

Supporting Information

For

Total Utilization of Miscanthus Biomass, Lignin and Carbohydrates, Using Earth Abundant Nickel Catalyst

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Instrumentation and Chromatographic Conditions

HPLC-MS analysis

All HPLC separations for mass spectrometry analysis were performed on a Surveyor Plus HPLC system from Thermo Scientific consisting of a quaternary pump, an autosampler, a photodiode array (PDA) detector, and a Zorbax SB-C18 column. A non-linear gradient of water (A) and acetonitrile (B) was used as follows: 0.00 min, 95% A and 5% B; 10.00 min, 95% A and 5% B; 30.00 min, 40% A and 60% B; 35.00 min, 5% A and 95 %; 38.00min, 5% A and 95% B; 38.50 min, 95% A and 5% B; 45.00 min, 95% A and 5% B. Flowrate of the mobile phase was kept at 500 μ L/min. PDA detector was set at the wavelength of 254 nm.

Mass spectrometric analysis (MS, MS^2 and MS^3) of HPLC eluent was performed using a Thermo Scientific linear quadrupole ion trap (LQIT) mass spectrometer equipped with an electrospray ionization (ESI) source. All mass spectrometry experiments were performed under negative ion mode. HPLC eluents were mixed via a T-connector with 1% sodium hydroxide water solution at a flow rate of 0.1 μ L/min before entering the ESI source. Addition of sodium hydroxide facilitates deprotonation of the analytes. ESI source conditions were set as: 3.5 kV spray voltage; 50 (arbitrary units) sheath gas (N_2) flow and 20 (arbitrary units) auxillary gas (N_2) flow.

MS^3 analysis was performed using the data dependent scan function of the Thermo Xcalibur software. The most abundant ion formed upon ESI was isolated and subjected to collision-activated dissociation (CAD). The most abundant fragment ion was further selected for isolation and fragmentation. For all MS^3 experiments, an

isolation window of 2 m/z units was used along with a normalized collision energy of 30 (arbitrary units). All mass spectrometry experiments were performed under negative ion mode.

HPLC/GC analysis

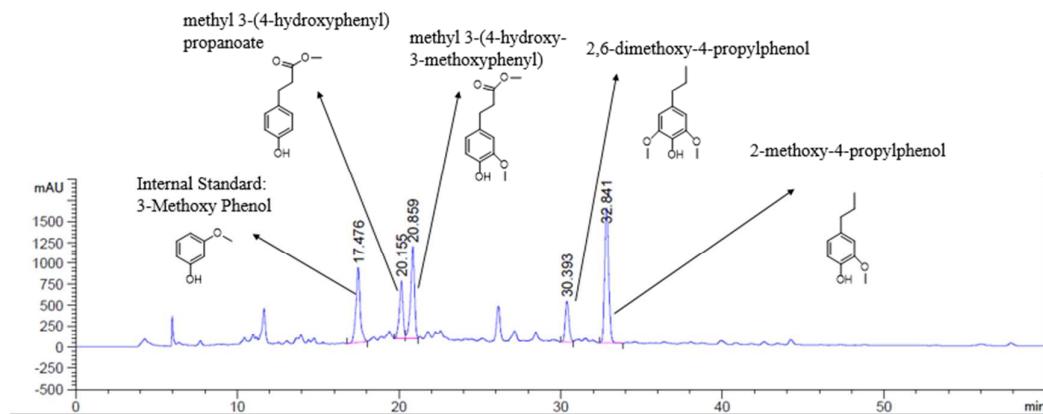
The liquid phase from lignin depolymerization reactions with methanol as solvent was analyzed with Agilent 1260 Infinity Quaternary High-Performance Liquid Chromatography (HPLC) system, using Zorbax Eclipse XDB-C18 Column (250 x 74.6mm) set at 30°C. The chromatography apparatus is equipped with G1315D Diode Array Detector (DAD). A mixture of H₂O (A) and acetonitrile (B) were used as the mobile phase at a flow rate of 0.5 mL/min. Nonlinear gradient was used (80% A and 20% B from beginning to 5% A and 95 % B at 55.0 min). A fixed amount (400 μ L) of internal standard 3-methoxy phenol (10 mM) was added into each sample for the quantification purposes. Standard curves for all the aromatic products were made by comparison of the products to internal standard. All results were analyzed and quantified according to standard curves. Before analyzing by HPLC, the liquid samples were filtered through a 0.22 μ m cutoff syringe filter (25mm diameter).

All sugar samples as well as aqueous phase after solid carbohydrate dehydration reaction were analyzed with Agilent 1260 Infinity Quaternary High-Performance Liquid Chromatography (HPLC) system, using Aminex HPX-87H column (300 x 7.8 mm) set at 70 °C. The chromatography apparatus is equipped with G1362A Refractive Index Detector (RID) calibrated with external standards. A 0.005 M sulfuric acid solution was employed as the mobile phase with flow of 0.6 mL/min. A fixed amount (400 μ L) of internal standard tert-butyl alcohol (10 mM) was added into each sample for quantification purposes. Standard curves (glucose, xylose, and arabinose) were made by comparison of the products with internal standard. All results were analyzed and quantified according to standard curves. Before analyzing by HPLC, the aqueous solutions were filtered through a 0.22 μ m cutoff syringe filter (25 mm diameter).

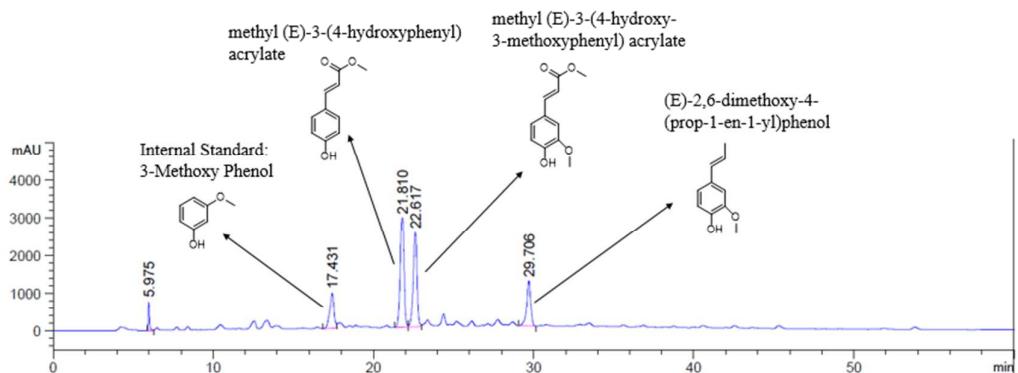
The organic phase after solid carbohydrate dehydration reaction was analyzed by Agilent Technologies 6890N Network Gas Chromatography (GC) System, equipped with a J&W Scientific DB-5 capillary column. Oven temperature was ramped from 50 °C to 250 °C at 15 °C min⁻¹, and from 250 °C to 300 °C at 25 °C min⁻¹ by using constant flow mode at 1.0 mL min⁻¹. Products (furfural and levulinic acid) were identified by retention time and compared to standards. Products were quantified by using a standard calibration curve. Before analyzing by GC, the organic solutions were filtered through a 0.22 μ m cutoff syringe filter (25 mm diameter).

Figure S1. Aromatic products after lignin depolymerizatoin reaction.

(a)



(b)



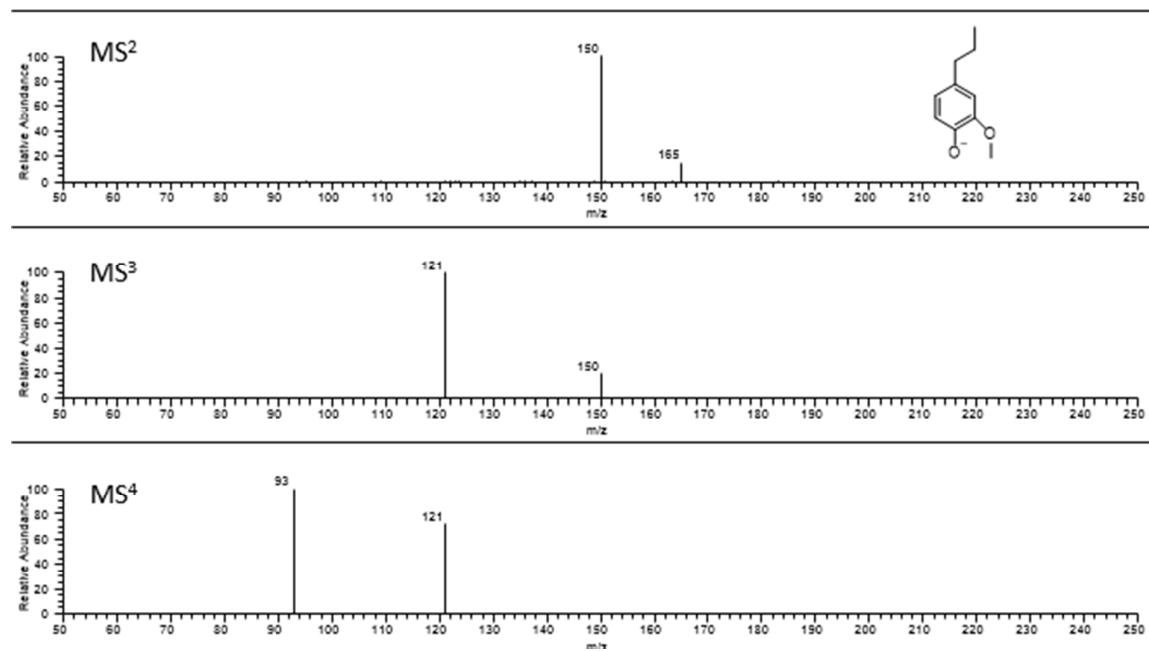
(a). HPLC/UV for single bond branch chain aromatic products from lignin depolymerization.

(b). HPLC/UV for double bond branch chain aromatic products from lignin depolymerization.

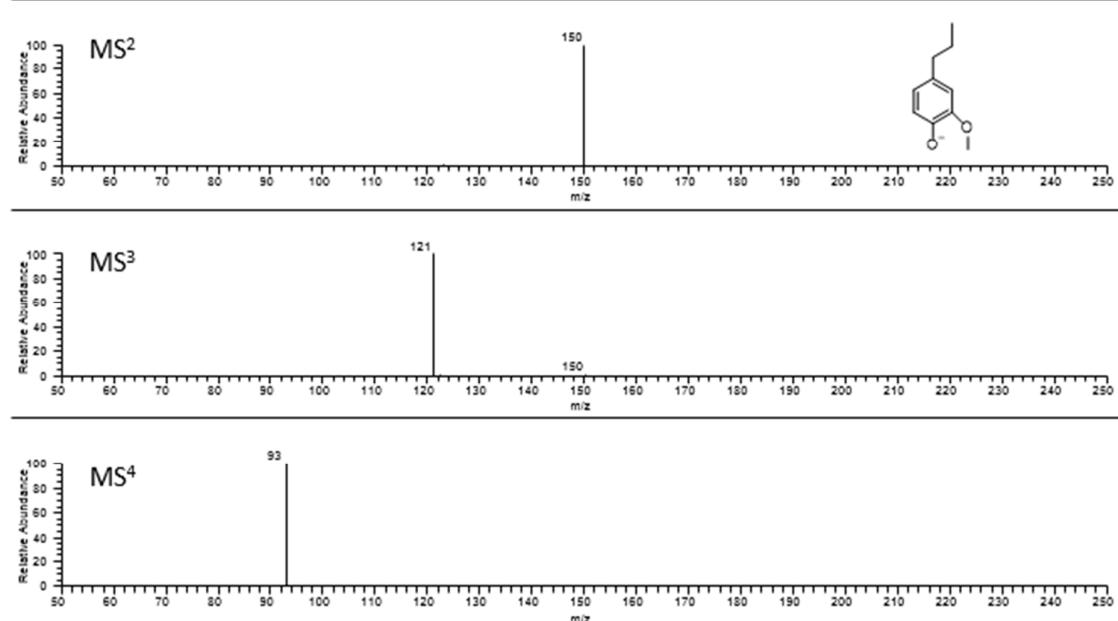
Figure S2. ESI(-)//MSⁿ analysis of standard compounds and aromatic products derived from catalytic lignin depolymerization reactions.

(a) CAD spectrum of pure 2-methoxy-4-propylphenol as well as m/z = 165 in lignin depolymerization products.

CAD spectrum of 2-methoxy-4-propylphenol (m/z=165)

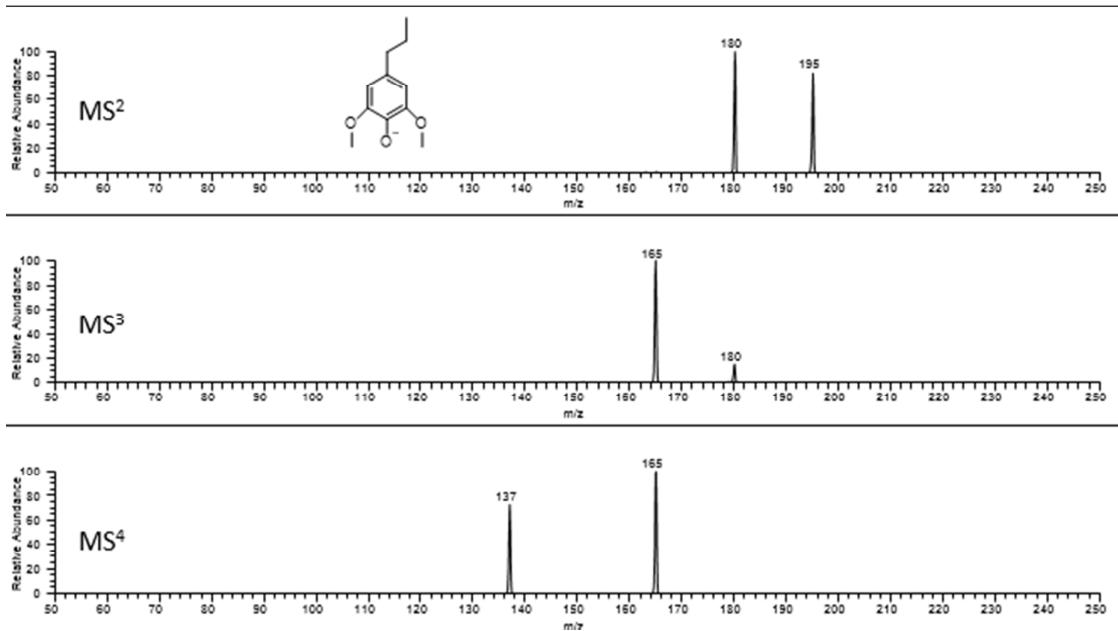


CAD spectrum of ion $m/z=165$ in lignin depolymerization products

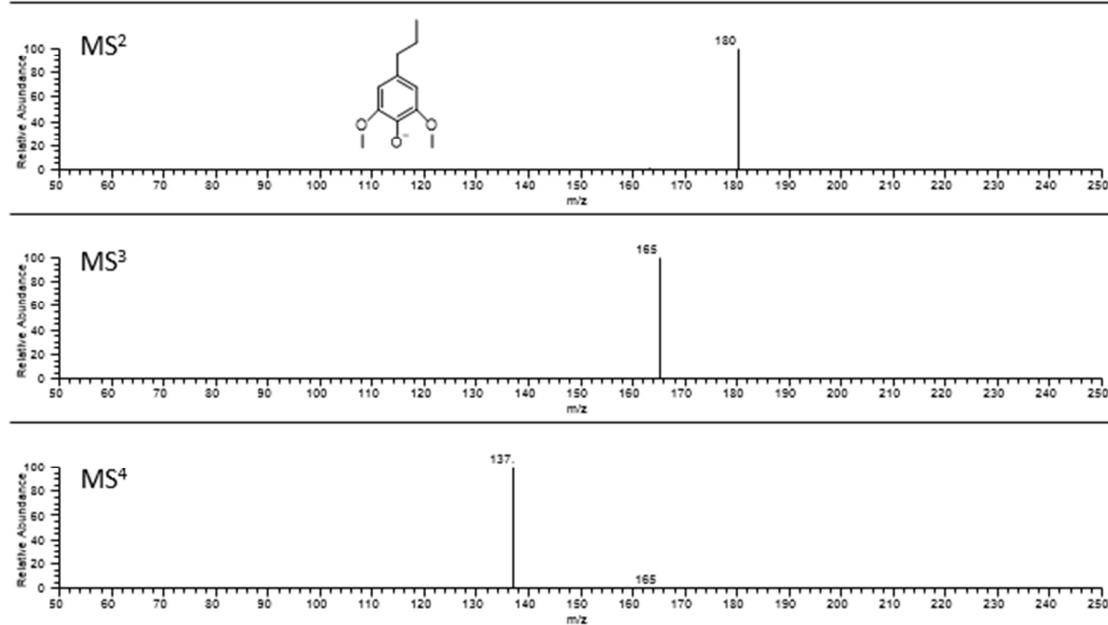


(b) CAD spectrum of pure 2,6-dimethoxy-4-propylphenol as well as $m/z = 195$ in lignin depolymerization products.

CAD spectrum of 2,6-dimethoxy-4-propylphenol ($m/z=195$)

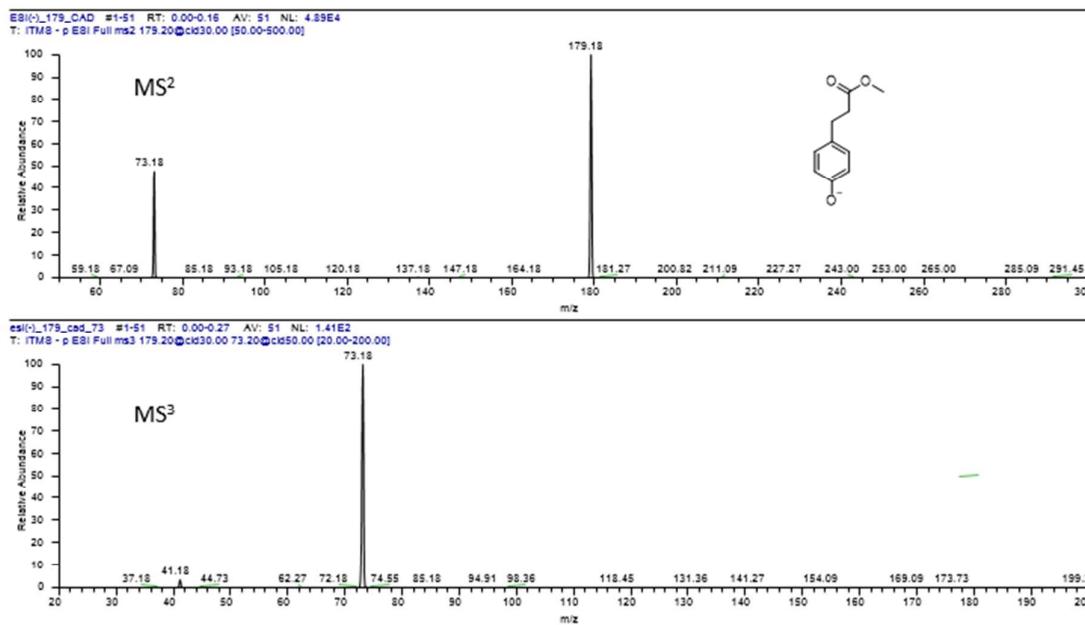


CAD spectrum of m/z=195 in lignin depolymerization products

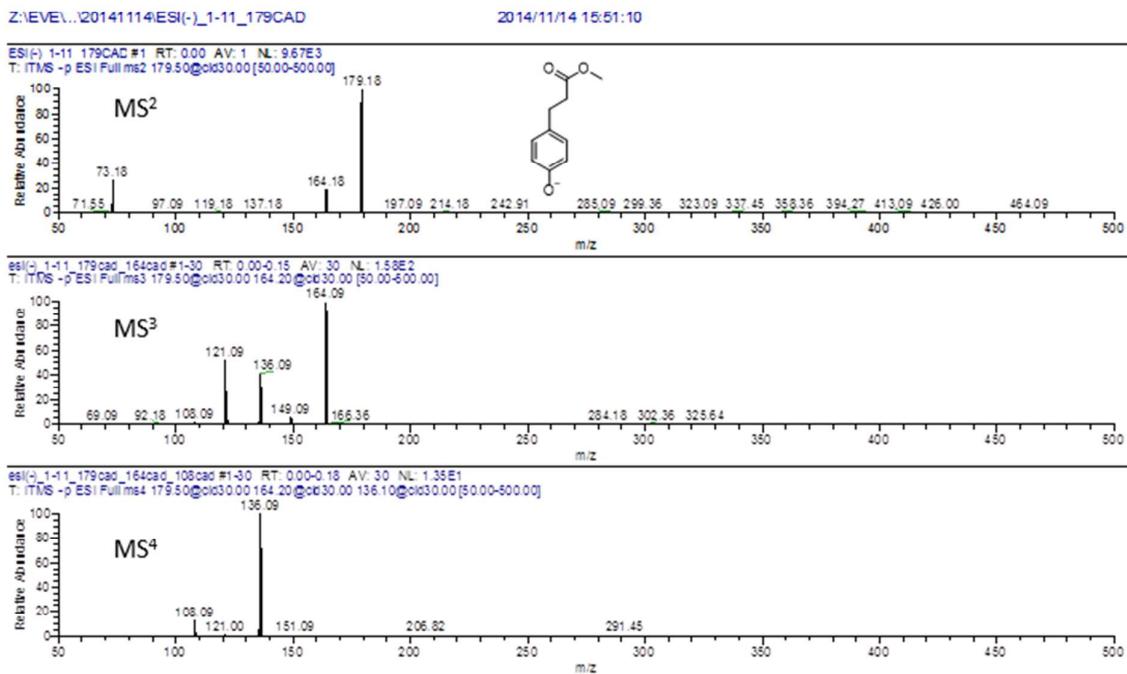


(c) CAD spectrum of pure methyl 3-(4-hydroxyphenyl) propanoate as well as m/z = 179 in lignin depolymerization products.

CAD spectrum of methyl 3-(4-hydroxyphenyl) propanoate (m/z=179)

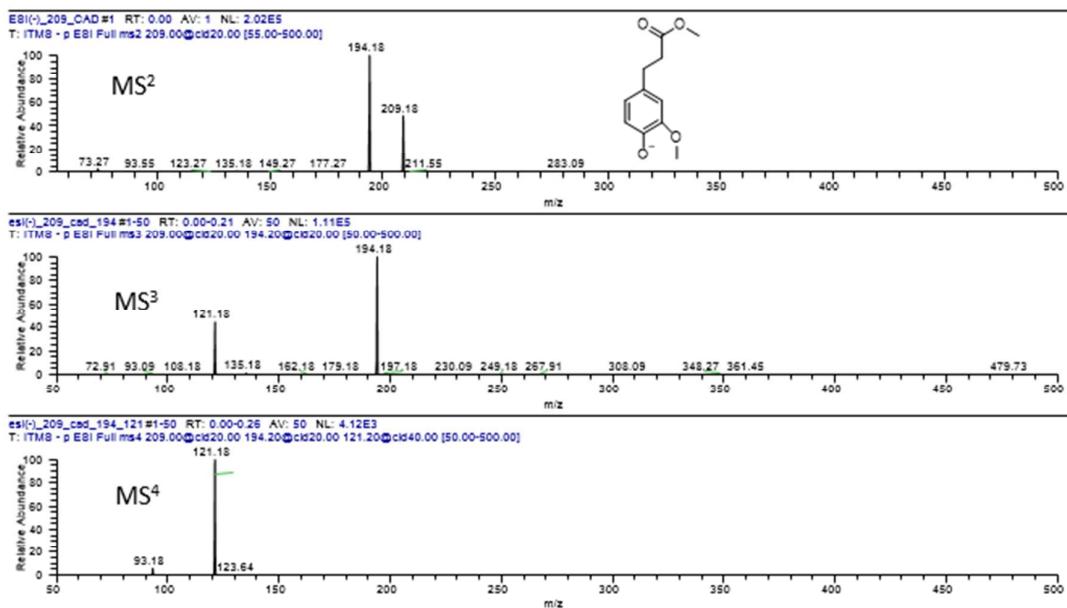


CAD spectrum of m/z=179 in lignin depolymerization products

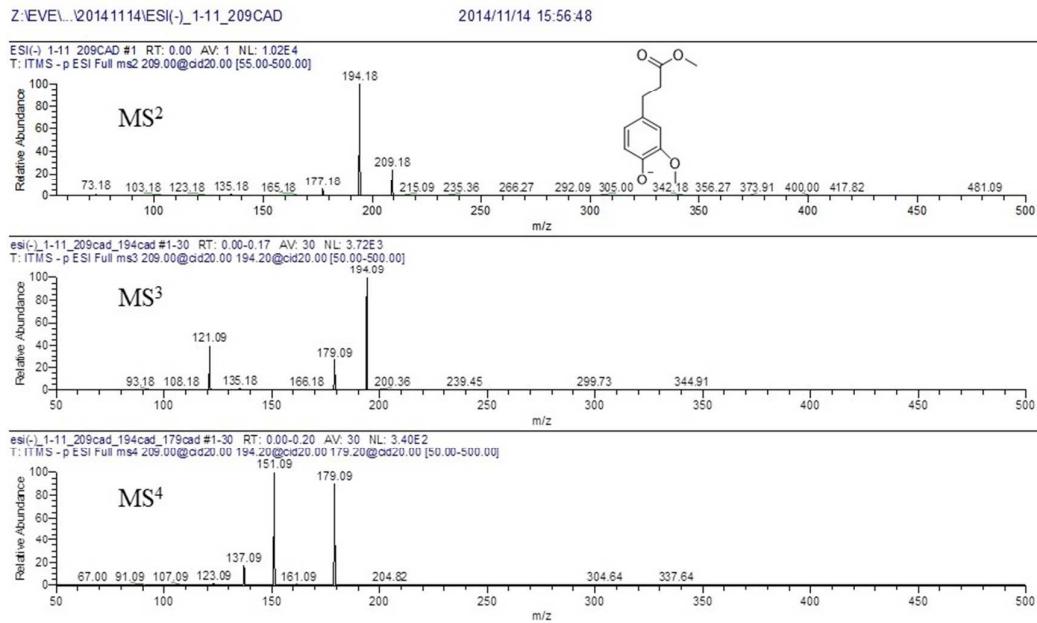


(d) CAD spectrum of pure methyl 3-(4-hydroxy-3-methoxyphenyl) as well as $m/z = 209$ in lignin depolymerization products.

CAD spectrum of methyl 3-(4-hydroxy-3-methoxyphenyl) (m/z=209)

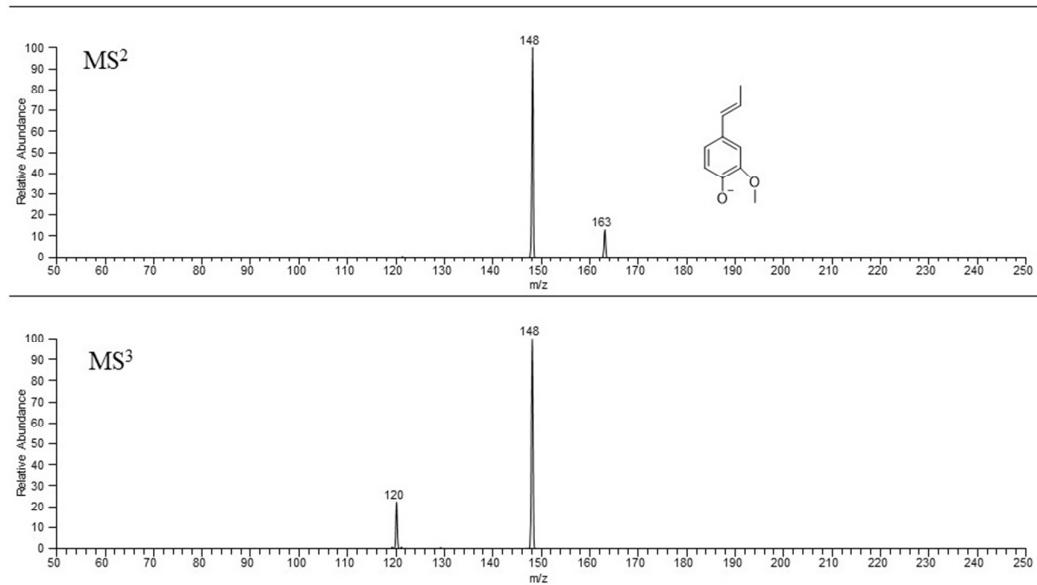


CAD spectrum of m/z=209 in lignin depolymerization products

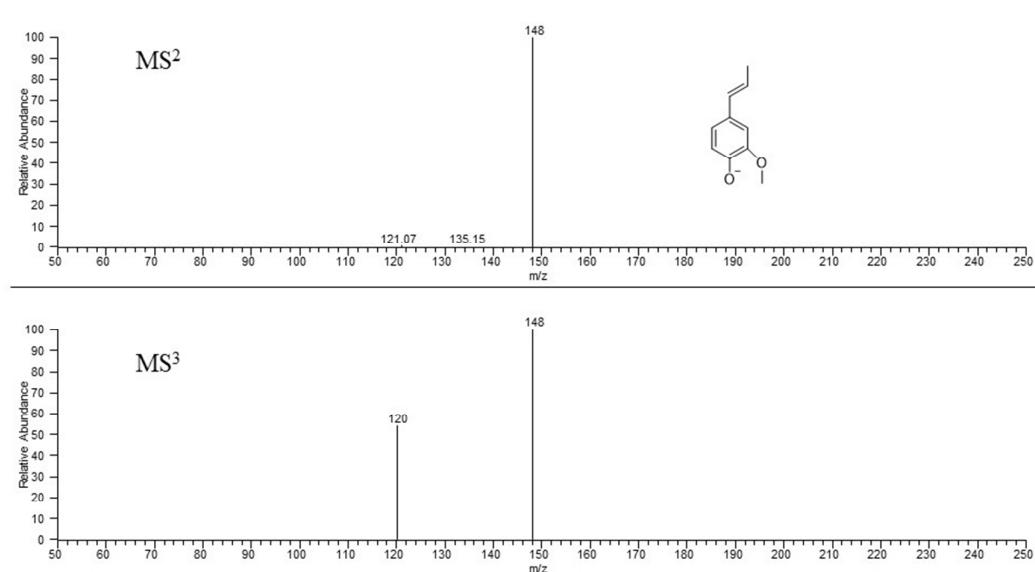


(e) CAD spectrum of pure isoeugenol as well as m/z = 163 in lignin depolymerization products.

CAD spectrum of isoeugenol (m/z=163)

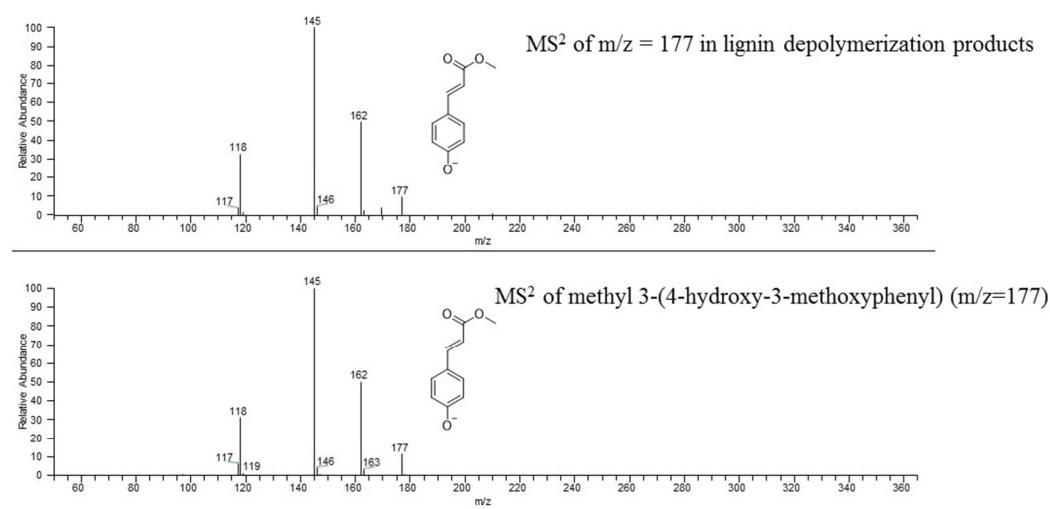


CAD spectrum of ion m/z=163 in lignin depolymerization products



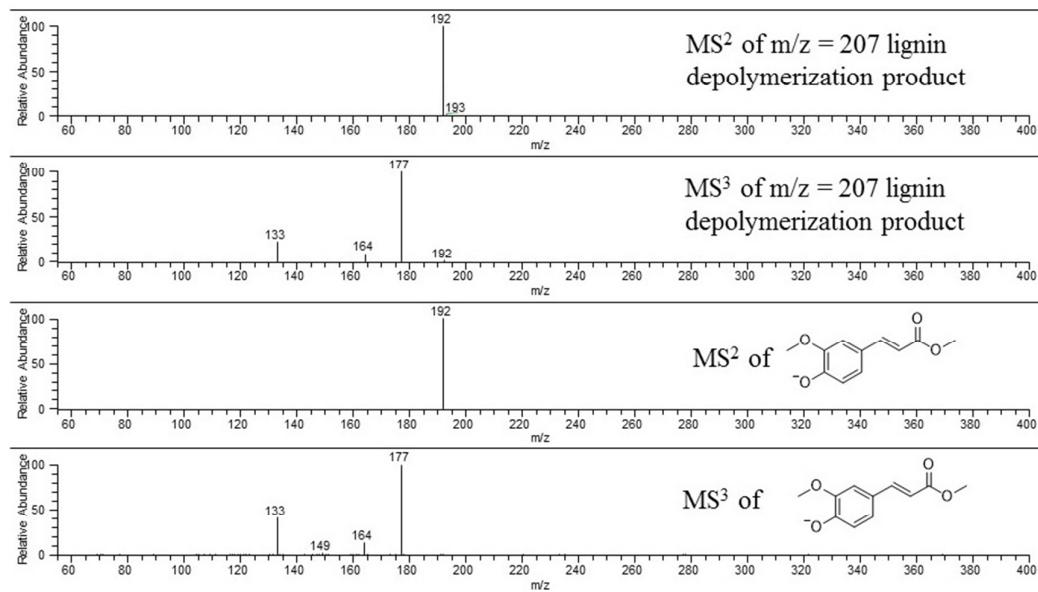
(f) CAD spectrum of pure methyl (E)-3-(4-hydroxyphenyl) acrylate as well as m/z = 177 in lignin depolymerization products.

CAD comparison of methyl 3-(4-hydroxy-3-methoxyphenyl) and lignin depolymerization product at m/z=177



(g) CAD spectrum of pure methyl (E)-3-(4-hydroxy-3-methoxyphenyl) acrylate as well as $m/z = 207$ in lignin depolymerization products.

CAD comparison of methyl (E)-3-(4-hydroxy-3-methoxyphenyl) acrylate and lignin depolymerization product at $m/z=207$



Carbohydrate determination in methanol phase

In order to determine the sugar content in the methanol phase after LDP reaction, 20 mL H_2O was first added into 10 mL methanol solution. The resulting solution was then extracted with 20 mL diethyl ether three times to remove aromatic products. The methanol- H_2O layer was collected, and methanol was removed from H_2O under reduced pressure. The H_2O layer which contains carbohydrate was subjected to acid hydrolysis by following standard NREL procedures, and the solution was analyzed by HPLC.^[1]

Table S1. Sugar content in methanol phase after lignin depolymerization (LDP) reaction

Entry	Carbohydrates in liquid phase			LDP Reaction Conditions	
	Glucan (mg)	Xylan (mg)	Arabinan (mg)	Total (mg)	
1	1	9	6	16	15wt% Ni/C in cage, 35bar H_2
2	2	11	7	20	10wt% Ni/C in cage, 35bar H_2

3	2	13	6	21	10wt% Ni/C in cage, 10bar H ₂
4	2	12	6	20	10wt% Ni/C in cage, 35bar N ₂
5	1	9	8	18	No catalyst, 35bar H ₂
6	2	11	6	19	No catalyst, 35bar N ₂

Mass balance calculation

Table S2. Reaction mass balance calculation after catalytic LDP reaction by using Ni/C as catalyst.^a

Entry ^b	Liquid Phase %			Solid Phase%		Total%	
	Ether Soluble	Water Soluble	Others%	Sugars%	Ash%		
1	9	2	20	57	2	8	98
2	8	2	14	57	1	9	91
3	4	2	20	56	1	11	94
4	3	2	18	55	1	12	91
5	3	2	23	56	1	11	96
6	3	2	21	47	2	22	97

^a All calculations are based on weight percent wt% with regard to miscanthus substrate used for the reactions.

^b LDP reaction conditions of Entries 1-6 are in accordance with Entries 1-6 of **Table 4**

Ash and others analysis

Composition analysis of ash in the acid insoluble solid was performed by following standard NREL procedure.^[2]

References for Supplementary Material:

[1]. A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter and D. Templeton, *Determination of sugars, byproducts, and degradation products in liquid fraction process samples: Issue Date, 12/08/2006*, National Renewable Energy Laboratory, 2006

[2]. A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, and D. Templeton, *Determination of Ash in Biomass: Issue Date, 07/17/2005*, National Renewable

Energy Laboratory, 2008.