Electronic Supporting Information (ESI)

Valorization of native sugarcane bagasse lignin to bio-aromatic esters/monomers via one pot oxidation-hydrogenation process

Tana Tana,[a] Zhanying Zhang,[a] Jorge Beltramini,[a,b] Huaiyong Zhu,[c] Kostya (Ken) Ostrikov, [c] John Bartley[c] and William Doherty *[a]

a. Centre for Tropical Crops and Biocommodities, Queensland University of Technology, Brisbane, QLD 4001, Australia. E-mail: w.doherty@qut.edu.au

b. Chemistry Department, Kumamoto University, Kumamoto, Japan.

c. School of Chemistry, Physics and Mechanical Engineering, Science and Engineering Faculty, Queensland University of Technology, Brisbane, QLD 4001 (Australia).

Experimental

Materials and chemicals

The raw material, sugarcane bagasse and trash (i.e. tops and leaves) were collected from Racecourse Sugar Mill (Mackay Sugar Limited) in Mackay and air-dried at 45 °C for 24 h and milled and passed through a sieve having an aperture size of 1.0 mm to allow biomass extraction and composition analysis to be conducted. Pine wood and rice straw were collected from Centenary Landscaping Supplies Pvt Ltd, Darra, Queensland. Organosolv sugarcane bagasse lignin (BOL) was prepared according to a procedure described previously (Wanmolee et al. 2018). Briefly, bagasse (10 % (w/v)) was pulped in a ternary mixture (79% (v/v)) of ethyl acetate:ethanol:water (32:25:43) with 21% (v/v) of formic acid at 164 °C for 45 min with initial pressure of 20 bars N_2. The lignin was recovered from the organic solvent by filtration, solvent evaporation and drying to constant weight at 45 °C. Commercial organosolv lignin (COL) was obtained from Chemical Point UG, Germany.

Sulfuric acid (98%), methanol, ethanol, acetone, tetrahydrofuran (THF) and diethyl ether were analytical grade and purchased from Chem Supply (AU). The chemicals used for NMR samples preparation such as acetic acid (100%) purchased from Merck & Co. (US), acetyl bromide, DMSO-d_6 purchased from Sigma-Aldrich Co. LLC (US). The enzyme Accellerate® 1500 used for enzymatic hydrolysis was purchased from DuPont Co. (US), citrate buffer and sodium azide were purchased from Sigma-Aldrich Co. LLC (US). The standards used for products quantification, such as 4-ethyl phenol, 4-ethyl guaiacol, 4-propyl guaiacol, methyl 3-(4-hydroxyphenyl) propionate and methyl 3-(3-Hydroxy-4-methoxyphenyl) propionate were chromatography grade and also purchased from Sigma-Aldrich Co. LLC (US). Palladium on activated carbon (10 wt% Pd) and ruthenium on activated carbon (5 wt% Ru loading) were obtained from Sigma-Aldrich Co. LLC (US).

The 4-propyl syringol standard was prepared as described earlier (Kumaniaev *et al.*, 2017). 12.5g K_2CO_3 was suspended in 100 mL acetone in a flask and flushed with argon. Then 7.0 g syringol and 4.3 mL allylbromide was added. The mixture was refluxed for 10 h, filtered and concentrated. Ethyl acetate and 5% of K_2CO_3 solution were added on the residue, then the organic phase was washed with brine and dried with Na_2SO_4. 2.0 g of *O*-allylsyringol obtained from last step was heated in microwave at 200 °C for 3 h to form 4-allylsyringol. 0.10 g of 4-allylsyringol was further dissolved in methanol in two-necked flask with 0.02 g of 5% Pd/C under argon flow. Then mixture was stirred under hydrogen flow for 2 h. After filtration and

concentration, the final product was purified by column chromatography ($CHCl_3$, SiO_2). 1H NMR ($CDCl_3$, 600 MHz): 0.94 (t, J = 7.3 Hz, 3H), 1.66-159 (m, 2H), 2.54-2.48 (m, 2H), 3.87 (s, 6H), 5.35 (s, 1H), 6.40 (s, 2H). The NMR data is consistent with reported in literature (Kumaniaev *et al.*, 2017).

Experimental method

General depolymerisation procedure*:* Before catalytic depolymerisation reaction, sugarcane bagasse and trash samples were further milled and passed through the 0.25 mm sieve, and dried overnight at 105 °C. Typically, lignocellulose depolymerisation experiment was carried out by mixing about 300 mg of dried lignocellulose with 10 mL methanol and 30 mg of 10 wt% Pd/C (or 60 mg of 5 wt% Ru/C with same metal loading) in a small glass beaker and stirred for 30 min. Then the reactant mixture was loaded in a 316-stainless steel tube reactor of 15 mm internal diameter (1.2 mm wall thickness) and 200 mm length with maximum volume of 25 mL, then bubbled with O_2 for 2 min and sealed. The reactor was heated to 250 °C in a fluidised sand bath (SBL-2D, Techne Inc., Burlington, NJ) for a predetermined amount of time. At the end of the reaction, the reactors were rapidly quenched in cold water to stop the reaction. The product mixture was subsequently subjected to filtration, following by washing the reactor and filter cake with methanol/ethanol several times. The filtrate and wash solution were mixed and the volume was recorded. The mixed solution was directly analysed by gas chromatography mass spectrometry (GC-MS) after filtration with a 0.45 μm syringe filter. The solid residue after filtration was vacuum dried at 45 °C for 48 h and stored for analysis.

$$Total\ oil\ yield = \left(\frac{Dried\ weight\ of\ alcohol\ fraction}{Total\ organic\ content\ in\ biomass}\right) \times 100\%$$

$$Solid\ residue\ yield = \left(\frac{Dried\ weight\ of\ solid\ residue\ -catalyst\ weight}{Initial\ weight\ of\ biomass}\right) \times 100\%$$

$$Aromatic\ products\ yield = \left(\frac{Quantified\ mass\ of\ Aromatic\ product}{Mass\ of\ lignin\ in\ biomass}\right) \times 100\%$$

Catalyst recovery and recycle*:* The recycling trial was started with larger amounts of Pd/C catalyst (100 mg) in order to quantitatively recover the catalyst. 100 mg of Pd/C, 500 mg of bagasse and 15 mL of methanol were mixed and loaded in 35 mL of stainless steel tube reactor, then heated to 250 °C for 4 h. The methanol fraction was separated from the solid residue by centrifuge at 3900 rpm for 10 min, and then analysed by GC-MS. The catalyst was separated from the solid residue by filtering through a sieve with an aperture of 53 μm size and washed with acetone. The acetone fraction containing the catalyst was collected in the flask and the

acetone was removed by using rotary evaporator. Then the catalyst was washed with acetone twice before dried in the vacuum oven at 45°C overnight.

The catalyst was regenerated by calcinating at 300 °C under air atmosphere for 5h to remove the residual biomass before the 5th recycle. Around 50 mg Pd/C was recovered after the calcination and the biomass loading for 5th cycle was adjusted according to the catalyst amount recovered (biomass: catalyst, 5:1).

Enzymatic hydrolysis: enzymatic hydrolysis was carried out in a 20 mL glass vial containing 0.1-0.2 g of bagasse or solid residues obtained at the end of the polymerisation reaction. The vial was added with an enzyme solution (pH 4.8, 0.05 M citrate buffer) to give a solid/liquid ratio of 1:49 (mass ratio). The enzyme solution also contained 0.02 wt% sodium azide and a cellulase (Accellerate® 1500, DuPont, US) loading of 10 FPU/g biomass. Enzymatic hydrolysis was conducted at 50 °C for 72 h in a rotary incubator (Ratek OM 11 Orbital Mixer, Australia) with a shaking speed of 150 rpm. The sampling times were 6, 24, 48 and 72 h with a sampling volume of 0.25 mL each time. The samples were centrifuged and the supernatants were diluted 4 times by deionised water. The diluted samples were filtered through 0.45 μm syringe membrane filters and analysed by HPLC. At the end of the hydrolysis, the enzymatic solutions were centrifuged at 3900 rpm for 5 min and the residual solids containing heterogeneous Pd/C or Ru/C catalyst was washed by deionised water and dried 45 °C for overnight for recycling experiments.

$$Glucose\ yield = \left(\frac{Quantified\ mass\ of\ Glucose}{Mass\ of\ cellulose\ in\ biomass}\right) \times 100\%$$

$$Xylose\ yield = \left(\frac{Quantified\ mass\ of\ xylose}{Mass\ of\ hemicellulose\ in\ biomass}\right) \times 100\%$$

Analytical methods

Biomass compositional analysis: compositional (cellulose, hemicelluloses, lignin and ash) analysis of the starting materials (Table S1) were conducted according to the standard methods developed by National Renewable Energy Laboratory (NREL), US (Sluiter *et al.*, 2008). The maximum standard deviation was no more than 1.9% using this method. Subsamples of lignocellulosic biomass were dried overnight at 105 °C to constant weights to determine moisture content prior to biomass composition analysis.

Aromatic product analysis: The methanol fraction samples were analysed by GC/MS using an Agilent 6890 series gas chromatograph and a HP 5973 mass spectrometer detector with helium as carrier gas. The installed column was a HP-5; cross-linked methyl siloxane, 25 × 0.32 mm

× 0.17 μm. The temperature program was set to 70 °C and subsequently heated to 320°C at a rate of 5 °C/min, and then held for 5 min. Compounds were identified by means of the Wiley library HP G1035A and NIST library of mass spectra and subsets HP G1033A.

Enzymatic hydrolysis products analysis*:* A Waters HPLC system equipped with a Phenominex RPM monosaccharide column (300 mm × 7.8 mm, Shodex, Japan) and a refractive index (RI) detector (Waters 410, US) was used to quantify the sugars obtained from enzymatic hydrolysis. The column temperature was 85 °C and the mobile phase was water with a flow rate of 0.5 mL/min.

NMR analysis: approximately 150 mg lignocellulose and solid residues after depolymerisation reaction (ground <0.5 mm) was added to acetic acid/acetic anhydride (4:1, 10 mL) for stirring over 12 h until a clear solution was obtained. The solution was then concentrated in vacuo and further dried under a stream of air for 15 min. The sample was then dissolved in 1 mL of DMSO-d_6, centrifuged and the solution transferred to an NMR tube (a diameter of 5 mm) for NMR analysis immediately (Lancefield et al, 2017). The oil products (methanol fraction) were vacuum-dried and dissolved in DMSO-d_6. ^{1}H-^{13}C correlation 2D HSQC NMR spectra were recorded using a 400 MHz NMR Bruker Avance spectrometer (Agilent, US) at room temperature. The spectral widths were 5 kHz and 20 kHz for the ^{1}H and ^{13}C dimensions, respectively. The number of the collected complex points was 1024 for the ^{1}H dimension with a recycle delay of 1.5 s. The number of transients was 128 for lignocellulose and solid residue samples and 64 for oil samples, and 256 time increments were recorded in ^{13}C dimension. The central solvent (DMSO-d_6) peak was used as an internal chemical shift reference point (δC/δH 39.5/2.49) (del Rio et al., 2015).

GPC analysis: The instrument used for this analysis incorporated a GPC Water Breeze system model 151 with an isocratic HPLC pump. Eluted fractions were detected with UV light (250 nm) and a RI detector (Water model 2414). Three Phenomenex phenogel columns (500, 104, and 106 Å porosity; 5μm bead size) were used for size exclusive separation. The mobile phase was tetrahydrofuran (THF) with a flow rate of 1 mL/min at 30 °C. Oil fraction samples were prepared at a concentration of 1−2 mg/mL in THF and filtered through a 0.45 μm Teflon syringe filter. A 100 μL solution was injected into the instrument. The approximate molecular weight range associated with a given elution time was determined via calibration with polystyrene standards of known molecular weight profile. Fifteen polystyrene standards were used of weight average molecular weight (M_w) ranging from 380 to 1.9 × 10^5 g/mol.

FTIR analysis*:* The functional groups in the oils and solids obtained after depolymerisation were obtained with an FTIR Nicolet 870 Nexus spectrometer that was connected to a continuum infra-red microscope equipped with a liquid N_2 cooled MCT detector and an ATR objective that has a Si internal reflection element (Nicolet Instrument Corp. Madison, WI). For each run, a spectrum was collected with 128 scans in the range 4000 to 650 cm^{-1} with 4 cm^{-1} resolution.

PY-GC/MS analysis: This was used to specifically determine the amounts of *p*CA and FA present in the biomasses. Each sample (0.1g) was pyrolysed at 500 ºC in an EGA/PY-3030D micro-furnace pyrolyser (Frontier Laboratories Ltd., Fukushima, Japan) connected to a GC 7820A (Agilent Technologies, Inc.,Santa Clara, CA) and an Agilent 5975 mass-selective detector (EI at 70 eV). The column used to separate the various components was a DB-1701 column (30 m × 0.25 mm, 0.25 μm film thickness) (J&W Scientific, Folsom, CA). It was placed in an oven that was heated to 50 °C and held at that temperature for 1 min before increasing the temperature to 100 °C at 20 °C min^{-1}. The temperature was then increased at rate of 6 °C min^{-1} to 280 °C and then held at this temperature for 5 min. The compounds were eluted with helium and were identified by comparison of their mass spectra with those contained within the Wiley and NIST libraries, those reported in the literature (Faix *et al.*, 1990) and, whenever possible, with retention times and mass spectra of standards. Molar peak areas for each degradation product were calculated, the summed areas were normalized, and the data for two replicates were averaged and expressed as percentages.

SEC-ESI: Spectra were recorded on a Q Extractive Plus (Orbitrap) mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with an HESI II probe. The instrument was calibrated in the m/z range 74-1822 using premixed calibration solutions (Thermo Scientific) and for the high mass mode in the m/z range of 600-8000 using ammonium hexafluorophosphate solution. A constant spray voltage of 3.5 kV, a dimensionless sheath gas and a dimensionless auxiliary gas flow rate of 10 and 0 were applied, respectively. The capillary temperature and was set to 320 °C, the S-lens RF level was set to 50, and the aux gas heater temperature was set to 125 °C. The Q extractive was coupled to an UltiMate 3000 UHPLC System (Dionex, Sunnyvale, CA, USA) consisting of a pump (LPG 3400SD), autosampler (WPS 3000TSL), and a temperature controlled column department (TCC 3000). Separation was performed on two mixed bed size exclusion chromatography columns (Agilent, Mesopore 250 × 4.6 mm, particle diameter 3 μm) with a precolumn (Mesopore 50 × 7.5 mm) operating at 30 °C. THF at a flow rate of 0.30 $mL \cdot min^{-1}$ was used as eluent. The mass

spectrometer was coupled to the column in parallel to an UV detector (VWD 3400, Dionex), and a RI-detector (RefractoMax520, ERC, Japan) in a setup described earlier (Gruendling *et al.*, 2009). 0.27 $mL \cdot min^{-1}$ of the eluent were directed through the UV and RI-detector and 30 $\mu L \cdot min^{-1}$ were infused into the electrospray source after post-column addition of a 50 µM solution of sodium iodide in methanol at 20 $\mu L \cdot min^{-1}$ by a micro-flow HPLC syringe pump (Teledyne ISCO, Model 100DM). A 100 µL aliquot of a polymer solution with a concentration of 2 $mg \cdot mL^{-1}$ was injected into the SEC system.

Table S1 Biomass compositional properties of the starting lignocellulose

Components wt% (dry basis)	Bagasse	Trash	Rice Straw	Pine wood
Lignin	24.0	26.3	14.2	37.6
Carbohydrates	59.1	46.3	52.1	49.8
Glucan	36.8	28.5	34.2	32.8
Xylan	18.5	14.3	13.0	10.1
Arabinan	2.5	2.0	3.1	4.7
Galactose	1.3	1.4	1.8	2.2
Ash	3.5	10.0	22.5	0.0

Note: the maximum standard deviation was less than 1.9%.

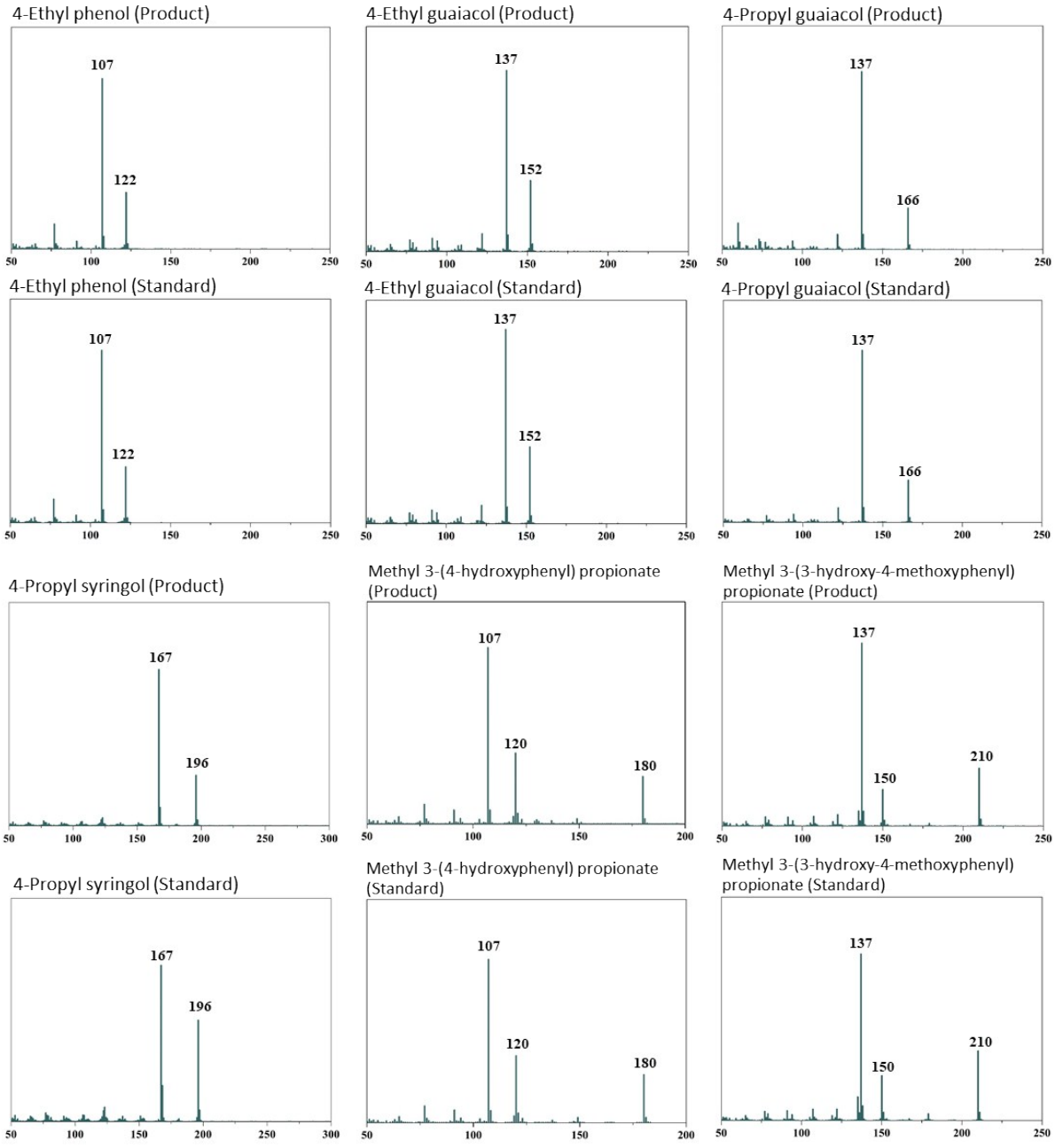

Fig. S1 Mass spectrum of the main aromatic components from direct depolymerisation of sugarcane bagasse in methanol and their commercial standards.

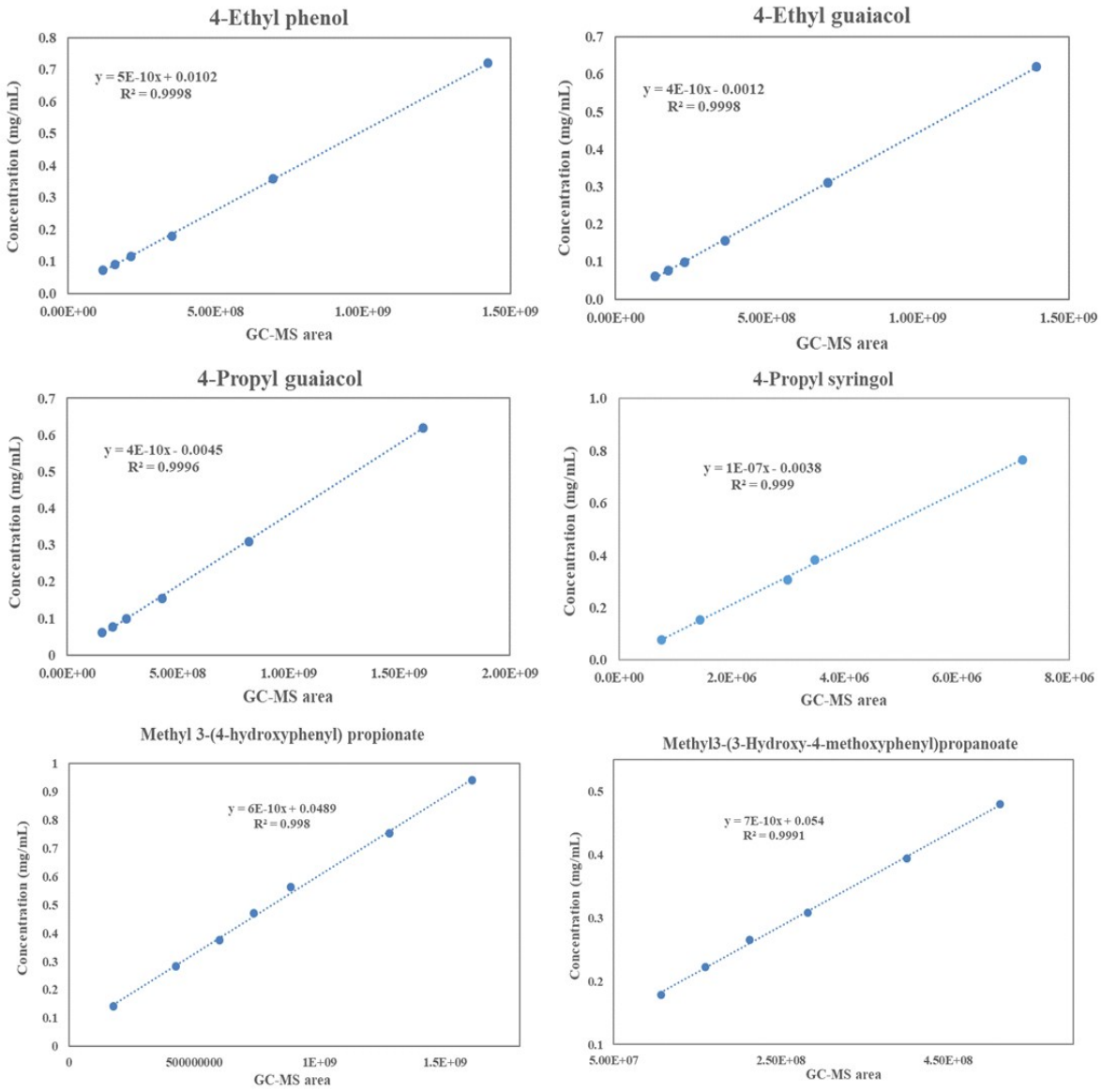

Fig. S2 GC-MS calibration curve of main aromatic components from direct depolymerisation of sugarcane bagasse based on commercial standards.

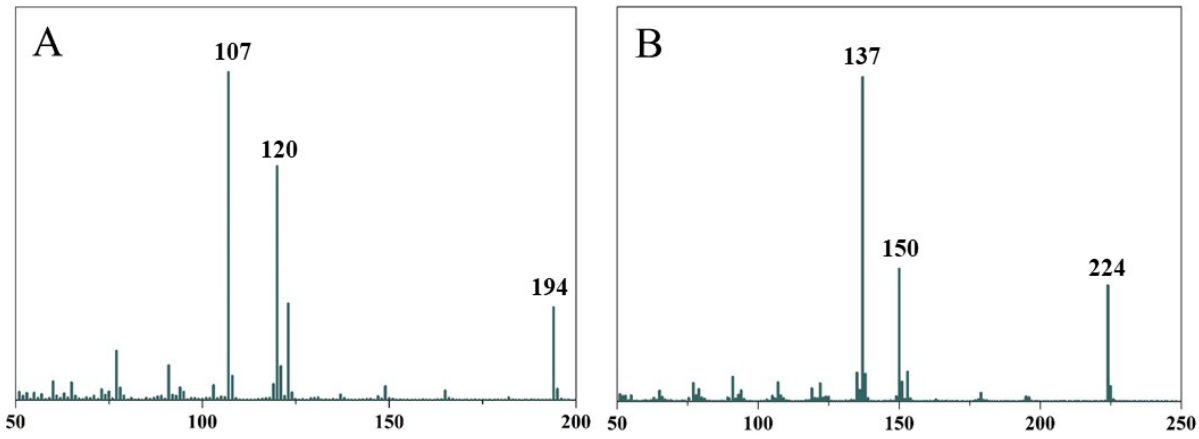

Fig. S3 Mass spectrum of A) ethyl 3-(4-hydroxyphenyl) propanoate and B) ethyl 3-(3-hydroxy-4-methoxyphenyl) propanoate from direct depolymerisation of sugarcane bagasse in ethanol.

Table S2 GCMS analysis of bagasse lignin oil from control experiment without catalysts (entry 2, Table 1)

R.T.	Library/ID	Pct Total	Qual
11.47	Cyclopentasiloxane, decamethyl-	0.82	90
12.52	2-Dodecene, (Z)-	0.96	97
15.49	.beta.-D-Ribopyranoside, methyl	5.85	78
19.81	trans-Isoeugenol	2.06	96
21.02	Cycloheptasiloxane, tetradecamethyl-	1.13	91
21.56	Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester	0.83	93
23.16	Cetene	0.92	97
24.97	Cyclooctasiloxane, hexadecamethyl-	0.134	93
25.73	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	2.04	93
27.38	Benzoic acid, 4-hydroxy-3,5-dimethoxy-, hydrazide	1.19	94
27.56	5-Octadecene, (E)-	1.19	99
28.86	2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester	7.47	98
30.30	Hexadecanoic acid, methyl ester	1.51	99
31.56	9-Tricosene, (Z)-	0.48	99
33.62	Octadec-9-enoic acid	0.48	94
34.11	Methyl stearate	0.49	99
35.23	Heptafluorobutyric acid, pentadecyl ester	0.24	87

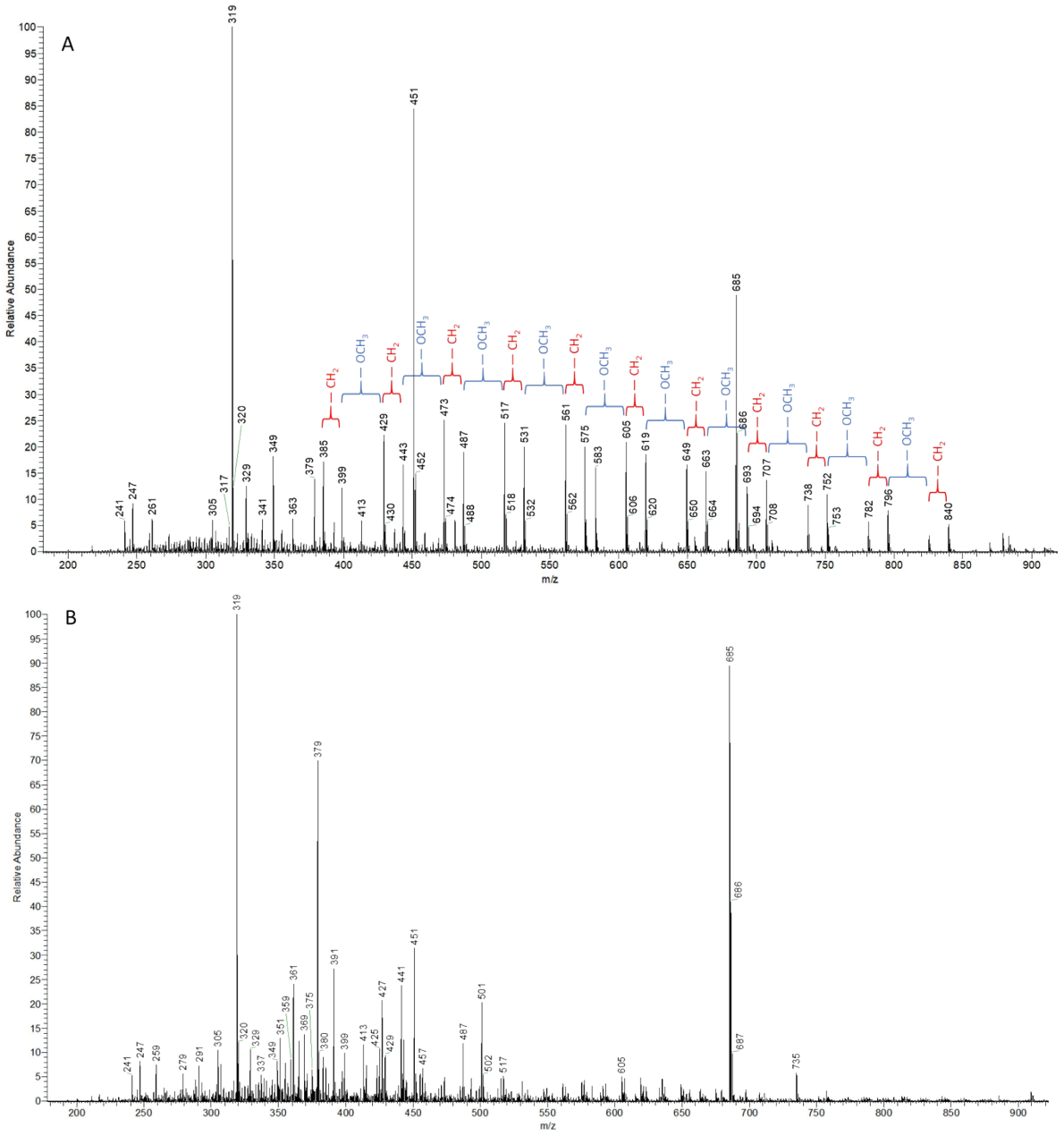

Fig S4 SEC-ESI spectra of oil from bagasse liquefaction without catalyst (A) and with Pd/C catalyst (B) in methanol for 8 h. The peak at 685.43 associated with the solvent THF used for ESI analysis.

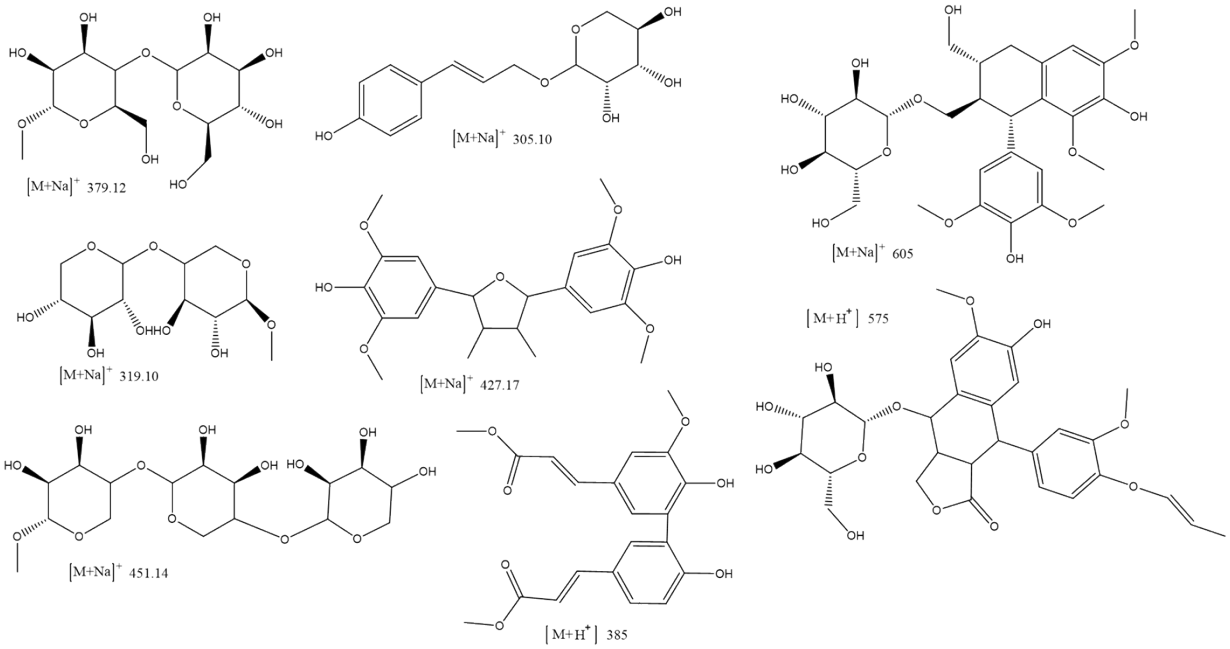

Fig S5 Some proposed dimers and oligomers structures based on ESI results and 2D NMR spectra**.**

Table S3 **The proposed formula and type of molecules in the oil obtained with and without catalysts based on ESI results.**

	Without catalysts				With Pd/C		
No.	**Formula**	**M/Z (Na^+)**	**Type**	**No.**	**Formula**	**M/Z (Na^+)**	**Type**
1	C14H18O6	305	monomer/dimer	**1**	C14H20O5	291	monomer/dimer
2	C11H20O9	319	monomer/dimer	**2**	C14H18O6	305	monomer/dimer
3	C18H17O4	320	dimer	**3**	C11H20O9	319	dimer
4	C12H22O10	349	dimer	**4**	C18H17O4	320	dimer
5	C13H24O11	379	dimer	**5**	C12H22O10	349	dimer
6	C20H25O6	385	dimer	**6**	C19H36O4	351	dimer
7	C21H28O6	399	dimer	**7**	C13H22O10	361	dimer
8	C22H30O7	429	dimer	**8**	C20H26O5	369	dimer
9	C23H32O7	443	dimer	**9**	C13H24O11	379	dimer
10	C16H28O13	451	oligomer	**10**	C21H20O6	391	dimer
11	C24H35O8	473	oligomer	**11**	C21H28O6	399	dimer
12	C25H37O8	487	oligomer	**12**	C21H26O7	413	dimer
13	C26H40O9	517	oligomer	**13**	C22H28O7	427	dimer
14	C27H42O9	531	oligomer	**14**	C22H26O8	441	dimer
15	C28H45O10	561	oligomer	**15**	C16H28O13	451	oligomer
16	C29H47O10	575	oligomer	**16**	C23H30O8	457	oligomer
17	C19H31O15	583	oligomer	**17**	C25H37O8	487	oligomer
18	C30H50O11	605	oligomer	**18**	C25H34O9	501	oligomer
19	C31H52O11	619	oligomer	**19**	C26H40O9	517	oligomer
20	C32H55O12	649	oligomer	**20**	C30H50O11	605	oligomer
21	C33H57O12	663	oligomer				
22	C34H60O13	693	oligomer				
23	C35H62O13	707	oligomer				
24	C36H65O14	738	oligomer				
25	C37H67O14	752	oligomer				
26	C38H70O15	782	oligomer				
27	C39H72O15	796	oligomer				
28	C41H77O16	840	oligomer				

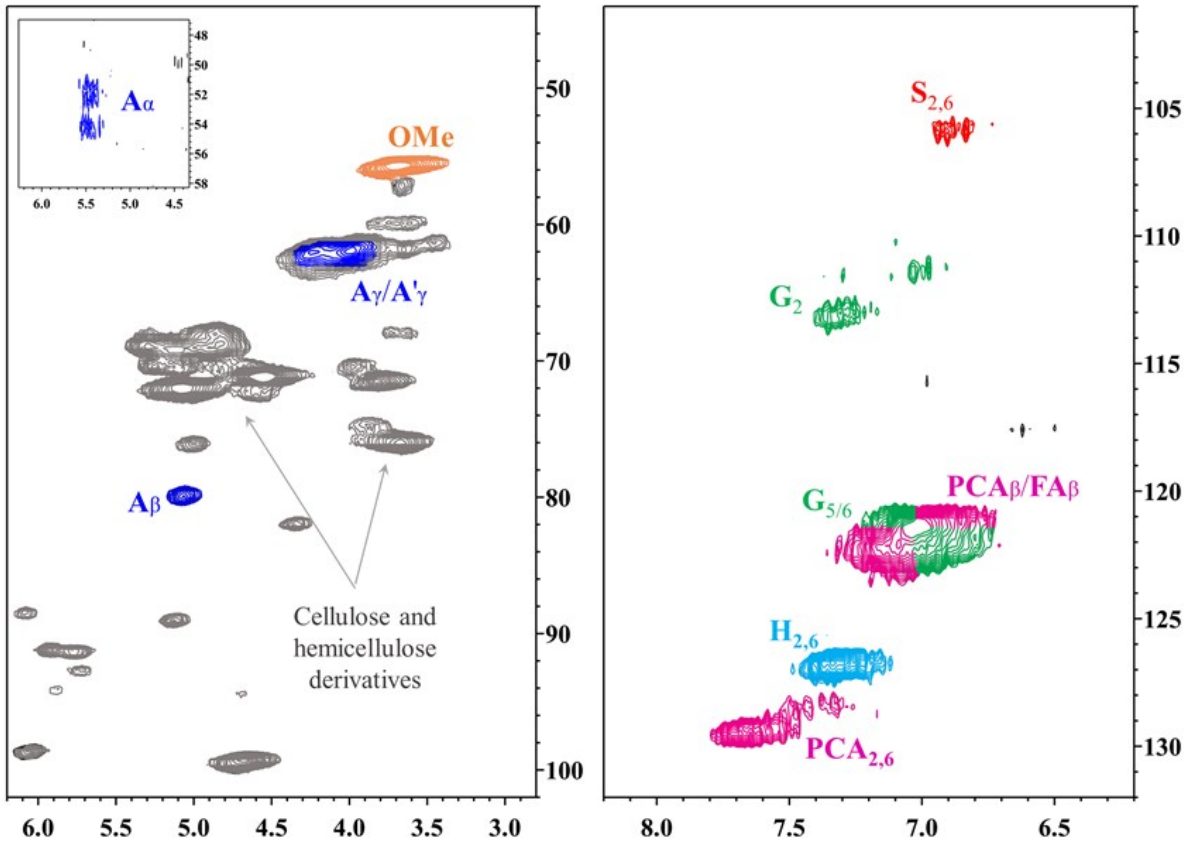

Figure S6 2D NMR spectra of sugarcane trash.

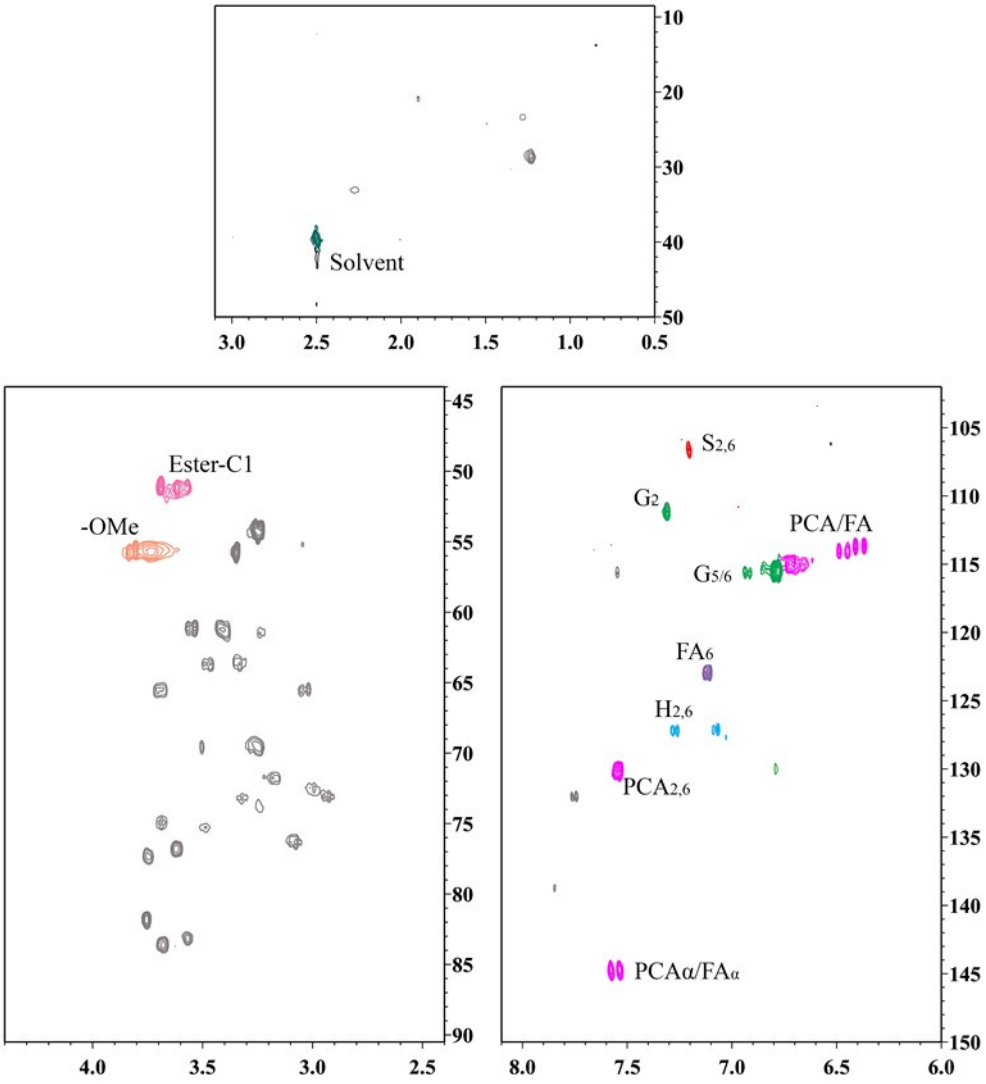

Figure S7 2D NMR spectrum of oil obtained without catalyst (entry 2 in Table1).

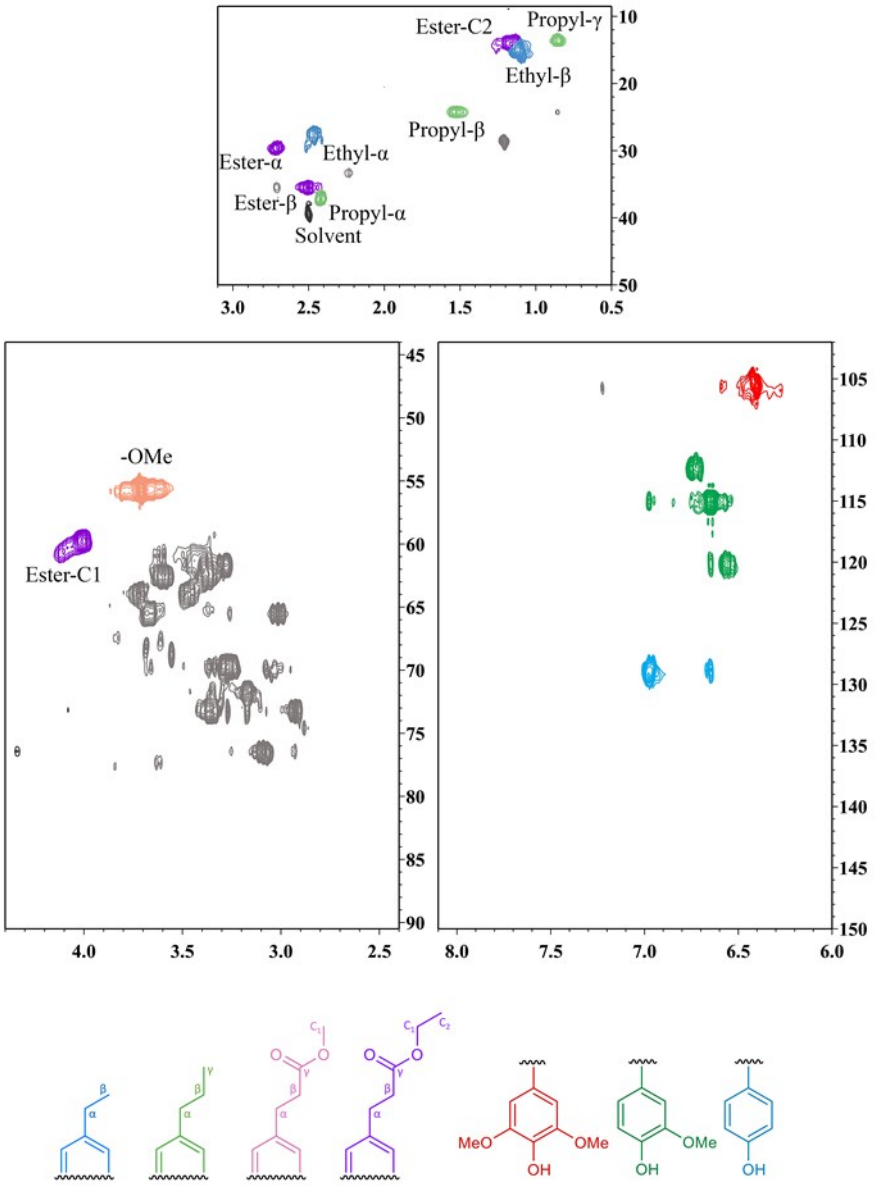

Figure S8 2D NMR spectrum of oil obtained from delignification of sugarcane bagasse with Pd/C and ethanol used as solvent.

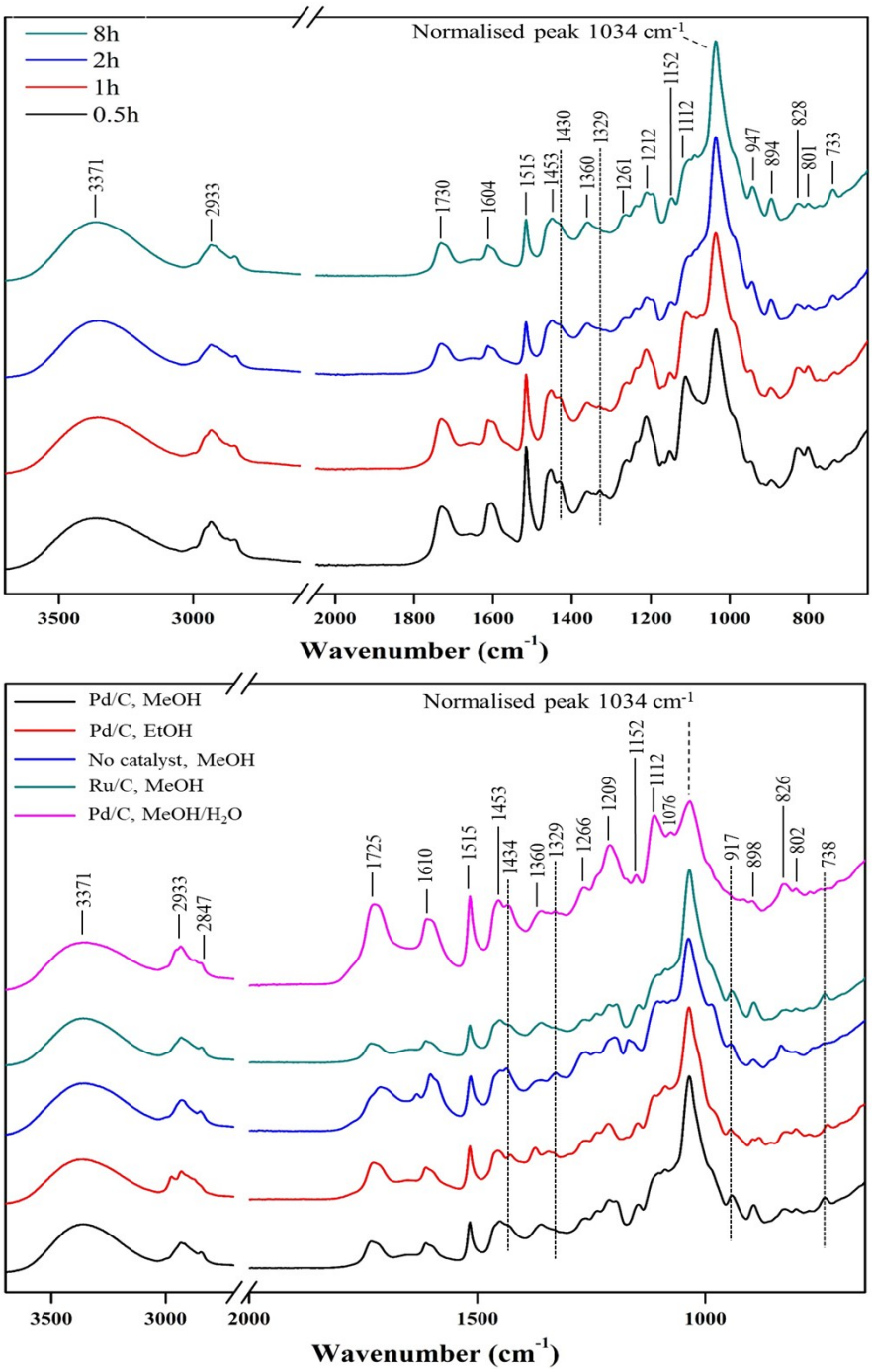

Fig S9 FTIR spectra of oil products obtained under different reaction time (0.5 h -8 h) and different reaction conditions.

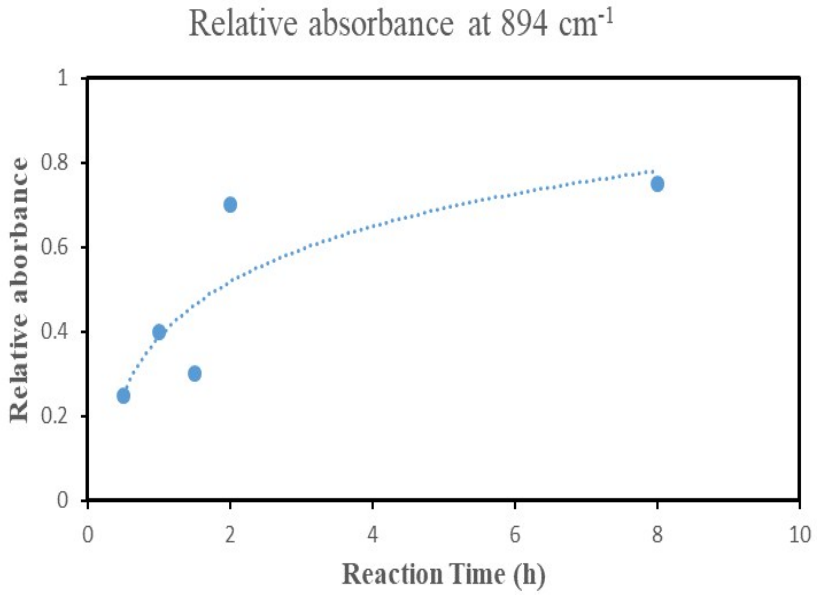

Fig S10 Relative absorbance at 894 cm^{-1} for oil fractions obtained with different reaction time.

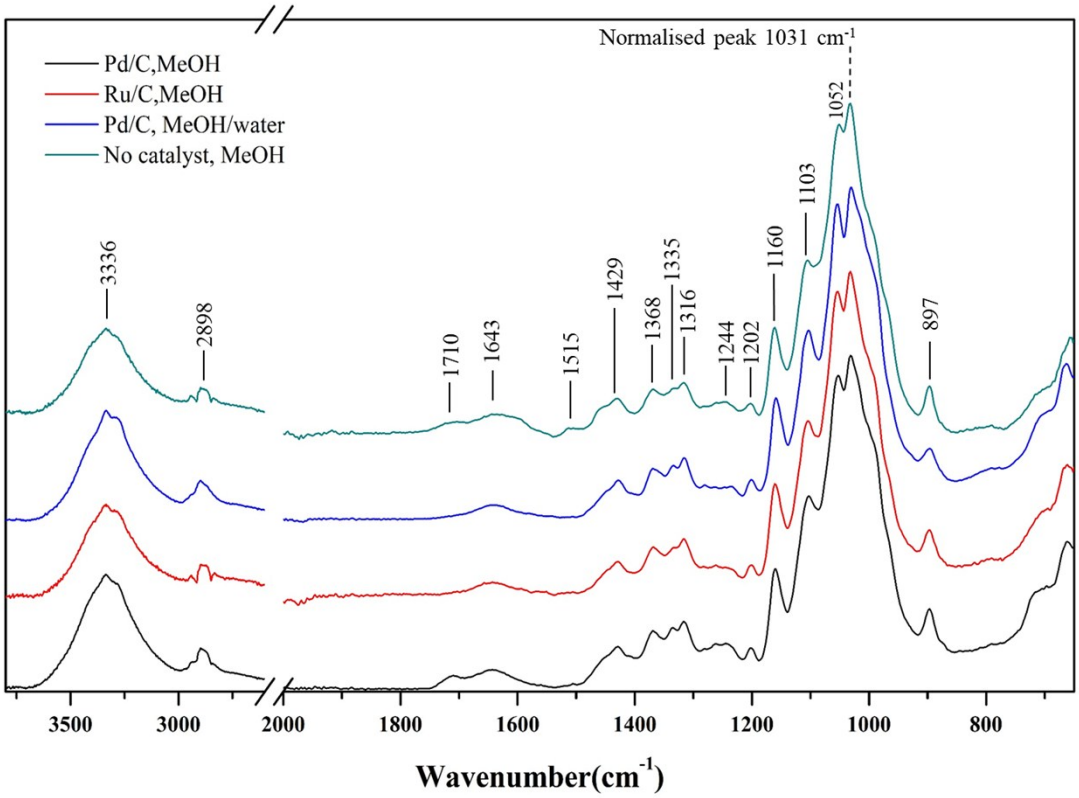

Fig. S11 FTIR spectra of solid residue from bagasse liquefaction under different reaction conditions.

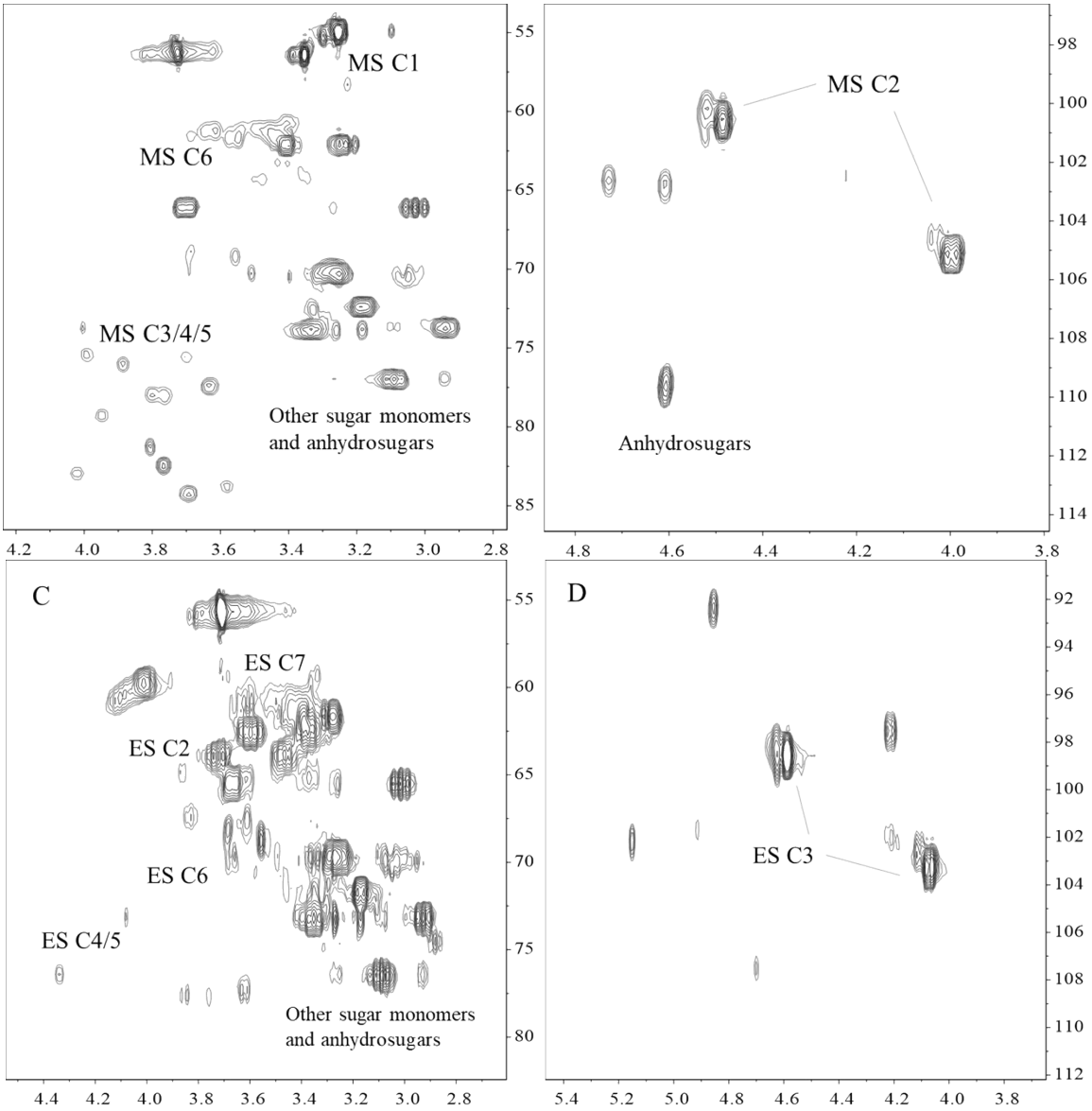

Fig S12 2D HSQC NMR spectra of the chemical correlation in the structure of mono-sugars alcohol derivatives from hemicellulose. A and B present for methylated mono-sugars, C and D present for the ethylated mono-sugars, C1-7 refer to the carbon position in the sugar derivatives structure.

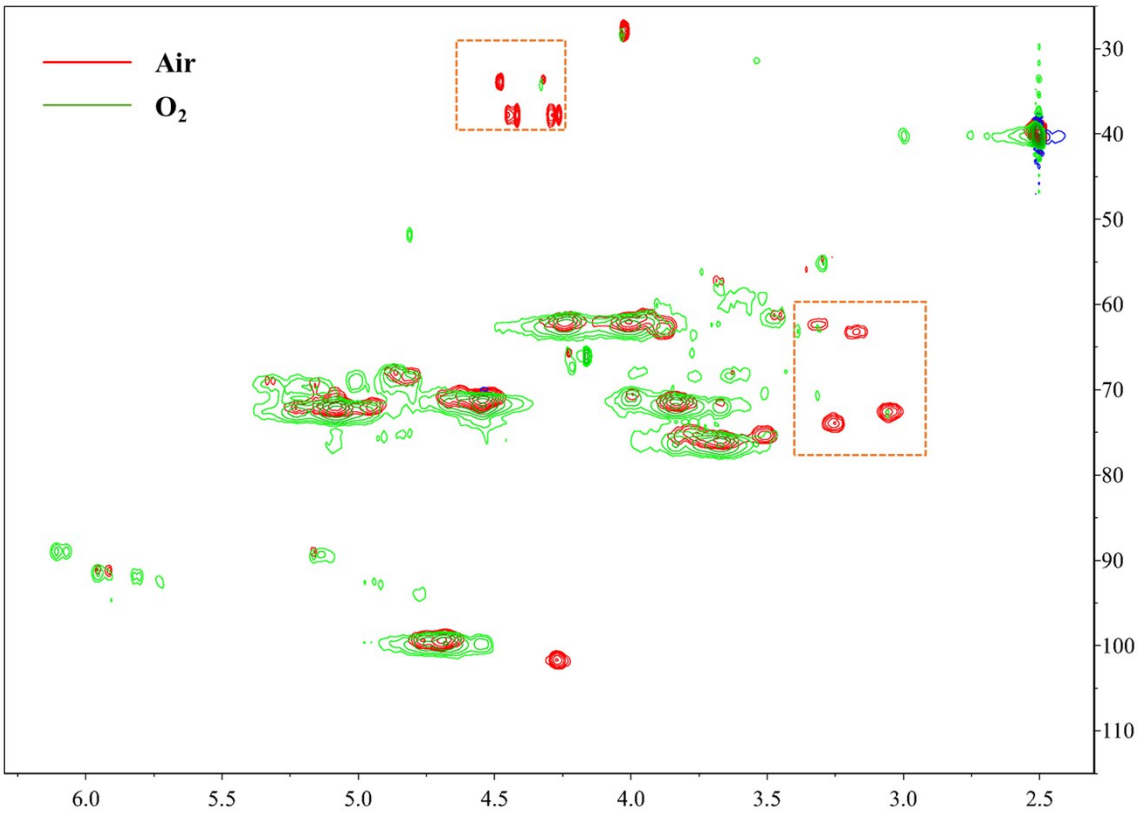

Fig S13 Comparison of 2D HSQC NMR spectra of solid residue obtained from direct conversion of bagasse under O_2 (green line) and air (red line) atmosphere.

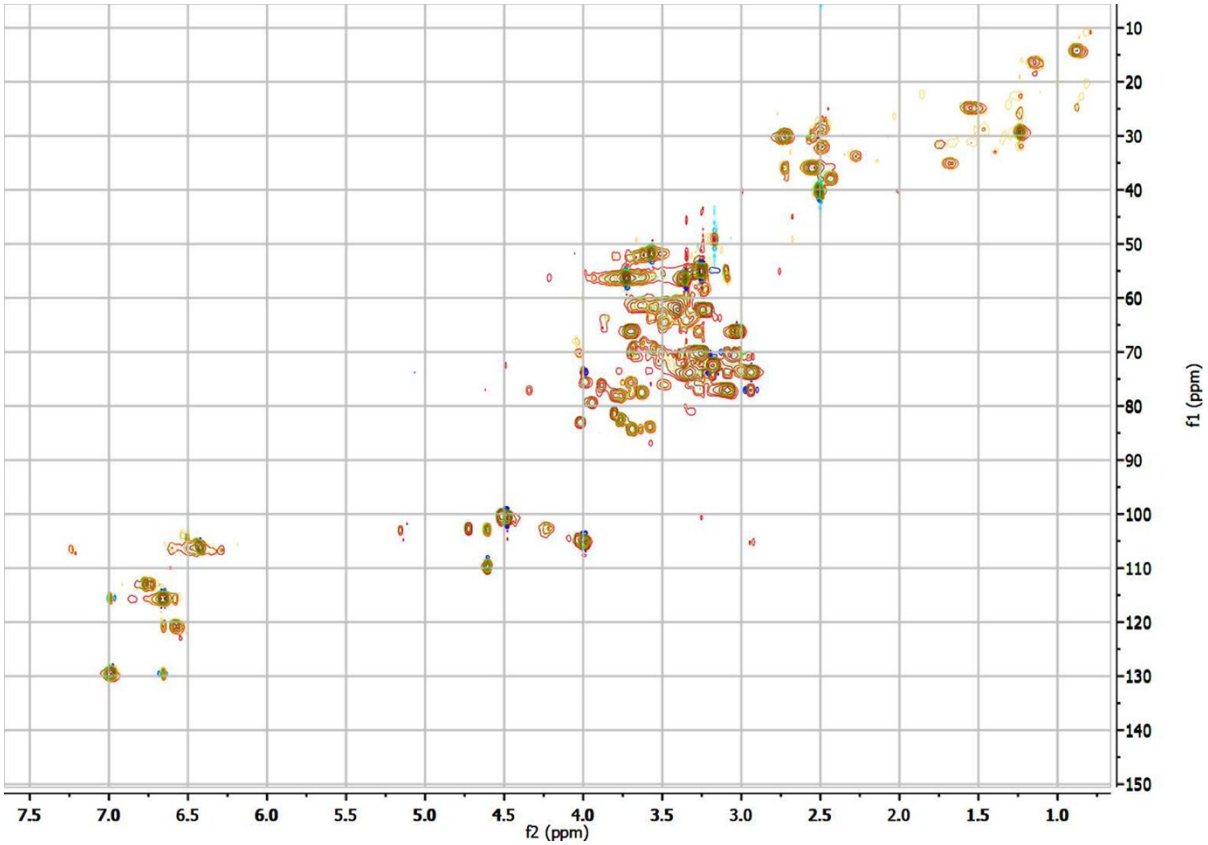

Fig S14 Comparison of 2D HSQC NMR spectra of oil fraction obtained from direct conversion of bagasse under O_2 (green line), Ar (yellow line) and air (red line).