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Characterization of Whole Biomasses in Pyridine Based Ionic Liquid at Low Temperature by ^{31}P NMR: An Approach to Quantitatively Measure Hydroxyl Groups in Biomass As Their Original Structures

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In this study, the dissolution of biomass components—cellulose, hemicellulose, and lignin, and two whole biomasses—switchgrass and poplar in a pyridine based ionic liquid at a low temperature—50°C has been examined, which will provide an opportunity to explore the original structures of biomass components. The following phosphitylation, and ^{31}P NMR measurement could provide quantitative results for various hydroxyl groups, including aliphatic, condensed phenolic, guaiacyl phenolic, *p*-hydroxyl phenyl and carboxylic hydroxyl groups in the biomass components, and whole biomass. By employing various biomass model compounds (glucose, cellotriose, and cellohexose), artificial mixtures of biomass components (cellulose, hemicellulose, and lignin), and computational simulation for the assignments by using density functional theory calculation in Gaussian, reliability and accuracy of this method have been examined as well, which indicated that this method is a reliable and accurate way to quantitatively characterize five different types of hydroxyl groups in biomass and its components.

Keywords: ionic liquid, biomass, lignin, cellulose, hemicellulose, ^{31}P NMR

INTRODUCTION

Biomass is a renewable resource for the sustainable production of fuels and chemicals that, to date, have been made primarily from fossil resources. Because of its carbon neutrality, relative abundance and non-food competition (David and Ragauskas, 2010), the use of biofuels and biochemicals could increase economic growth and provide environmental benefits. Lignocellulosic biomass contains three major constituents: cellulose, hemicelluloses, and lignin. Several reviews have summarized the distribution of these three major biopolymers in several hardwoods, softwoods, and agricultural residue species (Ragauskas et al., 2006; Huang et al., 2011).

Although lignocellulosic resources are readily becoming available for bioethanol production, their processing requires an aggressive pretreatment step to overcome their natural recalcitrance toward biological deconstruction to simple sugars. Clearly, the characterization of plant cell wall structure and its three major components has become a crucial research topic (Foston and Ragauskas, 2012). The traditional methods for the characterization of lignin, cellulose, and hemicellulose in the whole biomass always involve the isolation of the individual components by separation procedures followed by spectroscopic techniques (Hallac et al., 2009; Hu et al., 2010; Huang et al., 2011; Ben and Ragauskas, 2012). Furthermore, the traditional methods may change the original chemical structures of biomass components due to the relatively high-temperature treatment, oxidation, and hydrolysis. All of these limitations for the traditional methods are calling for new ways to quantitatively characterize the biomass without separation procedures, such as NMR characterization especially performed in a whole biomass solution (Ben and Ferrell Iii, 2016; Hao et al., 2016; Yoo et al., 2016; Li et al., 2018). A solvent system, which has the ability to dissolve whole biomass and could keep all the original chemical structures intact, appears to be very pragmatic. A DMSO-based system reported in the literature is a promising approach (Lu and Ralph, 2003). Most recently, some researchers used ionic liquid as a tool for dissolution and pretreatment of whole biomass (da Costa Lopes et al., 2013a,b) and employed various analytical methods including FT-IR and NMR to characterize the biomass. Argyropoulos' group (Kilpeläinen et al., 2007; Xie et al., 2007; King et al., 2008, 2009, 2010; Sadeghifar et al., 2014) has developed various imidazole-based ionic liquid to dissolve and characterize whole biomass and biomass components. Characterization of biomass in ionic liquid solutions have been reported including the use of ^{1}H, ^{31}P NMR, and FT-IR. Kishimoto's group (Qu et al., 2012, 2013) has investigated imidazole based ionic liquid to dissolve several hardwood, softwood, and bamboo samples, and HSQC NMR was also employed to characterize biomass solutions. Similarly, Muhammad (Muhammad et al., 2011) and Sun's groups (Wen et al., 2012; Yang et al., 2013) also used the imidazole family ionic liquid to dissolve various biomasses and biomass components, including lignin, cellulose, hemicellulose, and holocellulose. FT-IR, ^{13}C, and HSQC NMR have been used to characterize these ionic liquid solutions. All the imidazole type ionic liquids reported (Kilpeläinen et al., 2007; Xie et al., 2007; King et al., 2008, 2009, 2010; Muhammad et al., 2011; Qu et al., 2012, 2013; Wen et al., 2012; Yang et al., 2013) in the literature need a relatively high temperature (~100°C) to dissolve biomass. However, on the basis of reported TGA results (Yang et al., 2007), some biomass components, such as cellulose and hemicellulose, could be slightly decomposed even at ~80°C (Figure S3 in Supplementary Material). Ragauskas' group (Jiang et al., 2009, 2010; Samuel et al., 2011; Foston et al., 2012) has developed various pyridine ionic liquids and employed various NMR analytical methods, including ^{1}H, ^{13}C, and HSQC NMR, to characterize the chemical structures of biomass. Based on the screening of various pyridine-based ionic liquids (with different side chains), 1-allyl-3-butylpyridinium chloride is the most potential one, which could dissolve the whole biomass and biomass components at a relatively low temperature (50°C). To preserve as much as original chemical structures of biomass, this study will report a dissolution of biomass and its components at 50°C. Normally, ^{1}H and HSQC NMR characterizations of ionic liquid solutions require deuterated ionic liquids, which are relatively expensive. Nevertheless, the phosphitylation of hydroxyl groups using 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP) has been developed to quantitatively determine hydroxyl functional groups in various substrates including coal pyrolysis condensates (Wroblewski et al., 1988), coal extracts (Wroblewski et al., 1991), lignin, and pyrolysis oils (Ben and Ragauskas, 2011; Kosa et al., 2011). These hydroxyl functional groups which include aliphatic, condensed phenolic, guaiacyl phenolic, *p*-hydroxyl phenyl, and carboxylic OH groups are very important to understand the chemical structures of biomass and will also affect the following conversion process. In this study, ^{31}P NMR will be employed to analyze biomass solutions, which could provide quantitative structure information for various hydroxyl groups and will not require deuterated ionic liquids.

EXPERIMENTAL

Synthesis of 1-Allyl-3-Butylpyridinium Chloride

Allyl chloride (4.23 g, 55 mmol), 3-butylpyridine (6.75 g, 50 mmol), and anhydrous toluene (4.0 g) was added to a 25-ml

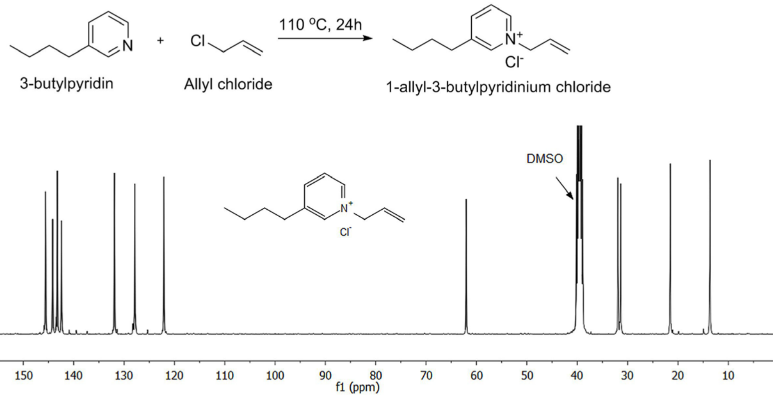

FIGURE 1 | Synthesis pathway and 13 C NMR result for the 1-allyl-3-butylpyridinium chloride ionic liquid.

vial. The mixture was heated at 110°C for 24 h under nitrogen. After decanting the toluene, the mixture was placed under direct vacuum at 80°C for 48 h. **Figure 1** shows the synthesis pathway and ^{13}C NMR spectrum (see Figure S1 in Supplementary Material for the detailed assignments. Based on ^{31}P NMR analysis, the water content for the ionic liquid is <0.1 wt%).

Biomass Components and Biomasses Preparation

Holocellulose was isolated from extractives-free Poplar by treatment with $NaClO_2$ and acetic acid at 70°C for 2 h (see Supplementary Material for detail). The process was repeated twice to ensure maximum lignin removal.

Ball-milled lignin was isolated by ball-mill extractive-free Poplar in a porcelain jar with ceramic balls using a rotatory ball-mill running at 96 rpm for 14 days under N_2. The ball-milled cell wall powder was then extracted with *p*-dioxane/water (96:4, v/v) and concentrated by evaporation and freeze–dried (Huang et al., 2011).

Two different types of hemicellulose are used in this study, one was commercially purchased (Sigma Aldrich) hemicellulose (xylan) produced from oak wood (hemicellulose #1) and the other was isolated by KOH extraction from Poplar holocellulose (hemicellulose #2). The differences from the natural and isolation process can lead to some different chemical structures for hemicellulose #1 and #2. Glucose, cellotriose, cellohexose, and cellulose were commercially purchased.

Baseline *populous* (*Populous trichocarpa* × *deltodies*) and lowland cultivar Alamo switchgrass (*Panicum virgatum*) samples were first Wiley-milled to pass 0.13-cm screen and then ball-milled with a Retsch MM 200 Mixer Mill equipped with a 10-ml stainless steel jar and two 7-mm stainless steel grinding balls, and milled for 20 min × 6 times at 25 s^{-1} frequency. The detailed information about the component analyses of feedstock has been reported in the literature (Foston and Ragauskas, 2010).

Dissolution and ^{31}P NMR Analysis of Biomass and Biomass Components in Ionic Liquid

Biomass and biomass components (5 mg, which is the maximum loading amount for the whole biomasses. For biomass components, the maximum dissolved amount could be even higher than 20 mg, for lignin it can be ~100 mg, however, to be consistent with the whole biomass samples, all the reported solution in this study have the similar concentrations) were dissolved in 1-allyl-3-butylpyridinium chloride ionic liquid (300 mg) at 50°C under nitrogen for 48 h. With 100–200 µl anhydrous pyridine added into the biomass solution, 80 µl 2-chloro-4, 4, 5, 5-tetramethyl-1,3,2-dioxaphospholane was carefully added to the solution. For whole biomass and cellulose samples, anhydrous 1-methyl-2-pyrrolidinone (NMP) and anhydrous *N*,*N*-dimethylformamide (DMF, 200 µl, each) were added to improve the dissolution of phosphitylation products. After fully dissolution and phosphitylation, an internal standard (50 µl, 20 mg/ml, cyclohexanol/$CDCl_3$) and relaxation reagent [50 µl, 20 mg/ml $Cr(acac)_3/CDCl_3$] were added into the final solution. (Note: The normally used internal standard—endo-*N*-hydroxy-5-norbornene-2,3-dicarboximide will be decomposed in this type of ionic liquid.)

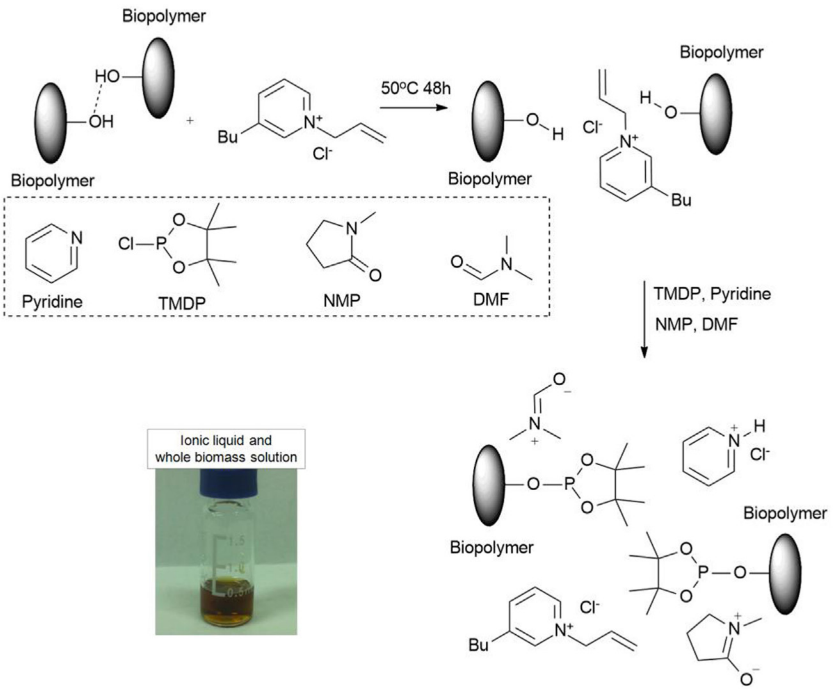

FIGURE 2 | Possible dissolution and phosphitylation of biomass components and whole biomass in the ionic liquid.

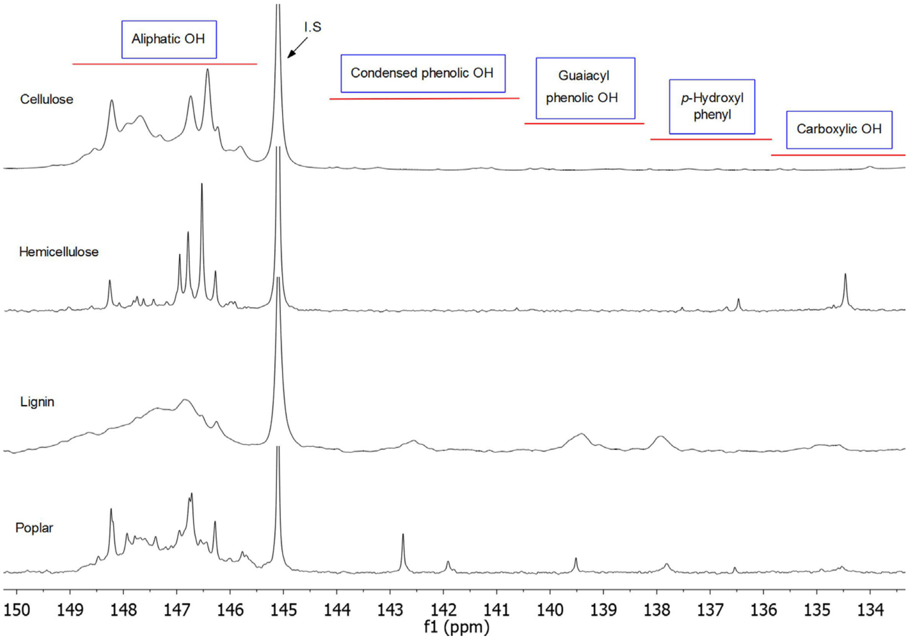

FIGURE 3 | ^{31}P NMR spectra for cellulose, hemicellulose, lignin, and poplar whole biomass after dissolution and phosphitylation with TMDP in ionic liquid.

^{31}P NMR was recorded at room temperature with a 5-mm BBO probe in the Bruker Avance/DMX 400 MHz NMR spectrometer and used an inverse gated decoupling pulse sequence, 90° pulse angle, 25 s pulse delay [optimized based on literature report (Zawadzki and Ragauskas, 2001; Ben and Ragauskas, 2011; Pu et al., 2011)], and 512 scans with a LB of 4.0 Hz. Spectral width is 200 ppm, and the acquisition time is 0.98 s. The cyclohexanol peak signal at 145.1 ppm was used as reference.

RESULTS AND DISCUSSION

It has been reported (Pinkert et al., 2009) that the mechanism for dissolution of biomass and its components in ionic liquid involves the disrupture of hydrogen bonds between biopolymers and replacing these hydrogen bonds between ionic liquid and biopolymer. By employing the new developed pyridine-based ionic liquids, the biomass and its components can be dissolved at a low temperature (50°C) very well, which indicate the original hydrogen bond in the biomass has been destroyed and formed a new equilibrium with the ionic liquid. However, after phosphitylation (see Figure S2 in Supplementary Material for reactions) of biomass solutions, some precipitate particles may appear, which may be due to the TMDP reacted with all the OH groups in the biomass and broke the equilibrium between ionic liquid and biomass. It is also evident that TMDP can react with all the OH groups in the biomass ionic liquid solution, which will also change the polarity for the biomass and lead to precipitate. It has been reported (Rinaldi, 2011) that NMP could improve the dissolution of cellulose, and DMF (Pu et al., 2011) was used to dissolve lignin samples. Therefore, to regain the balance and equilibrium, two extra solvents such as NMP and DMF were added into the solution to improve the dissolution. The result indicated that NMP will work perfectly for cellulose sample but not for whole biomass samples. DMF is a necessary step for the total dissolution of whole biomass, but DMF will not work as a single enhancer even for cellulose sample. The final solution is transparent with an amber color. **Figure 2** shows the possible dissolution and phosphitylation pathways of biomass in ionic liquid. It has been found the commonly used internal standard NHND (endo-*N*-hydroxy-5-norbornene-2,3-dicarboximide) is not stable in this type of ionic liquid, with the peak for this internal standard disappeared after 24 h. The similar phenomena have been reported in the literature as well (Ben and Ferrell Iii, 2016); however, the peak for NHND still remained 80%

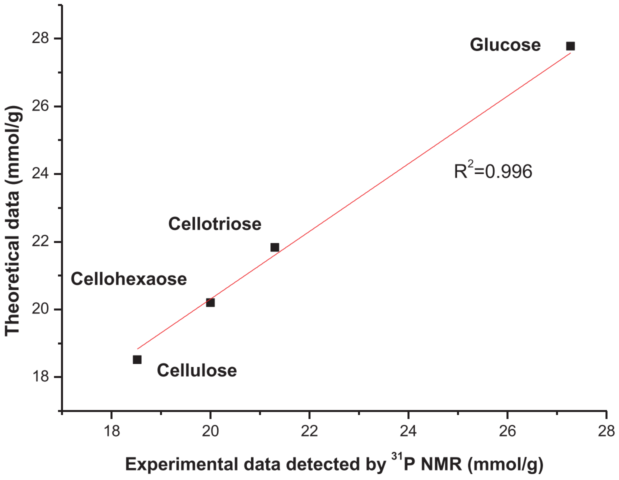

FIGURE 4 | The correlation between experimental and theoretical data of hydroxyl group contents of cellulose and cellulose model compounds.

TABLE 1 | Hydroxyl group contents of cellulose, hemicellulose, holocellulose, and lignin.

OH content (mmol/g)[a]	Cellulose	Hemicellulose #1	Hemicellulose #2	Lignin	Holocellulose
Aliphatic OH	18.52	3.72	5.81	3.83	13.88
Condensed phenolic OH	0.00	0.00	0.00	0.51	0.00
Guaiacyl phenolic OH	0.00	0.00	0.00	0.49	0.02
p-Hydroxyl phenyl	0.00	0.00	0.00	0.24	0.07
Carboxylic OH	0.00	0.59	1.40	0.22	0.46

Detected by ^{31}P NMR after dissolution and phosphitylation with TMDP in ionic liquid.

[a] *Duplicated tests have been done for all the samples, and the SD is lower than 2%.*

TABLE 2 | Hydroxyl group contents of two mixtures of cellulose, hemicellulose, and lignin.

OH content (mmol)	Mixture #1	Theoretical OH content	Mixture #2	Theoretical OH content
Aliphatic OH	64.51	60.92	90.06	94.01
Condensed phenolic OH	0.67	0.61	1.05	0.92
Guaiacyl phenolic OH	0.57	0.58	0.83	0.88
p-Hydroxyl phenyl	0.35	0.29	0.29	0.32
Carboxylic OH	1.93	2.2	7.01	7.12

Detected by ^{31}P NMR after dissolution and phosphitylation with TMDP in ionic liquid.

Mixture #1 made by lignin (~20 wt%), hemicellulose #1 (~45 wt%), and cellulose (~35 wt%).

Mixture #2 made by lignin (~20 wt%), hemicellulose #2 (~45 wt%), and cellulose (~35 wt%).

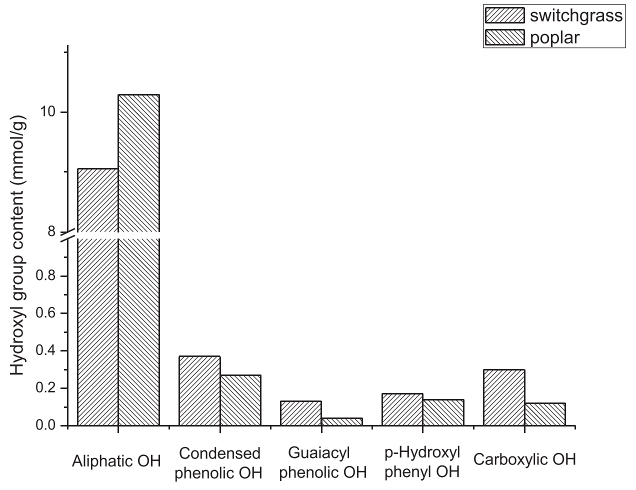

FIGURE 5 | Hydroxyl group contents of switchgrass and poplar. Detected by ^{31}P NMR after dissolution and phosphitylation with TMDP in ionic liquid. (Due to the possible overlap and shift, the content of syringyl type OH is only a preliminary number. There were ~65% of C5 substituted/condensed phenolic OH in poplar belong to syringyl OH, and there were ~40% of C5 substituted/condensed phenolic OH in switchgrass belong to syringyl OH.).

intensity after 14 days, which means this type of ionic liquid can accelerate the decomposition process for NHND. Therefore, for a precise and accurate data, in this study, cyclohexanol has been used as internal standard. By employing the same ionic liquid system, the obtained ^{31}P NMR spectrums for cellulose, hemicellulose, lignin, and poplar whole biomass are shown in **Figure 3**. Aliphatic OH, condensed phenolic OH (combined C5 substituted/condensed phenolic hydroxyl groups including β-5, syringyl, 4-*O*-5, and 5-5 structures. Table S1 for detailed assignment ranges and structures), guaiacyl phenolic OH, *p*-hydroxyl phenol, and carboxylic acid OH can be quantitatively characterized by this method.

To further evaluate the accuracy of this method, various oligomers of glucose and cellulose have been examined. Figure S2 in Supplementary Material demonstrates the dissolution and phosphitylation of aliphatic hydroxyl bonds with TMDP in ionic liquid, and **Figure 4** shows the correlation between experimental and theoretical data of hydroxyl groups in cellulose and oligomers of glucose, which is the evidence that this method is a reliable way to provide quantitative data for the contents of hydroxyl group for cellulose.

To explore more about this proposed ionic liquid system, two hemicellulose samples, lignin and holocellulose, were studied and the results are shown in **Table 1**. It was found that hemicellulose and lignin readily dissolved in this type of ionic liquid and will not precipitate after adding TMDP. Two cosolvents such as DMP and DMF were added for experimental consistency. As anticipated, the results show that there are no phenolic hydroxyl signal in cellulose and hemicellulose. The holocellulose sample contained a very small peak which can be assigned to guaiacyl and *p*-hydroxyl phenyl OH, which may be due to the residues of lignin. For lignin sample, the results of hydroxyl group contents between employing ionic liquid and routine ^{31}P NMR solvent are virtually identical. Therefore, this method can also provide detailed information of hydroxyl groups for all the three major biomass components—cellulose, hemicellulose, and lignin. To further evaluate this methodology, a set of biomass mixtures were prepared and examined as summarized in **Table 2**. The artificial mixtures of lignin, hemicellulose, and cellulose have the similar contents for each biomass components in the whole biomass tested in this study to represent the real biomass. The only difference for these two mixtures is the difference for the types of hemicellulose. Since the OH groups contents for all of these three biomass components have been fully characterized (**Table 1**), the artificial mixtures have the theoretical results for these different OH groups. By using the proposed ionic liquid system, the experimental and theoretical results for these two artificial mixtures are consistent with each other, which indicated that the proposed system can provide quantitative data for complex mixtures, which is the preapproval for this system to perform precise analysis for the real whole biomass.

Having successfully characterized major biomass components and artificial mixtures of these components, this method has also been used to analyze two whole biomasses—switchgrass and poplar. The ^{31}P NMR results are shown in **Figure 5**. The integration results indicated that compared to poplar, switchgrass has relatively less amount of aliphatic OH, but more aromatic and carboxylic OH. The carbohydrate and Klason lignin contents of these two biomasses (poplar: ~lignin 30%, ~cellulose 50%; switchgrass: ~lignin 28, ~cellulose 40%) also provide similar results (Foston and Ragauskas, 2010). Compare to the literature reported (Akim Leonid et al., 2001) lignin OH groups in the poplar, this study presents a relatively lower content. The reason for this difference is still unclear, which may be due to the benefit of low temperature treatment of biomass in this study or can be due to the differences between the biomass samples and characterization methods.

CONCLUSION

The dissolution of biomass components—cellulose, hemicellulose, and lignin, and two whole biomasses—switchgrass and poplar in 1-allyl-3-butylpyridinium chloride—was examined. The phosphitylation and ^{31}P NMR measurement provided quantitative results for various hydroxyl groups, including aliphatic OH, condensed phenolic OH, guaiacyl phenolic OH, *p*-hydroxyl phenyl OH, and carboxylic OH in the biomass components and whole biomass. Several evaluations of this process including the use of cellulose and various oligomers of glucose, and artificial mixtures of major biomass components have been examined. All the results show that this method is a reliable and accuracy way to quantitatively characterize hydroxyl groups in biomass and its components. The ^{31}P NMR results for the whole biomasses are also consistent with the traditional analysis method. Nevertheless, the established method in this work opens up a new way to dissolve whole biomass and biomass components at a low temperature (50°C), which provide an opportunity to explore the original structures of biomass (Figure S3 in Supplementary Material). The ongoing research in our group involves detailed assignments for cellulose (Figures S4 and S5 in Supplementary Material) and hemicellulose and evaluation of various pretreatment processes by this method.

SUPPORTING INFORMATION

Experimental details, ^{31}P NMR assignments and spectrums, computational simulations are available free of charge *via* the Internet at http://pubs.acs.org.

AUTHOR CONTRIBUTIONS

HB conducted all the major experiments and wrote the manuscript. GH provided the NMR facility in Qingdao University to finish some additional tests from China. YS did the sample preparation and helped with the manuscript preparation. WJ did some NMR tests and helped with the manuscript preparation. YP did the sample preparation, NMR tests in the US, and helped with the manuscript preparation. AR provided very valuable information on the whole idea and manuscript writing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fenrg.2018.00013/full#supplementary-material.

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