Supporting Information for

# Intensified Ozonolysis of Lignins in a Spray Reactor: Insights into Product Yields and Lignin Structure

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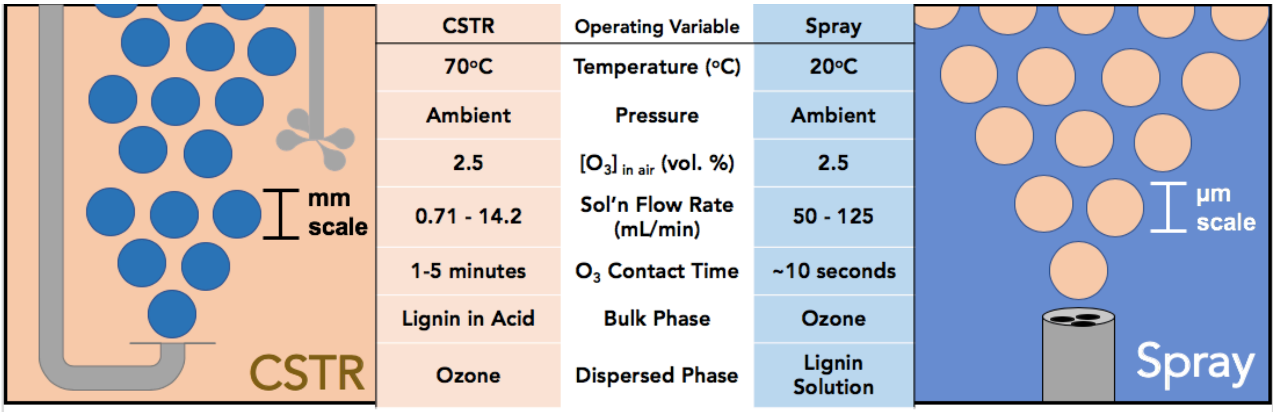
The following contents are arranged in the order they appear in the manuscript.

**Table S1: Comparison of continuous stirred tank and spray reactor operating conditions**



| CSTR | Operating Variable | Spray |
|---|---|---|
| 70°C | Temperature (°C) | 20°C |
| Ambient | Pressure | Ambient |
| 2.5 | $[O_3]_{\text{in air}}$ (vol. %) | 2.5 |
| 0.71 - 14.2 | Sol'n Flow Rate (mL/min) | 50 - 125 |
| 1-5 minutes | $O_3$ Contact Time | ~10 seconds |
| Lignin in Acid | Bulk Phase | Ozone |
| Ozone | Dispersed Phase | Lignin Solution |

**Calc S1: Estimation of ozone permeation time in droplets**

| Symbol | Value | Units | Description |
|---|---|---|---|
| $k_b$ | $1.38 \times 10^{-23}$ | J/K | Boltzmann Constant |
| $T$ | 293.15 | K | Temperature |
| $\eta$ | $1.391 \times 10^{-3}$ | kg/ms | Dynamic viscosity of 3:1 v:v acetic: formic acid solution at 20°C[1] |
| $r_{oz}$ | $1.40 \times 10^{-10}$ | m | Calculated radius of molecular ozone[2] |
| $D_{oz}$ | $1.10 \times 10^{-9}$ | $m^2/s$ | Calculated diffusion constant for ozone |
| $r_d$ | $3.5 \times 10^{-5}$ | m | Estimated droplet radius[3] |
| $t_p$ | 1.11 | s | Calculated permeation time |

The diffusion coefficient for ozone in acid solution ($D_{oz}$) at room temperature is determined using the following Stokes-Einstein equation.[2]

$$D_{oz} = \frac{k_b * T}{6\pi * \eta * r_{oz}}$$

The various physical and transport parameters in the foregoing equation are tabulated in the Table above. Based on the calculated diffusivity of ozone in acetic acid ($1.10 \times 10^{-9}$ $m^2/s$), it is estimated that for spherical liquid droplets with a radius of 35 µm, complete droplet permeation of the ozone will occur in approximately 1.11 seconds ($t_p$), based on the following equation.

$$t_p = \frac{r_d^2}{D_{oz}}$$

**Equation S1: Determination of liquid phase residence time in the spray reactor**

The spray reactor is operated such that there is no pooling (i.e., holdup) of the reaction mixture at the reactor bottom. Hence, the residence time is estimated based on the time a liquid stream enters the reactor via the spray and the time it exits the reactor as follows.

$$t_R = {[(t_1 - t_2) + (t_3 - t_4)]}/{2}$$

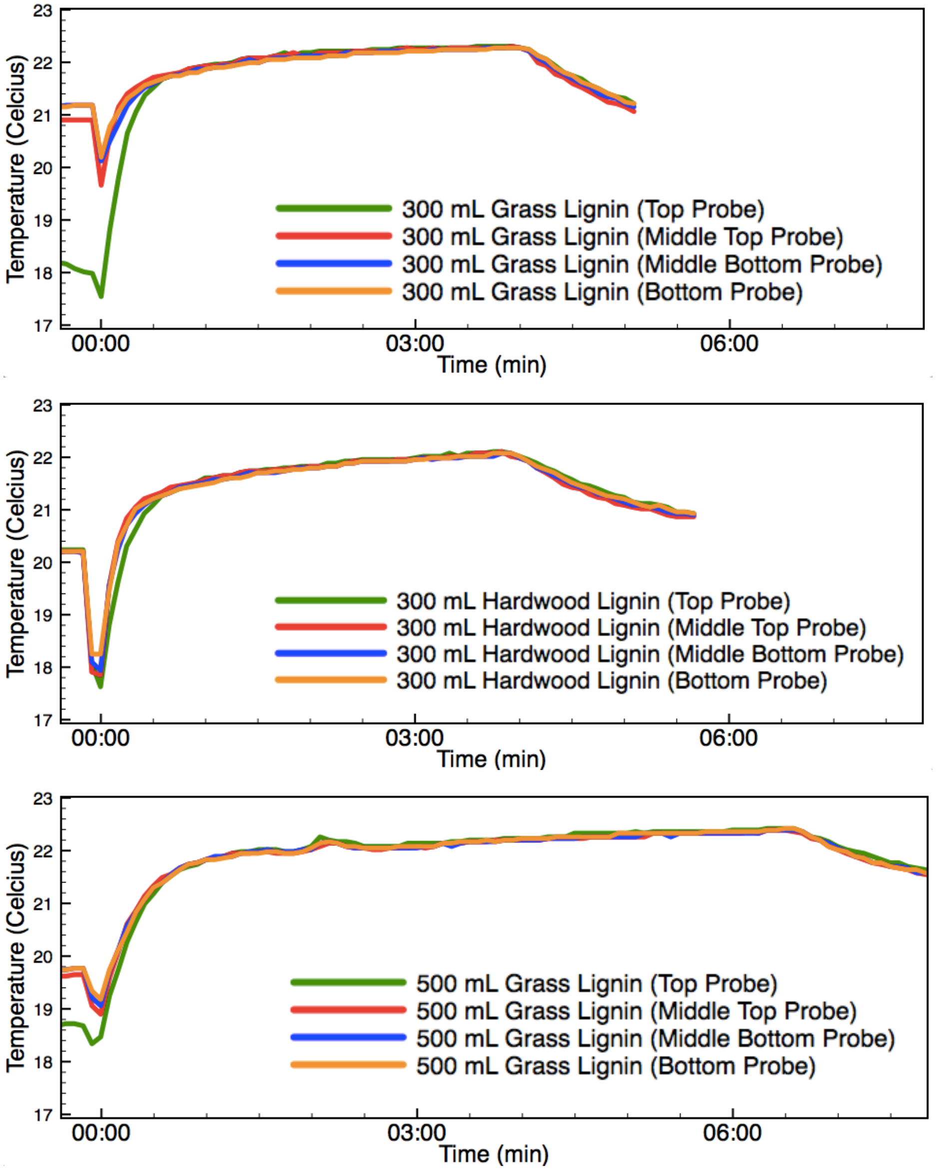
$t_R$ = residence time in reactor (s)

$t_1$ = time at which liquid first exits reactor (s)

$t_2$ = time at which liquid first enters reactor (s)

$t_3$ = time at which liquid stream exiting reactor breaks (s)

$t_4$ = time at which liquid stream stops spraying into reactor following cessation of spraying (s)



| Sample Fraction | [Vanillin] |
|---|---|
| (min) | wt. % of Lignin |
| Rxn Mixture | 0.24 ± 0.02 |
| 1:00 | 1.45 ± 0.04 |
| 2:00 | 1.55 ± 0.06 |
| 3:00 | 1.63 ± 0.05 |
| 4:00 | 1.62 ± 0.02 |
| 5:00 | 1.60 ± 0.04 |
| 6:00 | 1.59 ± 0.03 |

**Figure S1: Spray reactor temperature profiles during continuous ozonolysis of grass and hardwood lignins**. Temporal temperature profiles during the ozonolysis of G1 grass (top and bottom image) and H1 hardwood (middle image) lignins were measured with a 4-point thermocouple. In the 45 cm tall cylindrical reactor (Figure 2), the thermocouple (24 cm in length) has probes at the center of the reactor (“bottom probe”), 6 cm above that towards the gas stream inlet (“middle bottom”), a further 6 cm above that (“middle top probe”) and at the top of the reactor next to the gas stream inlet (top probe).The volume in the legends represents the total amount of sprayed solution. After 1 minute, the variation in temperature between the four probes is less than ± 0.1 $^{o}$C. As shown in the adjoining table above, temporal vanillin yields [defined as mass of vanillin produced (estimated from GC-FID data)/mass of lignin fed*100] show that the vanillin yield reaches a steady state after approximately 2 minutes.

## Calc S2: Comparison of aldehyde production rates from CSTR and spray reactor

Assuming (i) constant density ($\rho$) of the reaction mixture which is dominated by acetic acid (99 wt.%) (ii) negligible concentration of aldehydes in the feedstock, and (iii) fixed aldehydes yield ($Y$) from both CSTR and spray reactor, the production rate ($P$ mol/min) of aldehydes can be calculated as follows:

$$P\left(\frac{mol}{\mathrm{min}}\right) = C\left(\frac{g_{lignin}}{g_{solution}}\right)\rho\left(\frac{g_{solution}}{mL_{solution}}\right)Q\left(\frac{mL_{solution}}{min}\right)Y\left(\frac{g_{aldehydes}}{g_{lignin}}\right)M\left(\frac{mol_{aldehydes}}{g_{aldehydes}}\right)$$

The range of values used in the CSTR and spray experiments are provided in the Table below.

| Symbol | Value | Measure | Description |
|---|---|---|---|
| C | 1.0 | wt. % (g/g) | Feed concentration |
| $\rho$ | 1.05 | g/mL | Density of acetic acid |
| Q | 0.71-14.2<br>50 – 125 | mL/min | Range of flow rates for CSTR[7]<br>Range of flow rates for spray reactor |
| Y | 0.05 | g/g | Aldehyde yields |
| M | 129.6 | g/mol | Average molar mass of a 3:1 w:w 4-hydroxybenzaldehyde (122.1 g/mol) and vanillin (152.1 g/mol) mixture |

For the range of flow rates ($Q$) shown in the table above, the calculated production rates ($P$) for CSTR and spray reactor are shown in the following table. Clearly, the production rates in the spray reactor are between one to two orders of magnitude greater than in a CSTR.

| Reactor | ***P*** **(mol/min) corresponding to lowest flow rate used in the reactor** | ***P*** **(mol/min) corresponding to highest flow rate used in the reactor** |
|---|---|---|
| CSTR | 2.89 ($10^{-6}$) | 5.77 ($10^{-5}$) |
| Spray | 2.03 ($10^{-4}$) | 5.08 ($10^{-4}$) |

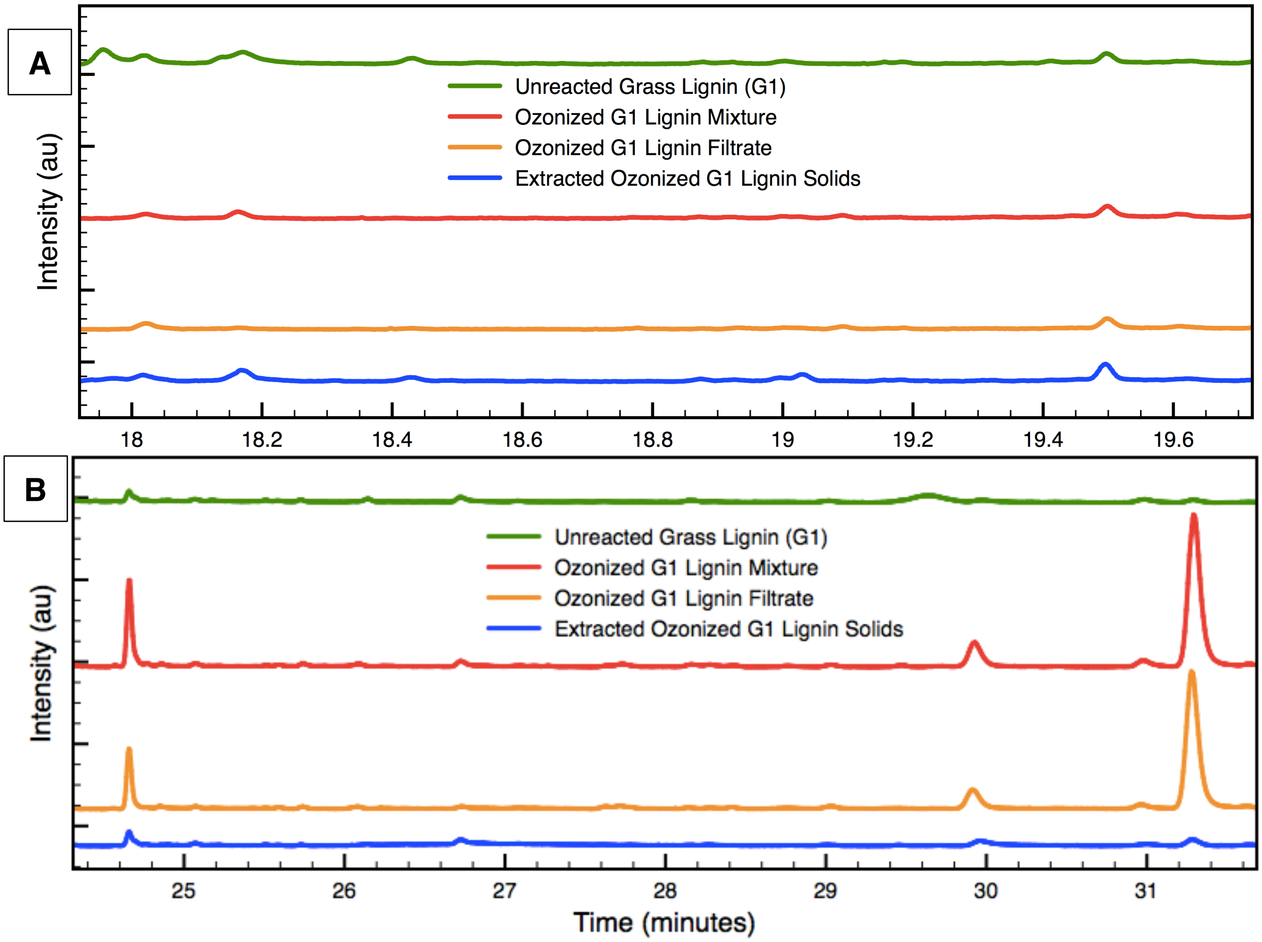


**Figure S2: GC/FID chromatograms of lignin solutions before ozonolysis and after ozonolysis following product fractionation.** Figure (A) shows byproducts vanillic acid (18.17 minutes) and 4-hydroxybenzoic acid (19.5 minutes) in low concentrations, while Figure (B) shows vanillin (24.6 minutes) and 4-hydroxybenzaldehyde (31.3 minutes) as major products. Reaction run at 20°C with 2.5 mol. % ozone in air flowing at 180 std L/h and 1.0 wt. % lignin feed solution in acetic acid flowing at 75 mL/min.

The quantitative aldehyde and acid yields obtained from the GC/FID chromatograms above are shown in the following table.

**Table S2: Aldehyde and acid yields from G1 lignin before ozonolysis and after ozonolysis following fractionation.**

| Component | Vanillic Acid wt. % | 4HBA wt. % | Vanillin wt. % | 4HB wt. % | Total Aromatics wt. % |
|---|---|---|---|---|---|
| Unreacted Lignin | 0.16 ± 0.01 | 0.07 ± 0.02 | 0.16 ± 0.04 | 0.06 ± 0.02 | 0.45 ± 0.05 |
| Ozonized Lignin | 0.07 ± 0.02 | 0.10 ± 0.05 | 1.1 ± 0.1 | 4.0 ± 0.1 | 5.2 ± 0.1 |
| LMW Extract | 0.03 ± 0.01 | 0.06 ± 0.05 | 0.73 ± 0.02 | 3.9 ± 0.1 | 4.7 ± 0.1 |
| HWM Solids | 0.10 ± 0.05 | 0.09 ± 0.02 | 0.21 ± 0.03 | 0.12 ± 0.02 | 0.57 ± 0.06 |

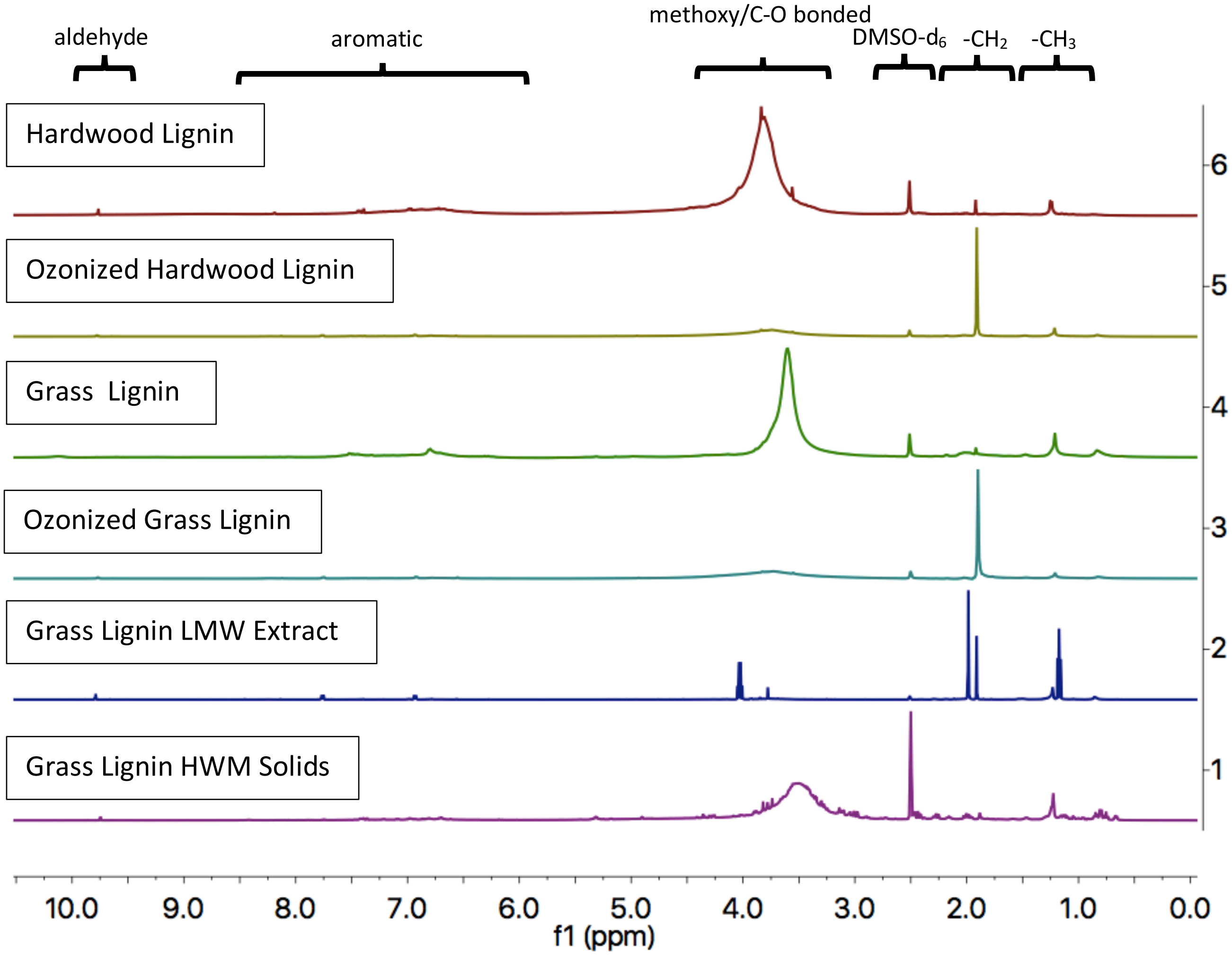


**Figure S3: Full $^1$H-NMR of Lignins before ozonolysis and after ozonolysis following product workup (Experimental conditions are identical to those shown in Figure S2 caption).**

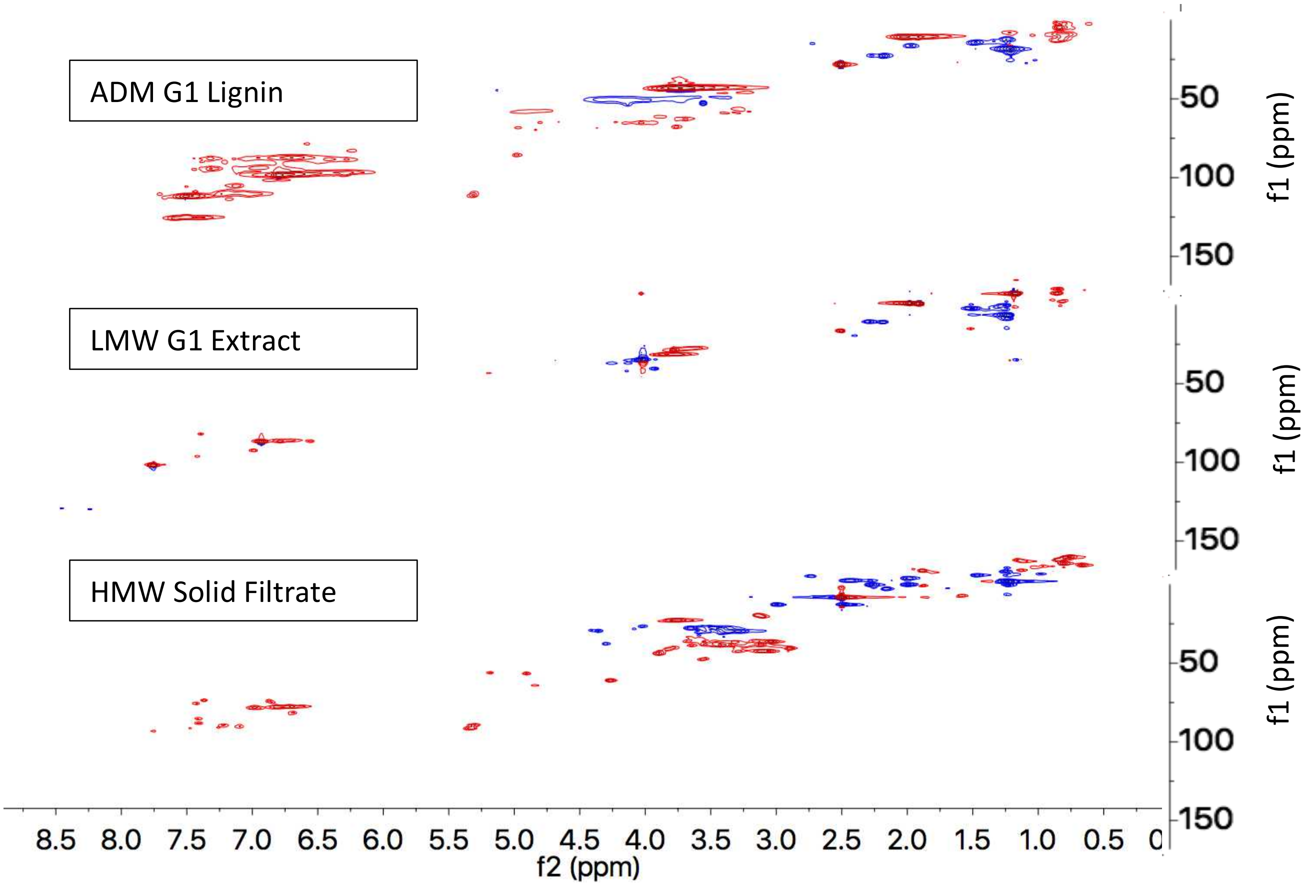


**Figure S4: $^1$H (f2) and $^{13}$C (f1) 2D-HSQC NMR analyses of grass lignin (G1) before ozonolysis and after ozonolysis following product workup corresponding to samples shown in Figure S3.**

**Table S3: Effect of ozone concentration on the yields of low molecular weight (LMW) and high molecular weight (HMW) fractions along with mass balance closure (Experimental conditions are identical to those shown in Figure S2 caption).**

| [$O_3$] in Air (Mol. %) | LMW Extract (wt. %) | HMW Solids Fraction (wt. %) | Mass Balance Closure (%) |
|---|---|---|---|
| 2.5 | 37 ± 1 | 59 ± 1 | 96 ± 1 |
| 5.0 | 45 ± 1 | 48 ± 1 | 93 ± 1 |
| 7.5 | 53 ± 1 | 38 ± 1 | 91 ± 1 |

Here, low molecular weight (LMW) fraction refers to the extracted fraction with peaks between ~150 Da and 22 kDa.

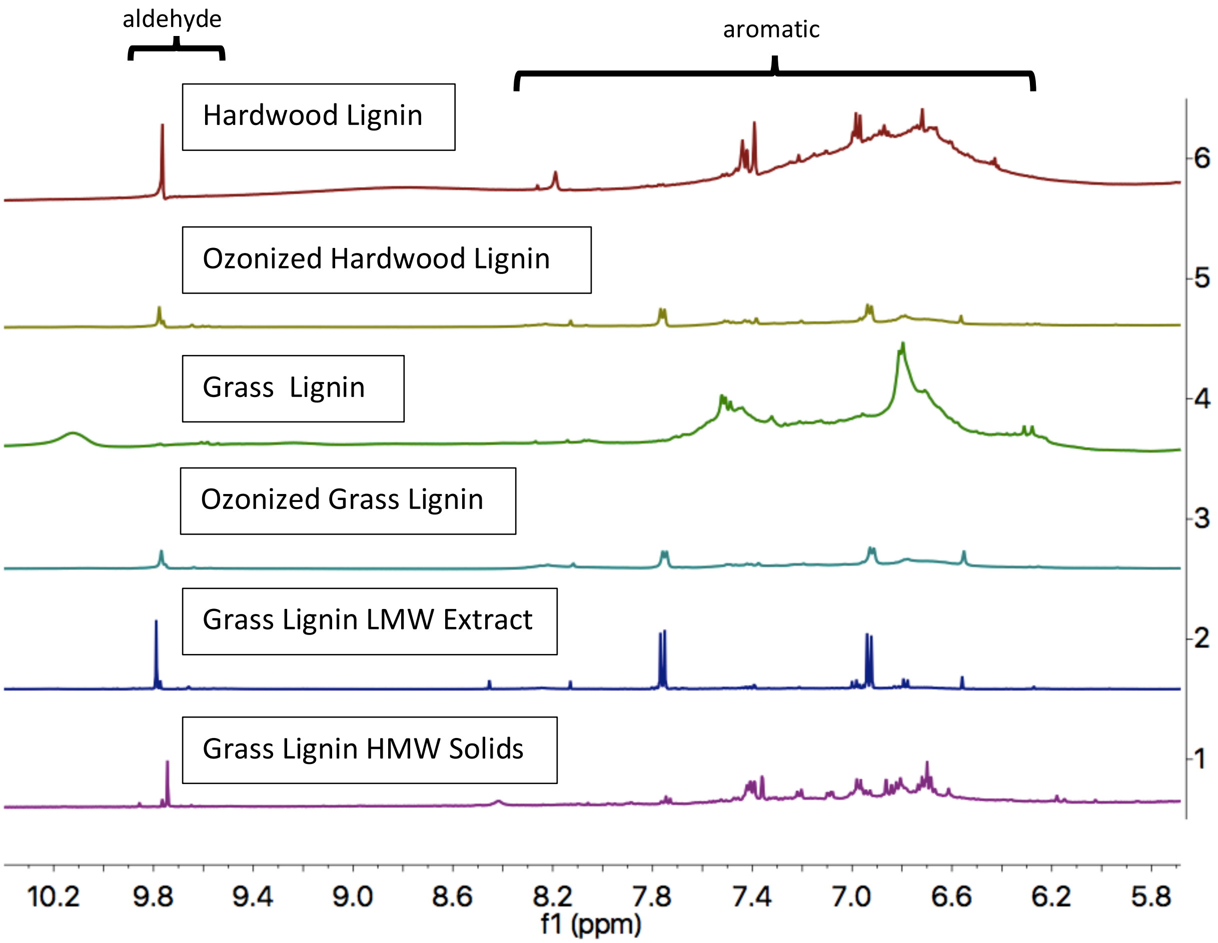


**Figure S5: Closeup $^1$H-NMR spectra of aromatic and aldehyde regions of lignin and product fractions shown in Figure S3.**

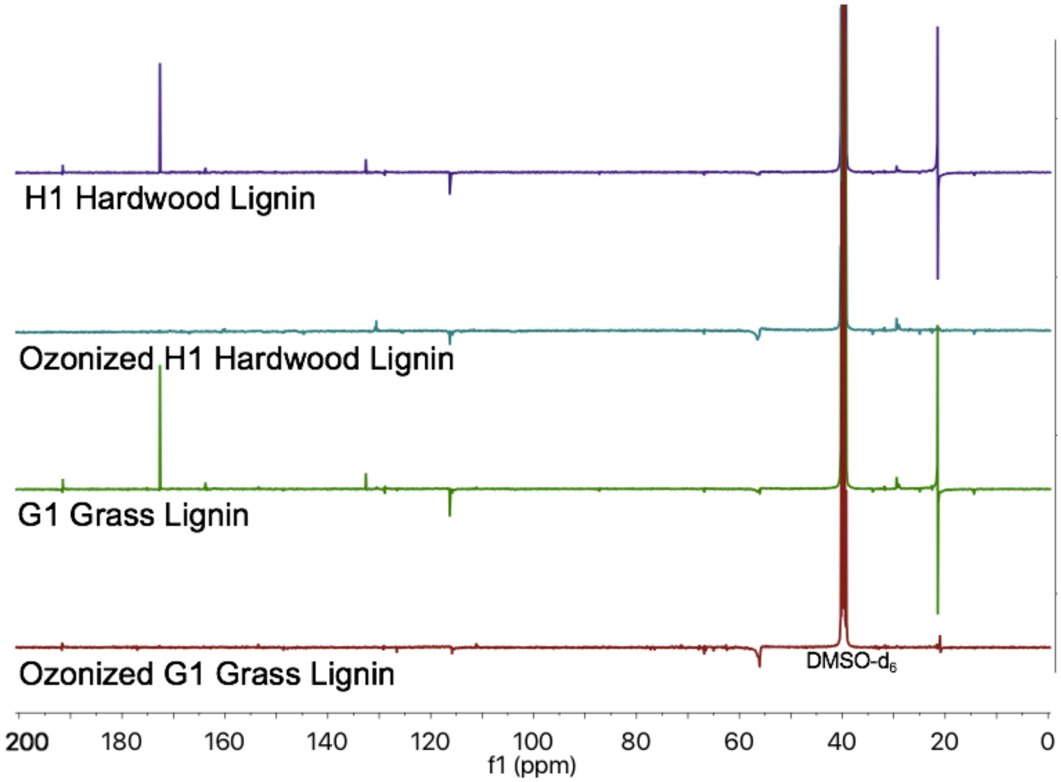


**Figure S6: DEPT $^{13}$C-NMR of lignins before ozonolysis and after ozonolysis following product workup (Experimental conditions are identical to those shown in Figure S2 caption).**

### SI Calc 3: Potential value of the aromatic aldehydes relative to lignocellulosic ethanol

It is assumed that the lignin content generated by one dry metric ton of corn stover is 18.5%.[4] Based on our experimental findings, the aromatic aldehydes (vanillin and 4-hydroxybenzaldehyde) recovered from the pendant aromatic groups constitute approximately 5 wt. % of the lignin content. Further, the ethanol production per dry metric ton of corn stover is reported to range from 47 to 76 gallons.[5] If one assumes that the average cost of the aromatic aldehydes is 12 US $/kg,[6] then the potential additional value created by these products per gallon of ethanol produced is estimated as follows:

$$\frac{5\,kg\ aldehydes}{100\,kg\ lignin} \times \frac{185\,kg\ lignin}{1000\,kg\ corn\ stover} \times \frac{1000\,kg\ corn\ stover}{76\,gal.ethanol} \times \frac{12\,US\,\$}{1\,kg\ aldehyde} = \frac{2.56\,US\,\$}{gal\ ethanol}$$

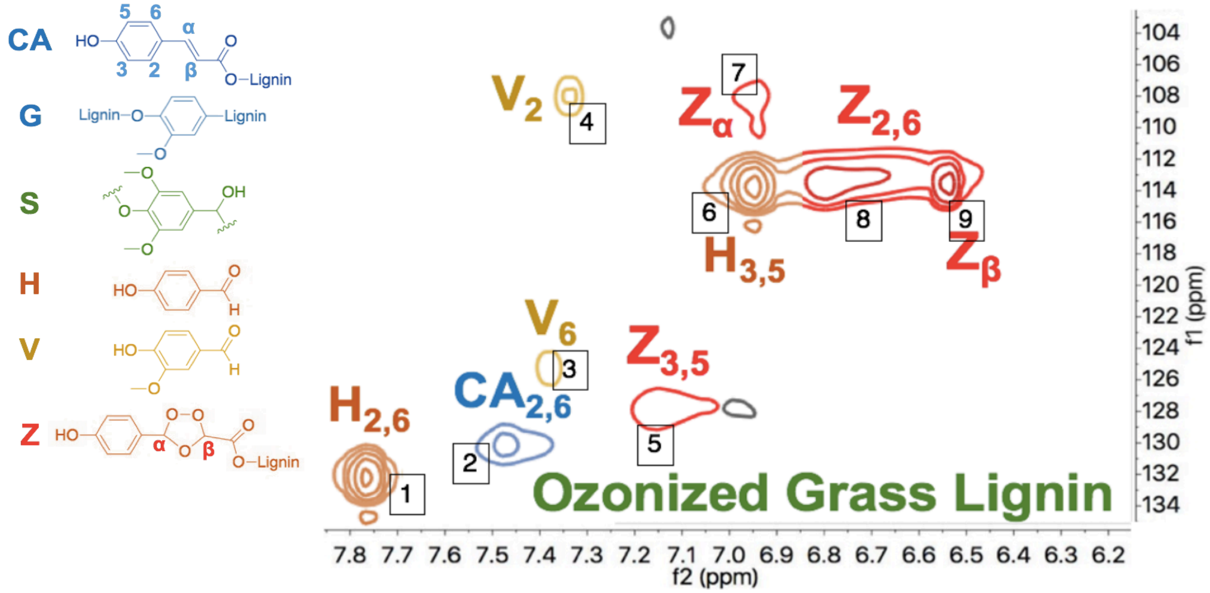


**Figure S7: Labeled $^{1}H$ (f2) and $^{13}C$ (f1) HSQC NMR peaks of ozonized grass lignin (G1) from Figure S4. Relevant parameters are given in Table S4 below.**

**Table S4: Integration parameters and data for Figure S7 from 2D NMR of ozonized grass lignin**

| Peak # | f2 ($^{1}H$) ppm | f1 ($^{13}C$) ppm | Width | Height | Normalized Peak Intensities | Absolute Peak Intensities |
|---|---|---|---|---|---|---|
| 1 | 7.75 | 132.48 | 68.36 | 462.28 | 2.00 | 729.65 |
| 2 | 7.51 | 130.43 | 58.59 | 317.82 | 0.38 | 139.47 |
| 3 | 7.42 | 126.43 | 19.53 | 231.14 | 0.09 | 31.19 |
| 4 | 7.38 | 111.04 | 19.53 | 288.92 | 0.12 | 44.76 |
| 5 | 7.14 | 128.84 | 115.87 | 288.92 | 0.51 | 185.17 |
| 6 | 6.95 | 116.06 | 78.12 | 491.17 | 2.56 | 933.45 |
| 7 | 6.94 | 111.86 | 53.18 | 490.87 | 0.34 | 123.75 |
| 8 | 6.74 | 115.75 | 126.95 | 462.28 | 2.49 | 908.38 |
| 9 | 6.56 | 115.75 | 58.59 | 462.28 | 1.21 | 439.76 |

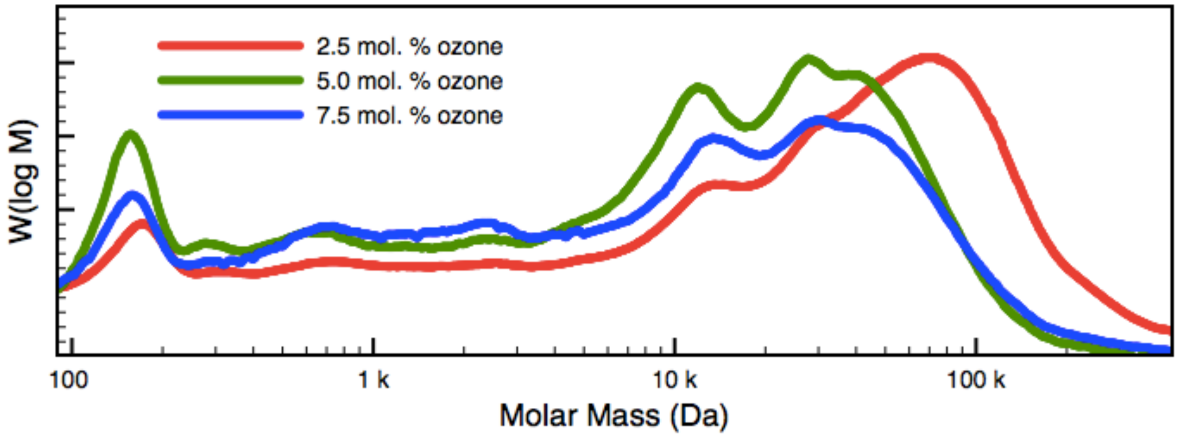


**Figure S8: Effect of ozone concentration on molecular weight distribution of product mixture obtained from G1 lignin (Other experimental conditions are identical to those shown in Figure S2 caption).**

**Table S5: Effect of ozone concentration on aromatic yields from G1 Lignin (Other experimental conditions are identical to those shown in Figure S2 caption)**

| Mol. % $O_3$ | Vanillic Acid | 4HBA | Vanillin | 4HB | Total |
|---|---|---|---|---|---|
| 2.5 | 0.13 ± 0.02 | 0.15 ± 0.02 | 0.73 ± 0.05 | 2.7 ± 0.1 | 3.7 ± 0.1 |
| 5.0 | 0.12 ± 0.04 | 0.18 ± 0.03 | 1.1 ± 0.1 | 4.4 ± 0.1 | 5.7 ± 0.1 |
| 7.5 | 0.12 ± 0.03 | 0.21 ± 0.06 | 0.80 ± 0.02 | 3.7 ± 0.1 | 4.9 ± 0.1 |

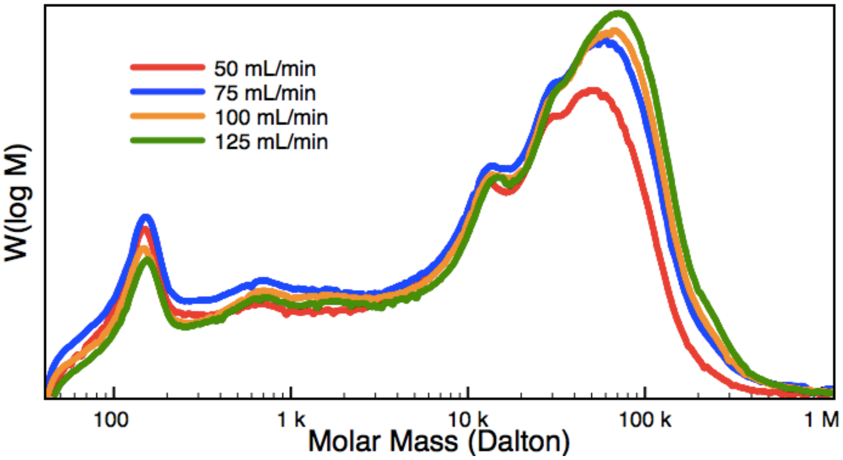


**Figure S9: Effect of feed flow rate on GPC profiles of product mixtures from G1 lignin following ozonolysis (Other experimental conditions are identical to those shown in Figure S2 caption).**

**Table S6: Effect of feed flow rate on aromatic product yields (wt.%) for G1 lignin following ozonolysis (Other experimental conditions are identical to those shown in Figure S2 caption).**

| **Flow Rate (mL/min)** | **[Vanillic Acid] (wt. %)** | **[4HBA] (wt. %)** | **[Vanillin] (wt. %)** | **[4HB] (wt. %)** | **Total (wt. %)** | **Residence Time* seconds** |
|---|---|---|---|---|---|---|
| 50 | 0.10 ± 0.03 | 0.16 ± 0.02 | 0.83 ± 0.03 | 4.56 ± 0.1 | 5.7 ± 0.1 | 9.2 ± 0.2 |
| 75 | 0.11 ± 0.02 | 0.15 ± 0.04 | 0.83 ± 0.01 | 4.40 ± 0.2 | 5.5 ± 0.1 | 8.3 ± 0.3 |
| 100 | 0.11 ± 0.06 | 0.14 ± 0.03 | 0.80 ± 0.01 | 4.15 ± 0.1 | 5.2 ± 0.1 | 7.9 ± 0.2 |
| 125 | 0.11 ± 0.04 | 0.13 ± 0.04 | 0.77 ± 0.03 | 3.80 ± 0.1 | 4.8 ± 0.1 | 7.6 ± 0.4 |

*see Equation S1 for definition

**Table S7: Macromolecular changes in molar mass from GPC spectra**

| **A** | **G1 Peaks (Da)** | | **Δ mass** | **G2 Peaks (Da)** | | **Δ mass** | **H1 Peaks (Da)** | | **Δ mass** | **H2 Peaks (Da)** | | **Δ mass** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Peak #** | **Unreacted** | **Oz.** | **(%)** | **Unreacted** | **Oz.** | **(%)** | **Unreacted** | **Oz.** | **(%)** | **Unreacted** | **Oz.** | **(%)** |
| **1** | 13500 | 12300 | -8.9 | 13200 | 10200 | -23 | 10900 | 10500 | -3.7 | 12300 | 10800 | -12 |
| **2** | 28800 | 27200 | -5.6 | 26200 | 23500 | -10 | 26250 | 25100 | -4.4 | 29250 | 24900 | -15 |
| **3** | 46500 | 41600 | -10.5 | 41400 | 34800 | -16 | 38600 | 36600 | -5.2 | 42800 | 37300 | -13 |
| **4** | | | | | | | 89500 | 60500 | -32 | 96400 | 67000 | -30 |
| **5** | | | | | | | 180000 | 175000 | -2.8 | 183000 | 169000 | -7.7 |

$$\frac{mass_{unreacted} - mass_{ozonized}}{mass_{unreacted}} * 100 = \Delta\, mass\ (\%) \tag{d}$$

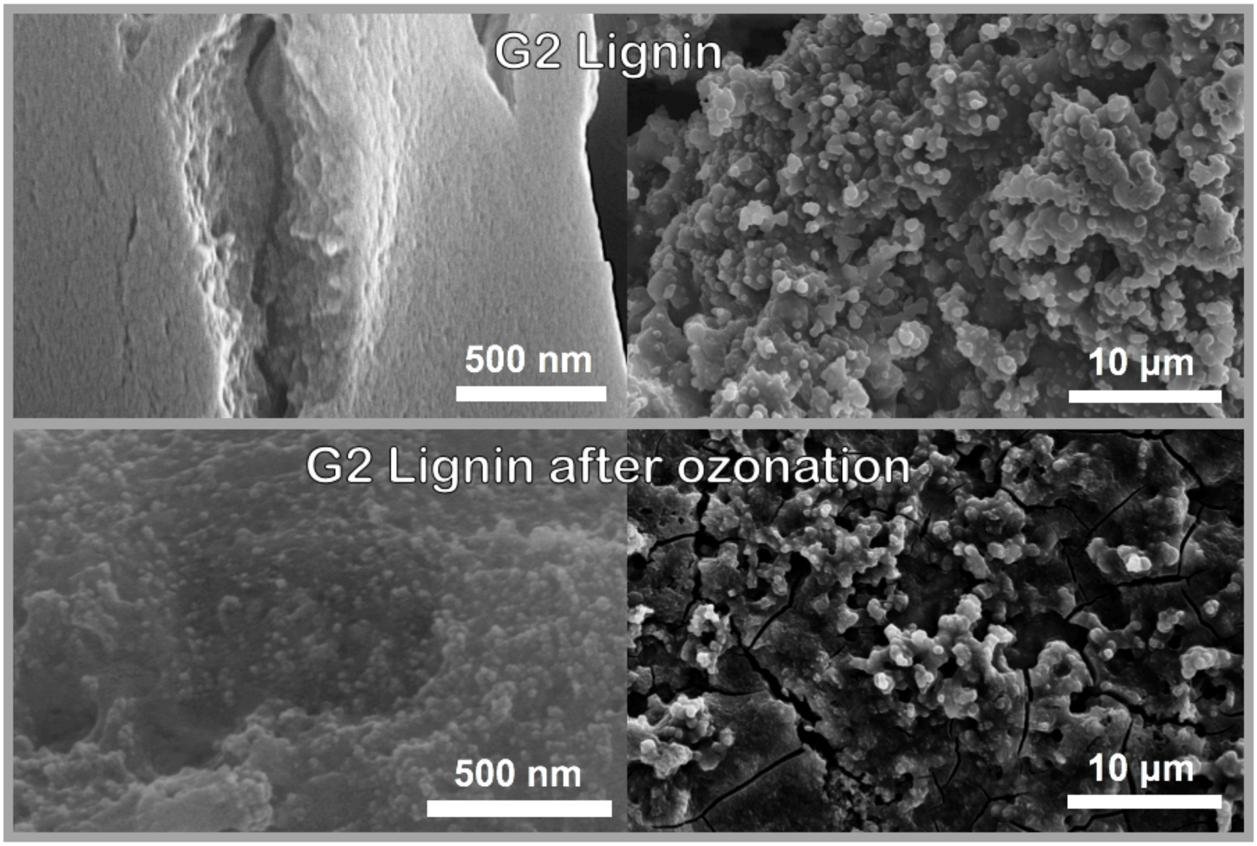


**Figure S10: Scanning Electron Micrographs of 1.0 wt. % G2 grass lignin before (top) and after (bottom) ozonolysis at the nanometer (left) and micron (right) scale highlighting the globular modular components of lignin. Experimental conditions are identical to those in Figure S2 caption.**

## SI Methods

### *Gas Chromatography*

The starting materials and recovered product solutions were analyzed by GC-FID by diluting 300 µL of recovered product solution with 1 mL of methanol. Purchased vanillin, 4-hydroxybenzaldehyde, vanillic acid, and 4-hydroxybenzoic acid were used as standards for the chromatographic analysis. The GC method used an HP-INNOWAX column on an Agilent 7890A GC and uses a carrier gas (He) flow of 1 std. $cm^3$/min, an inlet temperature of 250 °C, and an injection volume of 1 µL. The oven temperature was initially held at 40 °C for 5 min, then ramped at 10 °C per minute to 220 °C and held at this temperature for a further 20 min. Masses were scanned from 20 to 500 Da. Products were identified by comparison of retention times with known compounds and from their mass spectrometry fragmentation patterns. Quantification was performed using GC-FID analysis with calibration curves generated from known samples (0.01 – 5.0 wt. % solutions) employing previously reported methods.[7] After subtracting the solvent blank, the areas of the GC-FID peaks (at ~24.7 and ~31.5 minutes for aldehydes: vanillin and 4HB respectively, and ~18.1 and 19.5 minutes for vanillic and 4-hydroxybenzoic acid respectively) were integrated in Plot2, and converted to mass fractions based on calibration curves.

### *Size Exclusion Chromatography Gel Permeation Chromatography*

For GPC analysis, the solvent is evaporated from samples and replaced with dimethylformamide (1.0 mL). GPC was performed on an Agilent 1260 Infiniti GPC system fitted with an Agilent refractive index detector. Two columns, a 300 mm Polargel-M followed by a 300 mm Polargel-L, were used in series at 40 °C. The samples were eluted with dimethylformamide at a flow rate of 1.0 mL/min. Poly(methyl methacrylate) standards were used for calibration. Gel permeation chromatograms are calibrated against a polymethylmethacrylate standard before analysis in Plot2 for peak locations.

### *$^{1}$H-$^{13}$C HSQC Nuclear Magnetic Resonance Spectroscopy*

NMR spectra were recorded using a Bruker AVIII 500 MHz spectrometer with a multinuclear BFFO cryoprobe. Approximately 100 mg of lignin, ozonized lignin product, or model compound was dissolved in 0.7 mL dimethylsulfoxide-d$_6$. For the $^{13}$C NMR analysis, a power-gated decoupling sequence was used to screen out the Nuclear Overhauser Effect (NOE) with the following parameters: 150 ms relaxation delay, 64 K data points, and 1024 scans. 2D $^{1}$H-$^{13}$C HSQC NMR spectra were obtained using the hsqcedetgpsisp2.2 HSQC program. The central solvent peak was used as an internal chemical shift reference point (δC/δH 39.5/2.49). The spectral widths were 20 ppm and 234 ppm for $^{1}$H and $^{13}$C dimensions, respectively, along with the following parameters: 2 second relaxation delay, 8 scans, and 256 time increments were recorded in the $^{13}$C dimension. NMR spectra (.fid/.ser files) were analyzed in MestreNova following automated integration of 1D and 2D spectra.

### *Scanning Electron Microscopy*

Lignin and reaction products were dried directly placed on the head of an SEM pin. The samples were sputter coated with 5 nm of gold before being imaged with a FEI Versa 3D Dual Beam machine, at a voltage of 5 kV and a spot size of 4.5. Images were processed in ImageJ to determine average particle sizes.

Supplemental References:

(1) Davis, P. B.; Jones, H. C. The Viscosities of Binary Mixtures of the Associated Liquids, Water, Formic Acid and Acetic Acid. *J. Am. Chem. Soc.* **1915**, *37* (5), 1194–1198.

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(4) Stephen, J. D.; Mabee, W. E.; Saddler, J. N., Will Second-Generation Ethanol Be Able to Compete with First-Generation Ethanol? Opportunities for Cost Reduction. *Biofuels, Bioprod. Biorefin.* **2012,** *6* (2), 159-176.

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(7) Danby, A. M.; Lundin, M. D.; Subramaniam, B. Valorization of Grass Lignins: Swift and Selective Recovery of Pendant Aromatic Groups with Ozone. *ACS Sustain. Chem. Eng.* **2018**, *6* (1), 71–76.