds cause the phenolic groups to be sterically hindered, interfering with the analysis (Sarkanen and Ludwig, 1971).

Generally, peaks between 8.0 and 6.2 ppm were attributed to substituted aromatic rings (Xu et al., 2006). Syringylpropane and guaiacyl-propane units (signals between 6.8 and 6.2 ppm (Faix et al., 1992)) were detected in all lignin samples, but switchgrass and corn stover lignin signals were much stronger than that of prairie cordgrass lignin. Signals around 7.4–7.3 ppm can be assigned to *p*-hydroxyphenyl units, C_α=O groups, or *p*-coumaric and ferulic acids in switchgrass and corn stover lignin (not detected in prairie cordgrass lignin) (Seca et al., 2000). The presence of ethoxyl groups was confirmed by sharp signals at 3.7 ppm (Xu et al., 2006), which were strongly observed in switchgrass lignin, distinct in corn stover lignin, but weak in prairie cordgrass lignin. Solvent peaks (DMSO-d_6) were detected at 2.5 and 3.3 ppm (associated with protons from water present in DMSO). Signals between 2.0 and 0.8 ppm were assigned to protons in the aliphatic side chains of the lignin (Xu et al., 2006).

3.4. Nitrobenzene oxidation

Evaluation of the extent of oxidation showed that vanillin was produced in relatively high concentrations from all the examined lignin samples. According to literature, kraft, sulfite or organosolv lignin-based vanillin production can result in 10–30% yield (calculated per initial lignin input weight) (Billa and Monties, 1995; Gogotov et al., 1996; Sarkanen and Ludwig, 1971; Schulz, 1940). In another study, oxidation of lignin obtained from the steam explosion process resulted in vanillin yields lower than 10% (Wu et al., 1994). In this study, vanillin yields spanned approximately 40–60%w/w, and the highest yield (59.63%w/w) was obtained for organosolv lignin extracted from corn stover (Table 6). This suggests high guaiacyl unit content, and availability in all the extracted lignin samples, and thus their applicability for vanillin production.

When comparing oxidation results (Table 6) to the UV ionization difference spectra results (Table 5), it can be deducted that type 1 structures in corn stover were represented mainly by guaiacyl-propane units (which are oxidized to produce vanillin). Furthermore it can be concluded that prairie cordgrass and switchgrass contained large amounts of syringyl-propane groups, which resulted in lower vanillin yields produced from these lignins, even though the amounts of detected phenolic units were higher for these samples. This observation leads to a conclusion that corn stover-originated lignin is more suitable to produce vanillin than switchgrass- and prairie cordgrass-originated lignins.

Furthermore, it has been suggested by Ehara et al. (2002) that alkaline oxidation by nitrobenzene is a good measure of ether linkages present in the lignin structure. Therefore, high yield of oxidation products proves that little or no condensation-type linkages are present in the lignin structure (Ehara et al., 2002).

Table 6
Vanillin yields produced by nitrobenzene oxidation of the lignins.

Lignin origin	Vanillin produced [mg]	Vanillin yield [% w/w]
PCG	0.96	48.00
SG	0.78	39.00
CS	1.19	59.63

PCG is prairie cord grass; CS is corn stover; SG is switchgrass.

4. Conclusions

Analysis of the lignin extracted from prairie cordgrass, switchgrass and corn stover revealed that all the examined lignin samples contained over 50% Klason lignin, less than 5% of carbohydrate contamination, and less than 3% ash. Lignin extracted from switchgrass was characterized as the purest of the three. The highest solubility of the lignin samples was observed in dioxane and methanol. High yields of the vanillin were observed in all lignin types, suggesting high availability of guaiacyl units. Proton NMR analysis confirmed the presence of guaiacyl and syringyl units, and methoxyl groups in the acetylated lignin samples.

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