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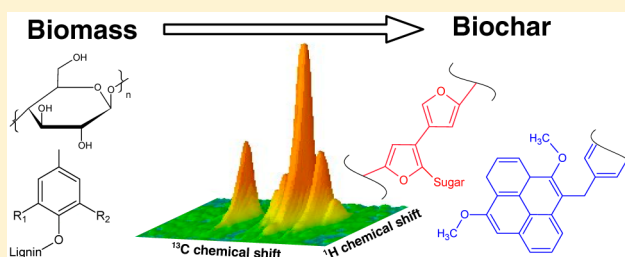
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High Resolution Solid State 2D NMR Analysis of Biomass and Biochar

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S Supporting Information

ABSTRACT: Solid state NMR methods are required to analyze biomass as a function of its chemical or biological treatment for biofuels, chemicals, or biochar production. The native polymers network in lignocellulosic biomass and other solid materials, such as coal, coke, or biochar, can hardly be analyzed by liquid state NMR due to their poor swelling ability without chemical modification. A ^1H – ^{13}C two-dimensional heteronuclear correlation (HETCOR) experiment with frequency-switched Lee-Goldburg (FSLG) irradiation is performed on a high field spectrometer (750 MHz). This method leads to previously unattained resolution for biomass and biochar and offers a unique ability to reveal their chemical composition. The formation of aromatic moieties from carbohydrates and lignin thermal conversion is clearly distinguished. This method can be applied to all other carbonaceous materials.



The worldwide growing demand of energy and the necessity to reduce greenhouse gas emissions lead to the increasing valorization of lignocellulosic biomass. Bio-ethanol from cellulosic feedstock and the thermochemical conversion of biomass are promising solutions to produce energy and green chemicals.^{1,2} Lignin, cellulose, and hemicelluloses form a complex network in the cell walls of biomass. There is a strong need for advanced analysis of the chemical functions involved in this network to better understand the composition of native biomass, as a function of growing conditions or genetic engineering,³ and the conversion mechanism of these materials, as a function of the thermochemical, biochemical, or chemical pretreatment.

NMR methods are nondestructive and have been used in many investigations for the analysis of coal, biomass polymers, or char.^{4–23} Some NMR characterizations were made by using isolated fractions of the plant cell wall polymers which requires tedious separation and isolation techniques of cellulose, lignin, and hemicelluloses. This approach not only is time-consuming but also is likely to alter the native structure of the polymers. Recently, the solution state NMR spectroscopy of whole plant cell was reported by employing ionic liquids,^{24,25} acetylated cell wall, or swelled cell wall in a solvent to produce gel.^{26,27} These methods require long preparation time and can alter the chemical structure of native polymers as well (e.g., lignin in ionic liquids). In addition, they are not adapted to study the chemical structure of cross-linked materials such as chars which cannot be swelled by a solvent without chemical modification. Solid state NMR is a promising method for the analysis of chemical structures of insoluble materials. Nevertheless, 1D

solid state NMR applied to whole plant material or to char is often limited by poor resolution and overlapping caused by the complex structure of the biomass. Advanced two-dimensional solid state NMR methods have been previously used on biomass in extensive works^{17,28–30} but at relative low field. The aim of the present work is to develop high resolution solid state 2D NMR methods which could be applied on biomass and biochar for revealing the mechanism of biomass thermal conversion.

Here, we show the outstanding benefit of using a high field (17.6 T) 2D ^1H – ^{13}C heteronuclear correlation (HETCOR) experiment with frequency-switched Lee-Goldburg (FSLG) irradiation during the evolution time³¹ for the analysis of biomass and biochar. The dramatic increase of resolution in the proton dimension obtained by FSLG homonuclear decoupling together with a high field makes this technique a unique choice for proton–carbon analysis in the solid state. De Groot and co-workers demonstrated the interest of this kind of approach on fully labeled ^{13}C bacteriochlorophyll.³² We show here that the FSLG ^1H – ^{13}C HETCOR technique can be successfully applied without ^{13}C labeling, leading to previously unattained resolution for biomass and biochar samples. In order to sort out even most of the unprotonated carbons, we found that a cross-polarization (CP) contact time of 4 ms was optimal. In those conditions, even the weakly proton dipolar coupled rare spins like carbonyls give rise to rather well detectable cross

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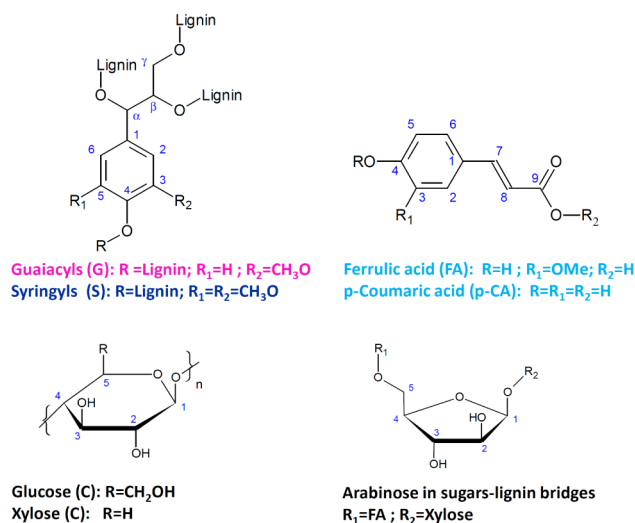
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peaks despite their expected reduction by the “dipolar truncation” phenomenon³³ due to the stronger dipolar interactions like CH involving the same protons.

The different kinds of sugar and lignin carbons analyzed by this method are named in a standard way (Chart 1). The whole

Chart 1. Schematic Representation of the Main Moieties of *Miscanthus Giganteus*² and Notations for Carbon Atoms



cell wall of miscanthus straw was analyzed in situ by solid state FSLG ¹H–¹³C HETCOR (Figure 1). The NMR procedure and chemical characterizations of biomass are presented in the Supporting Information.^{34–36} The spectrum was compared with those obtained from raw miscanthus and isolated lignin

through 1D, HSQC, HMQC, and HMBC spectra^{12,15,25,37,38} (see the Supporting Information).

The HETCOR NMR spectrum of untreated miscanthus exhibits characteristic cross peaks for cellulose, hemicelluloses, and lignin. The clusters of cross peaks at $\delta C/\delta H$ 64.4/3.6, 74.9–72.3/3.6, 83.6/3.6, 87.9/3.6, and 104.8/3.6 are assigned to C₆ (CH₂OH in glucose unit), C_{2,3,5}, C₄ disordered structure, C₄ ordered structure, and C₁ carbons of polysaccharides, respectively. These carbons seem to correlate with their own protons,²⁹ but in this range of ¹³C chemical shift (105–64 ppm), there is also ¹³C signal from aliphatic lignin.³⁹ Correlation between these carbons with O-alkyl protons and aromatic protons is shown in the area of $\delta C/\delta H$ 50.0–110/3–7 ppm. Concerning lignin, correlations are observed at $\delta C/\delta H$ 56.2/3.6 and 56.2/6.35–7 ppm corresponding, respectively, to O-alkyl protons from CH₃O and aromatic protons from lignin aromatic rings. According to the ¹³C chemical shift of 74 and 104 ppm, correlation with aromatic protons (6–7 ppm) could be due to interaction between the carbohydrates carbons and lignin aromatic protons⁴⁰ or into the lignin structure. Indeed, solid state HETCOR measurement allows correlations between carbon and proton through the space. These correlations indicate that carbohydrates are closely associated with lignin in biomass structures.

Cross signals corresponding to aromatic protons (C_{Ar}–H) at $\delta C/\delta H$ 110–122/6.3–6.9 ppm were observed in this region of the FSLG ¹H–¹³C HETCOR spectrum. These signals, corresponding to correlations between G₂, G₅, and G₆ to their own protons, were observed at $\delta C/\delta H$ 110–112/6.7–7.5, 113–115/6.5–7.5, and 118–120/6.3–7.0 ppm, respectively. Cross signal observed at 111.5/3.6 could be attributed to a correlation between G₂ and methoxyl protons. Para-coumarate

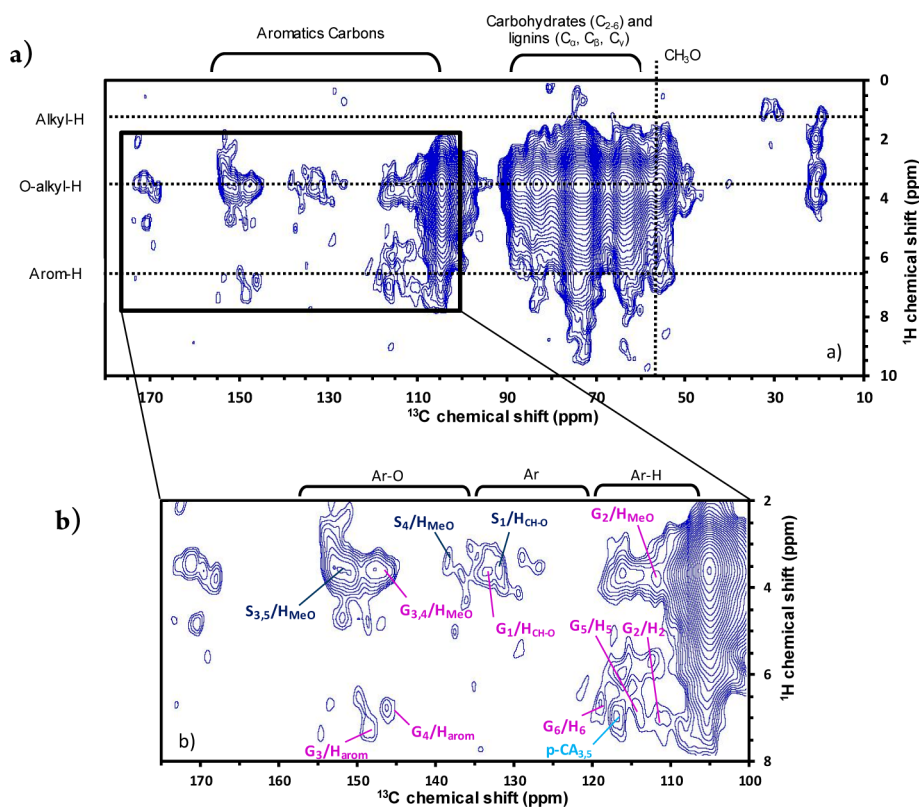


Figure 1. (a) ¹H–¹³C 2D HETCOR spectra of miscanthus; (b) zoom on the 175–100 ppm zone.

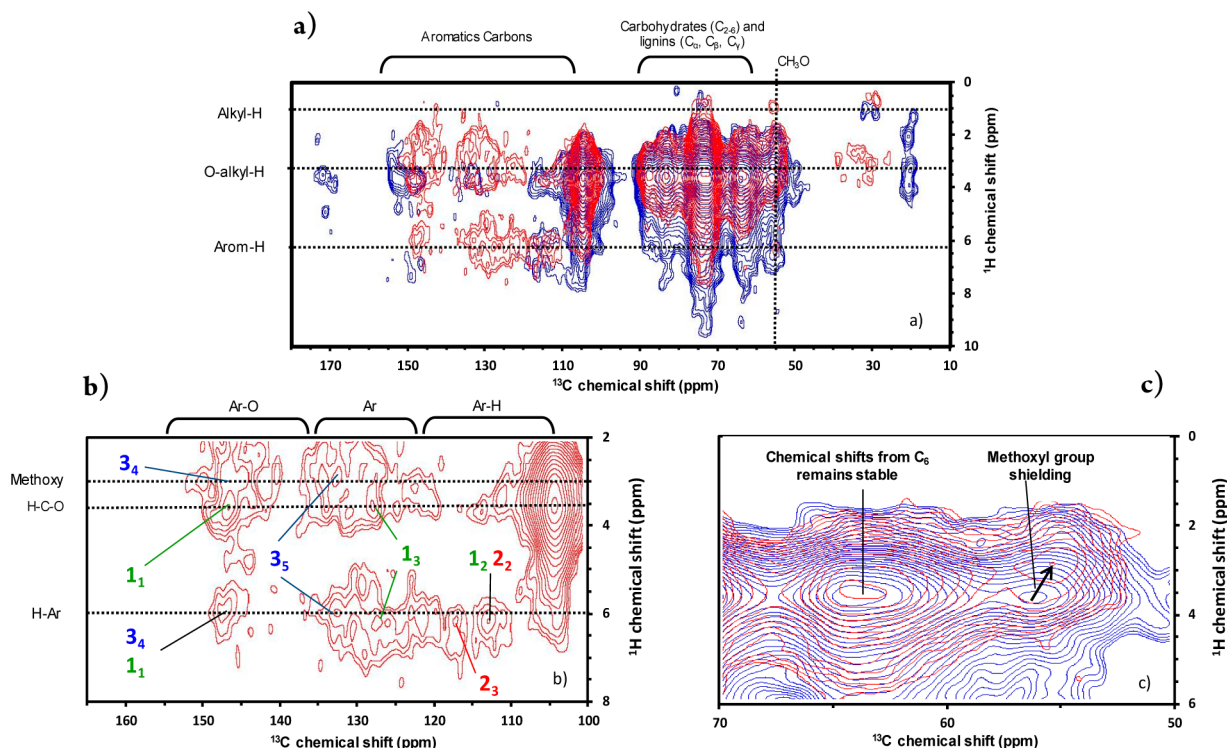


Figure 2. ^1H – ^{13}C 2D HETCOR spectra (a) comparison between native miscanthus (blue) and biochar produced at 300 °C (red), (b) zoom on the 165–100 ppm $\delta^{13}\text{C}$ zone of biochar, and (c) zoom on the 70–50 ppm $\delta^{13}\text{C}$ comparison between native miscanthus and biochar, highlighting the shielding of the methoxyl group in lignin-based aromatic clusters.

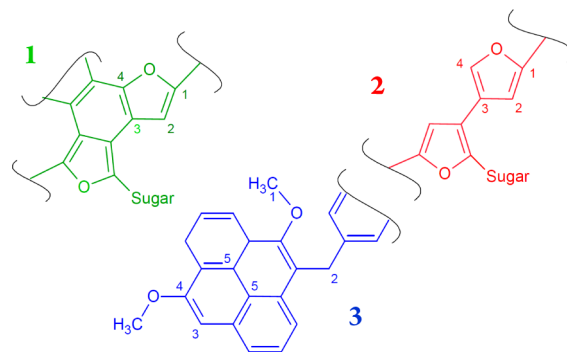
group was also detected at $\delta\text{C}/\delta\text{H}$ 116.9/6.9 ppm (p- CA_3 and p- CA_5). Signals for correlations of S_1 and G_1 units (aromatic carbons) with aliphatic CH–O protons were detected at $\delta\text{C}/\delta\text{H}$ 132–136/3.0–4.0 ppm. Cross signals of aromatic C–O carbons with aliphatic CH–O protons corresponding to $\text{S}_{4\text{r}}$, $\text{G}_{3-4\text{r}}$ and S_{3-5} etherified were observed at $\delta\text{C}/\delta\text{H}$ 138.0–140.0/3.2–4.0, 146.0–149.5/3.1–4.0, and 150.0–154.0/3.2–4.0 ppm, respectively. Long-range correlations between aromatic C–O carbons with aromatic protons were also observed at $\delta\text{C}/\delta\text{H}$ 145–147/6.8–7.2 ppm (G_4) and $\delta\text{C}/\delta\text{H}$ 147–149/7.0–7.5 ppm (G_3). The region at $\delta\text{C}/\delta\text{H}$ 168–174/1.5–7.0 ppm (Figure 1b) also gives valuable information concerning the carbonyl groups and their environment. The acetate group is unambiguously identified through the correlation signal at $\delta\text{C}/\delta\text{H}$ 172–173/1.9–2.1 ppm (CH_3CO). The chemical shifts of the cross signals at 167–168/3.6–4.0 ppm could be consistent with those of the carbonyl group with ferulate (Figure 1) involved in lignin–hemicelluloses cross-linking.⁴¹

To illustrate the capability of this analytical method to unravel biomass conversion as a function of process conditions, the spectra of native miscanthus and char produced at 300 °C are compared in Figure 2. In the Supporting Information, 1D ^{13}C CPMAS NMR spectra of different pyrolysis temperatures are presented and the choice of 300 °C for the 2D NMR investigation is justified.

Upon heating, the network of polymers in miscanthus undergoes competitive bond breaking and cross-linking reactions to form mobile species, volatiles, and char.⁴² The cross-linked network produced in char requires the use of solid state NMR to understand the chemical mechanisms involved in thermal conversion. The high resolution of our NMR method

enables one to reveal some typical chemical moieties in the char (Chart 2), in agreement with previous works.^{43,44}

Chart 2. Representation of Some Chemical Moieties in Char



It appears from Figure 2 that the correlation signals assigned to acetate moieties and lignin–ferulate–arabinose cross-links (172–173/1.9–2.1 and 167–168/3.6–4.0 ppm, respectively) are no longer detected in the char. This observation could reveal the cleavage (1) of the acetate groups from hemicelluloses or (2) of the bonds between ferulic acid in lignin and arabinose in the network (Figure 2). The shoulder at around 100 ppm on the C_1 sugars strongly decreases, pointing out the degradation of carbohydrates in agreement with previous works^{17,45} (Figure 2a). Concerning the aromatic carbons, signals from S_3 and S_5 are no longer present at 300 °C. It can be explained by the cleavage of $\beta\text{-O-4}$ functions or by demethoxylation.

The methoxyl groups undergo a shielding due to the formation of aromatic clusters in the char (Figure 2c).¹¹ Hence,

the $\delta^1\text{H}$ chemical shift from methoxyl groups (2.7–3 ppm) can be distinguished from other $\delta^1\text{H}$ from carbohydrates (3.2–3.6 ppm) (Figure 2b). This finding is of tremendous importance to understand the chemistry of lignin and carbohydrates pyrolysis in the network and is enabled by our high magnetic field combined with the FSLG method. The formation of aromatic moieties from carbohydrates and lignin conversion is thus clearly distinguished.

The peak at 146–147 ppm could be furanic moieties cross-linked from the conversion of carbohydrates.^{43,46,47} The furanic carbons correlate with aromatic (146–147/5.6–5.9 ppm) and aliphatic protons (146–147/3.3–3.7 ppm) (Chart 2, structure 1, carbon 1). This 1_1 carbon signal is overlapped by the 3_4 ^{13}C signal which correlates with aromatic ^1H from aromatic clusters and ^1H from methoxyl functions (146–147/6.0 and 3.0 ppm).

The important peak at 132/6.0 ppm related to aromatic clusters^{43,48} could be assigned to the aromatic carbons (3_5) formed from lignin⁴⁴ or from carbohydrates.⁴⁵ Correlations at 132–133/2.7–3 ppm highlight the methoxyl groups remaining in the char from lignin. Carbon peaks at 127.5–128.5 ppm could be assigned to aromatic carbons from carbohydrates⁴⁹ (structure 1) because these carbons seem to be correlated with aliphatic protons (3.2–3.6 ppm) from sugar and aromatic protons (6.1–6.4 ppm).

The signal at 117.5–118/6–6.5 ppm (Figure 2b) can be assigned to 2_3 carbons (correlation with aromatic ^1H from furanic moieties). It can be observed that this signal is not correlated with any aliphatic or methoxyl protons, demonstrating the absence of aliphatic moieties in the structure 2. The signal at 113–114/6–6.3 ppm could be assigned to 1_2 and 2_2 with a direct correlation with aromatic ^1H .

All these structures evidenced in this work reveal the different mechanisms of aromatization during carbohydrates and lignin pyrolysis in the network of biomass cell walls. This 2D NMR method is powerful to analyze solid materials hardly soluble or with labile moieties without any sample preparation, thanks to the high magnetic field and the FSLG method. It can be combined with quantitative 1D NMR for solid analysis¹⁸ and high resolution mass spectrometry for liquid products analysis⁵⁰ to achieve a complete outlook of products from the chemical or biological conversion of any kind of carbonaceous material.

■ ASSOCIATED CONTENT

■ Supporting Information

Methods for biomass pyrolysis, analysis of samples, NMR procedure, assignments, ^{13}C 1D NMR spectra and effect of contact time, and TGA. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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