Electronic Supplementary Material (ESI) for Green Chemistry.

Supplementary Information

Reductive catalytic fractionation of agricultural residue and energy crop lignin and application of lignin oil as antimicrobials

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Table S1a. Physicochemical composition of herbaceous feedstock.

	%Structural Ash	%Structural Protein	%Extractives	%Lignin	%Glucan	%Xylan	%Galactan	%Arabinan	%Acetate
Corn stover	3.46	1.58	8.96	16.52	37.52	21.77	1.66	3.37	2.45
Switchgrass	1.88	1.51	14.34	16.24	33.21	21.65	1.43	3.27	3.07
Miscanthus	0.52	0.47	5.21	20.41	40.79	22.01	1.11	2.86	3.87
Sugarcane bagasse	6.93	1.06	5.06	25.6	41.59	18.05	0.71	1.3	1.84
Wheat straw	5.5	3.07	14.56	16.27	32.24	16.95	1.6	3.17	1.7

Table S1b. Composition of ash of herbaceous feedstock.

	%Al as Al_2O_3	%Ca as CaO	%Fe as Fe_2O_3	%K as K_2O	%Mg as MgO	%Mn as MnO	%Na as Na_2O	%P as P_2O_5	%Si as SiO_2	%Ti as TiO_2	%S as SO_3
Corn stover	3.51	7.73	1.27	13.01	3.64	0.13	0.61	1.22	65.31	0.15	1.27
Switchgrass	0.25	7.37	1.63	17.55	9.79	0.19	1.61	4.45	53.53	0.01	2.73
Miscanthus	0.29	18.34	1.2	6.44	9.03	1.11	0.18	3.58	52.31	0.02	3.15
Sugarcane bagasse	7.2	2.74	2.4	4.46	1.3	0.06	1.3	0.95	79.19	0.4	0.57
Wheat straw	2.77	10.83	2.99	15.45	2.69	0.07	1.16	2.15	58.16	0.11	2.34

Table S2. NMR characterization of isolated oligomer oils

Relative integrated contour area (V_i/V_{ar})									
	$H_{2,6}$	G_2	$S_{2,6}$	$S^{ox}_{2,6}$	Methoxy	β-β', γ	β-O-4', γ	α-6', α	β-β', β
Corn Stover	0.42	0.43	0.01	0.15	2.23	0.02	0.91	3.00	-
Switchgrass	0.34	0.47	0.01	0.17	2.14	0.09	0.26	3.31	0.11
Miscanthus	0.22	0.51	0.27	-	1.99	0.02	0.12	1.04	0.05
Sugarcane Bagasse	0.4	0.27	0.02	0.3	1.61	0.03	0.22	1.17	-
Wheat Straw	0.09	0.44	0.12	0.35	5.81	0.22	0.54	7.83	-
$V_i = V_j$/ (# of C-H Bonds), I = j ; $V_{ar} = V_{G2} + (V_{S2,6} + V_{S2,6})/2$									

Table S3. Monomer yield, average molecular weight, relative α-6' content of the obtained lignin oils and ferulate content of feedstock.

	Monomer Yield (%)	Average oil molecular weight (g/mol)	%Ferulate	α-6', α
Corn stover	45.8	275	14.0	0.76
Miscanthus	43.8	275	18.0	0.81
Switchgrass	41.5	290	27.0	0.84
Sugarcane bagasse	33.7	360	34.0	0.83
Wheat straw	20.0	365	84.0	0.91

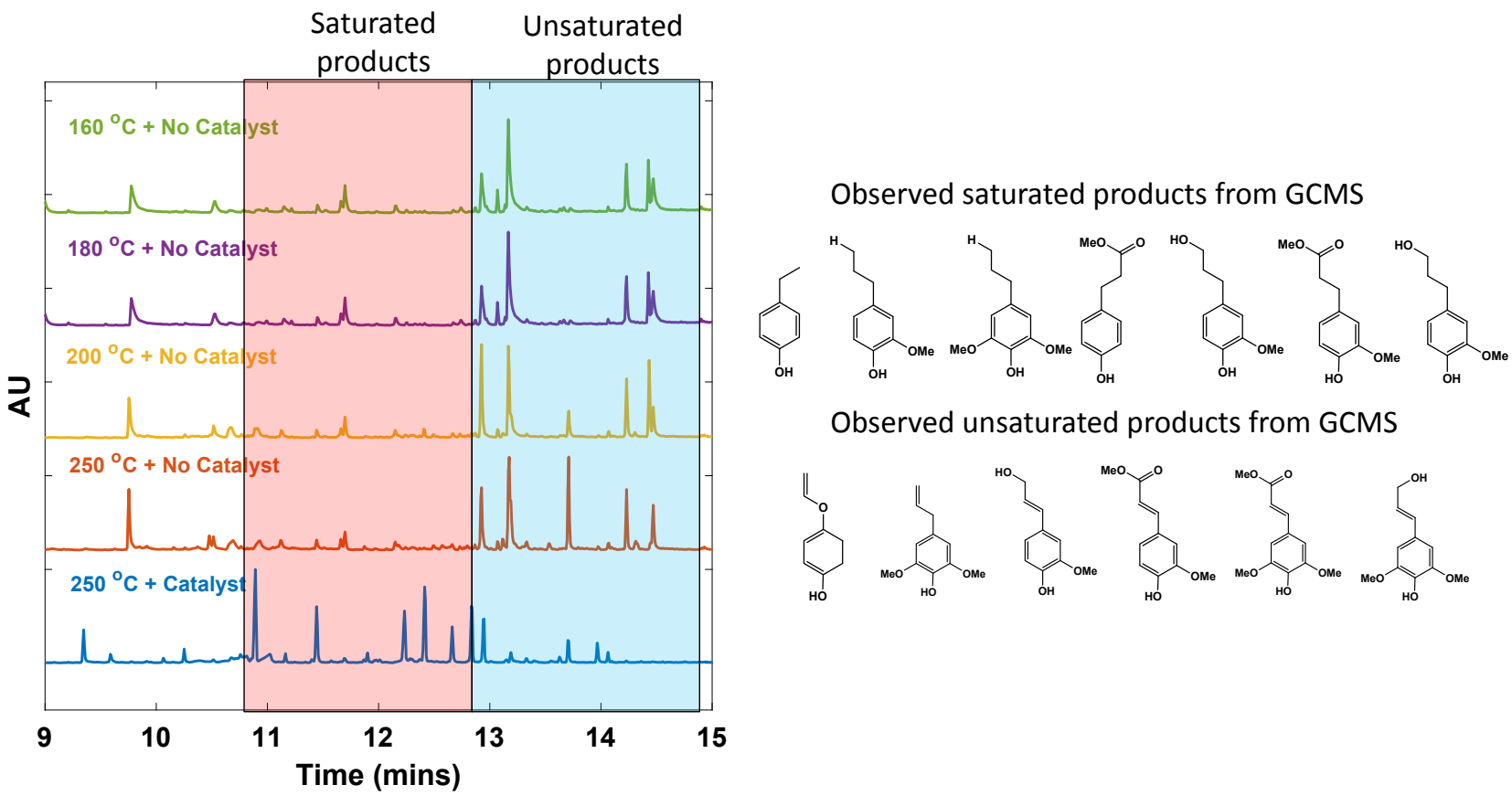

Figure S1. GCMS spectra of depolymerized miscanthus at different temperatures in methanol, with and without Ru/C + H_2 catalyst. Reactions with no catalyst mean no Ru/C and N_2 gas instead of H_2 gas.

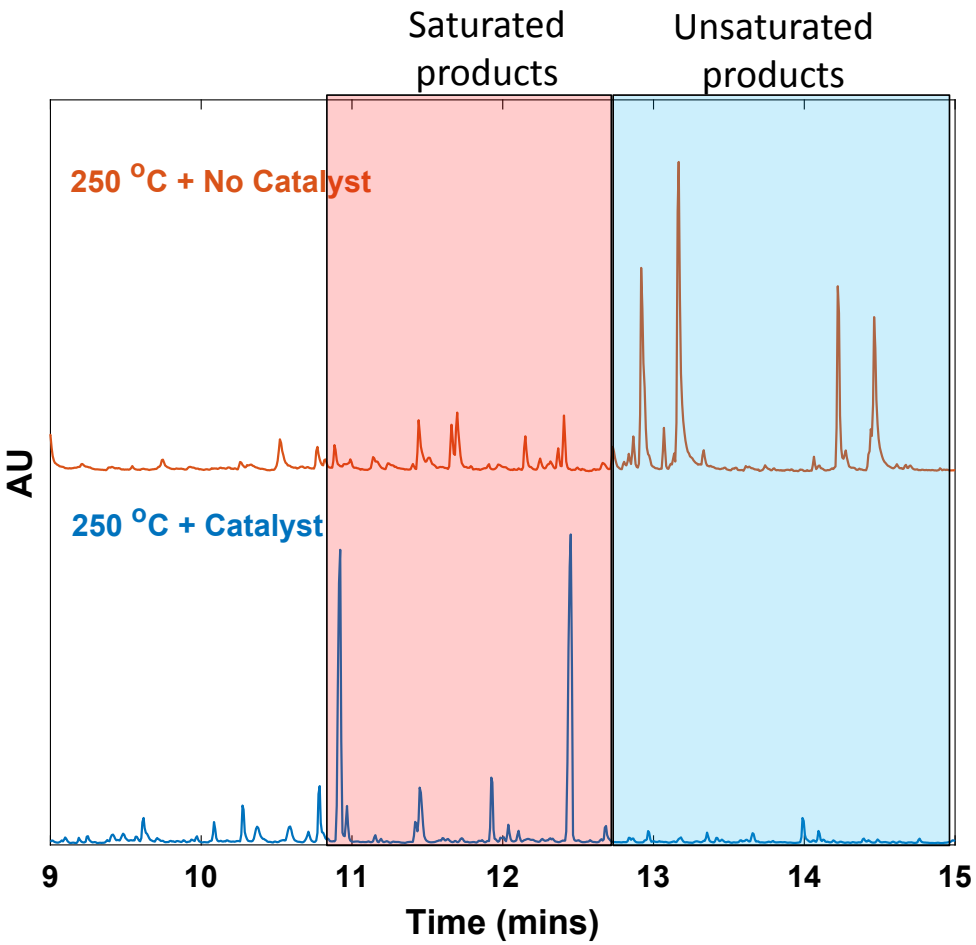

Figure S2. GCMS spectra of depolymerized PW in methanol, with and without Ru/C + H_2 catalyst. Reaction with no catalyst mean no Ru/C and N_2 gas instead of H_2 gas.

Table S4. Composition of gas phase after lignin depolymerization. Reaction conditions: 1 g miscanthus, 0.1 g Ru/C, 20 ml methanol, 250 °C, 5 hrs.

Gas composition (%)		
Component	Without *ex-situ* H_2	With *ex-situ* H_2 (40 bar)
H_2	48.9	93.8
CO	34.5	4.2

CO_2	3.4	0.3
CH_4	2.7	1.4
O_2	10.5	0.3

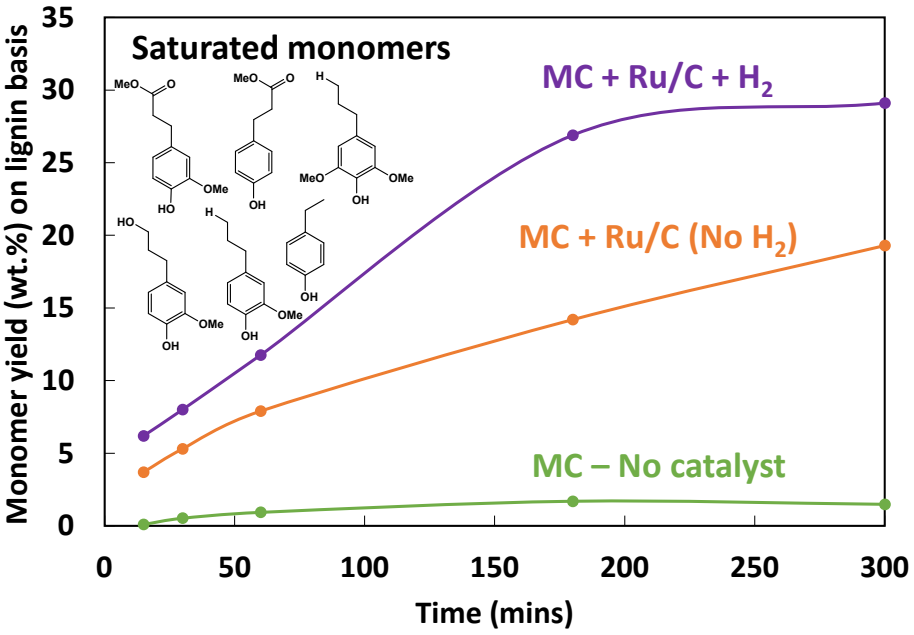

Figure S3. Time dependent profile of saturated monomers during miscanthus depolymerization in methanol. Reactions with no catalyst mean no Ru/C and N_2 gas was used instead of H_2 gas. Fitted curves are added to guides for the readers' eyes.

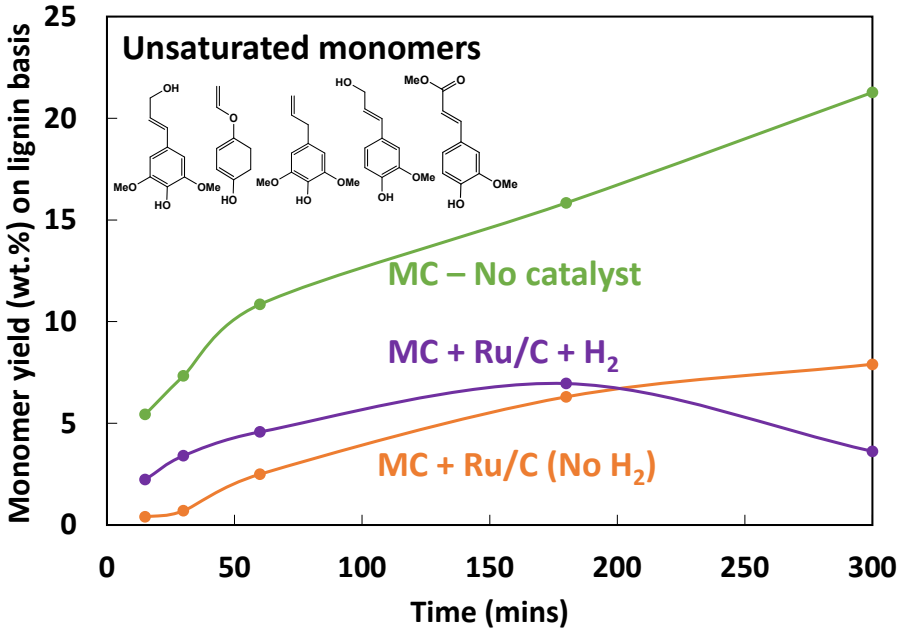

Figure S4. Time dependent profile of unsaturated monomers during miscanthus depolymerization in methanol. Reactions with no catalyst mean no Ru/C and N_2 gas instead of H_2 gas. curves are added to guides for the readers' eyes.

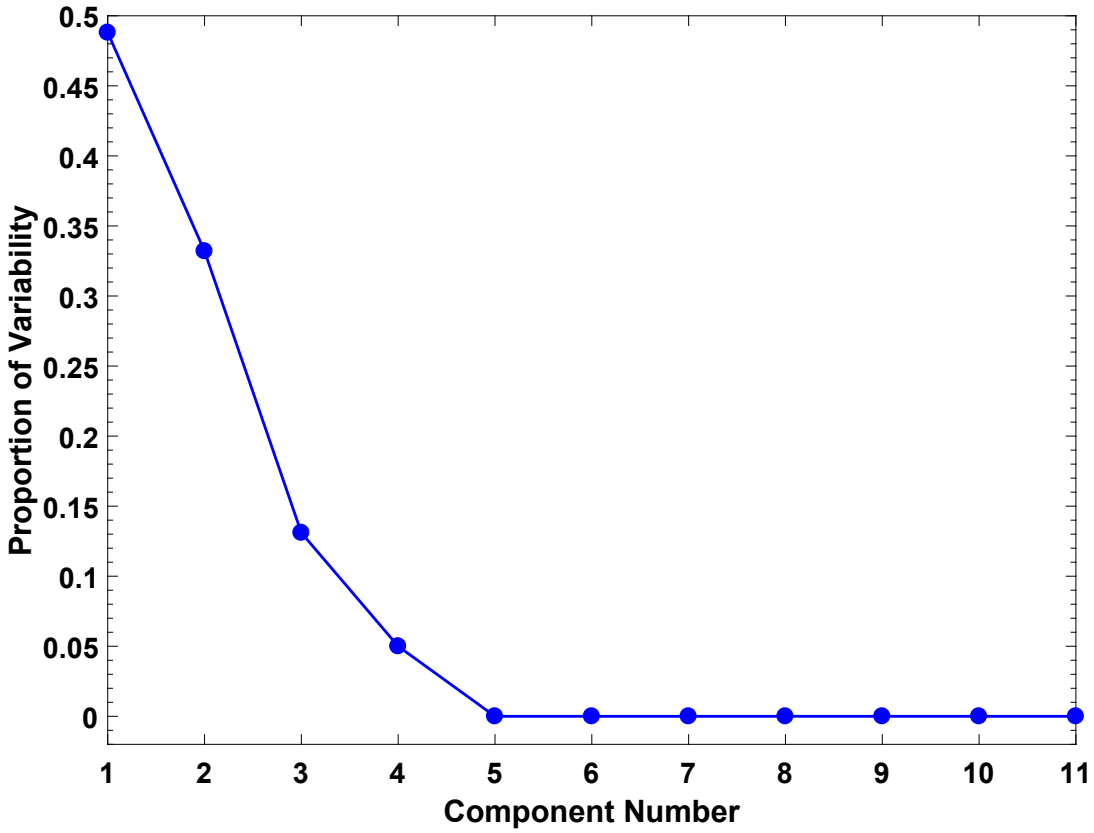

Figure S5. Scree plot showing the contributions of each principal component towards explaining the variance of the dataset.

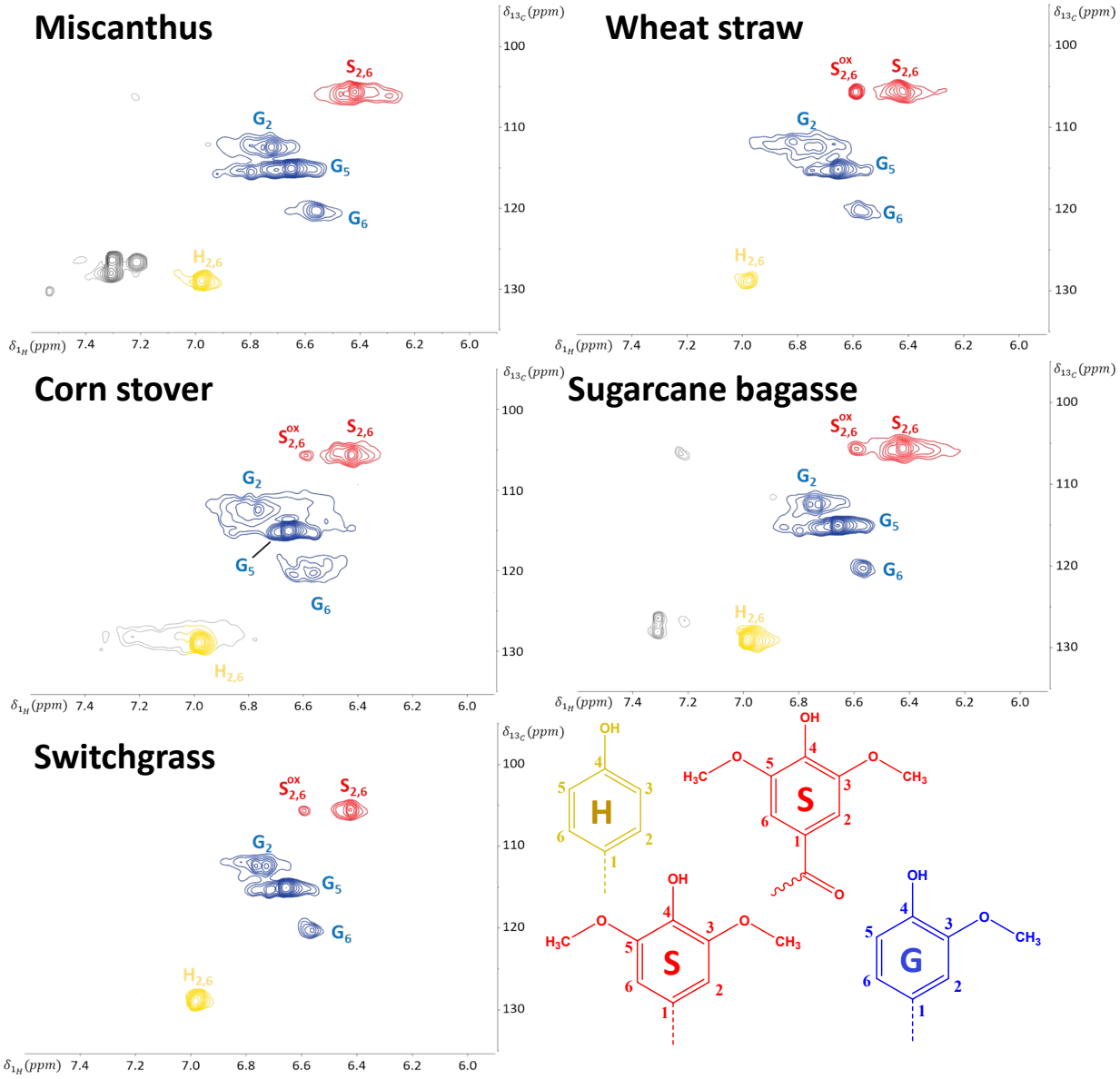

Figure S6. 2D HSQC NMR of the isolated oligomer lignin oils (aromatic region).

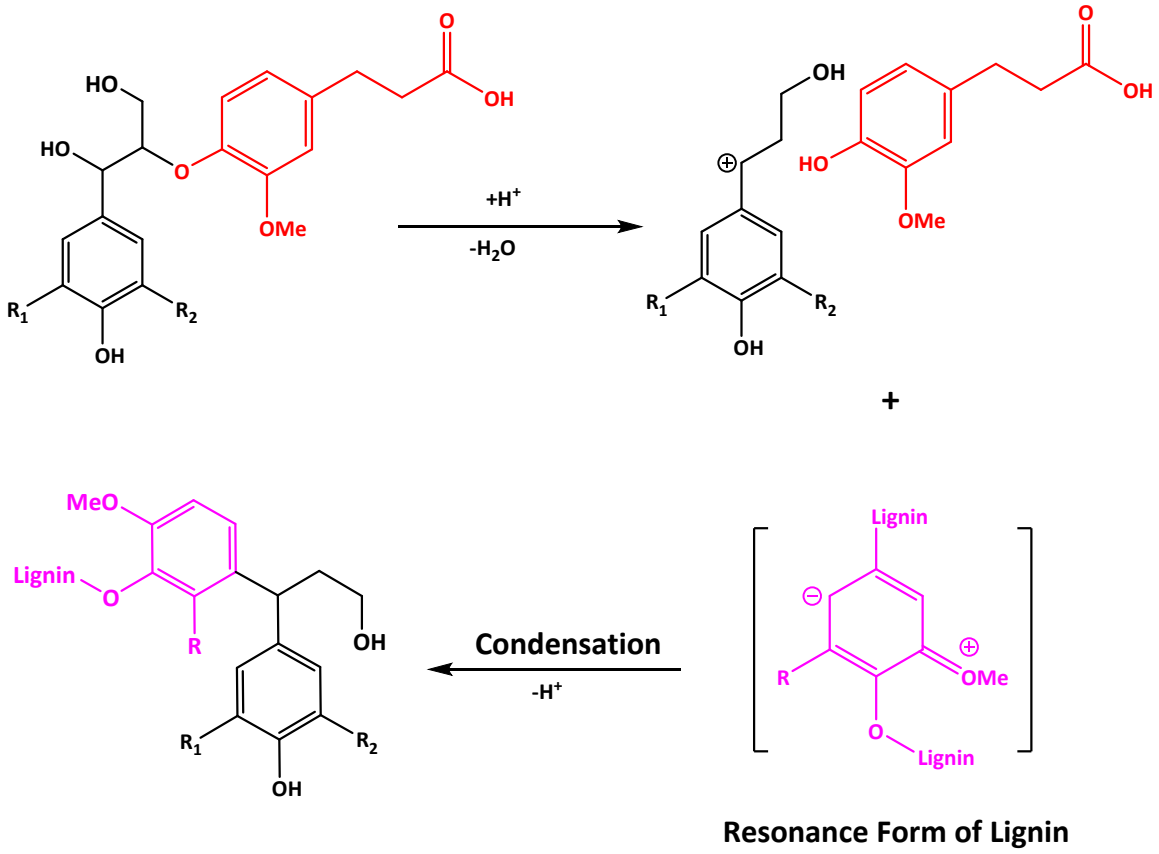

Figure S7. Lignin condensation pathway to form α-6 oligomers in the presence of an acid.

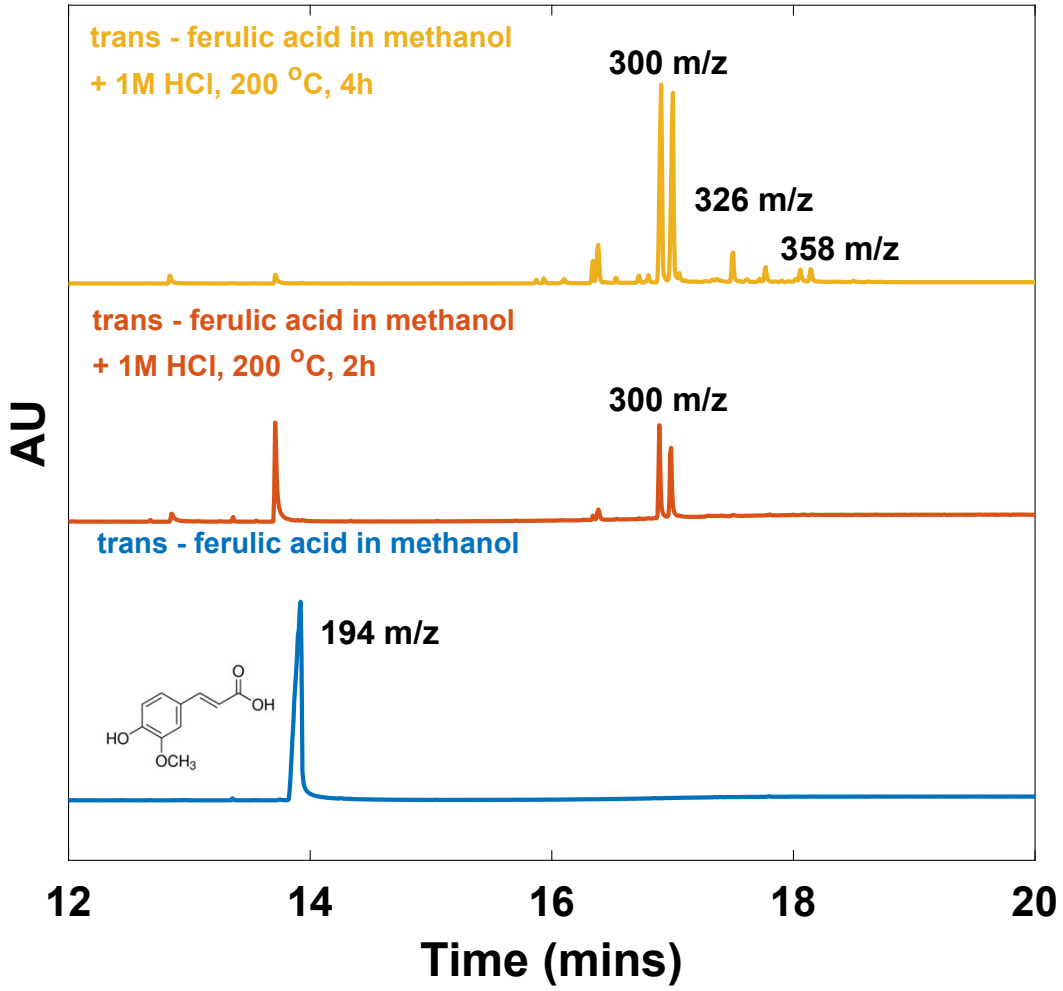

Figure S8. GCMS product distribution of trans ferulic acid in methanol after heating in acid.

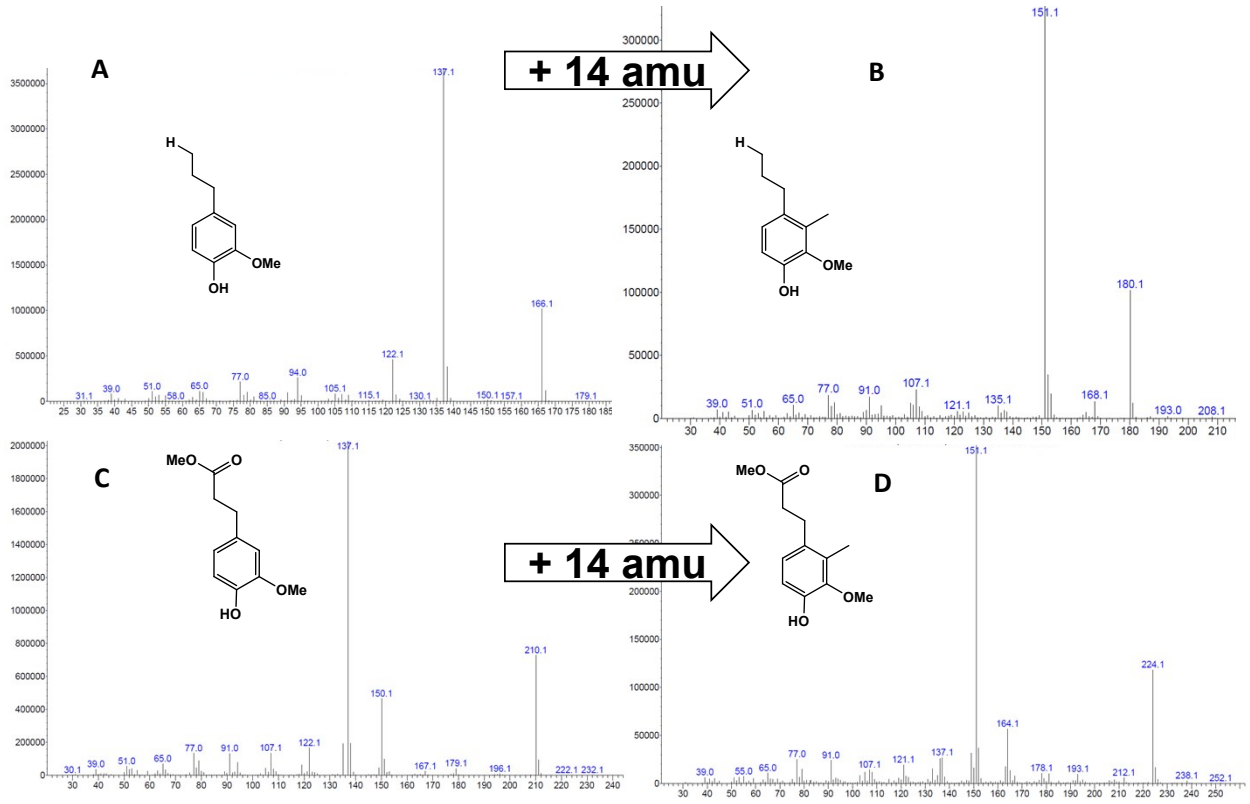

Figure S9. GCMS ionization spectra for A) propyl guaiacol, B) methylated propyl guaiacol, C) ferulic acid, and D) methylated ferulic acid identified in the reaction products from miscanthus RCF with formaldehyde stabilization. Consistent increase of 14 amu in base peak indicating the addition of a methyl group to the benzene ring.

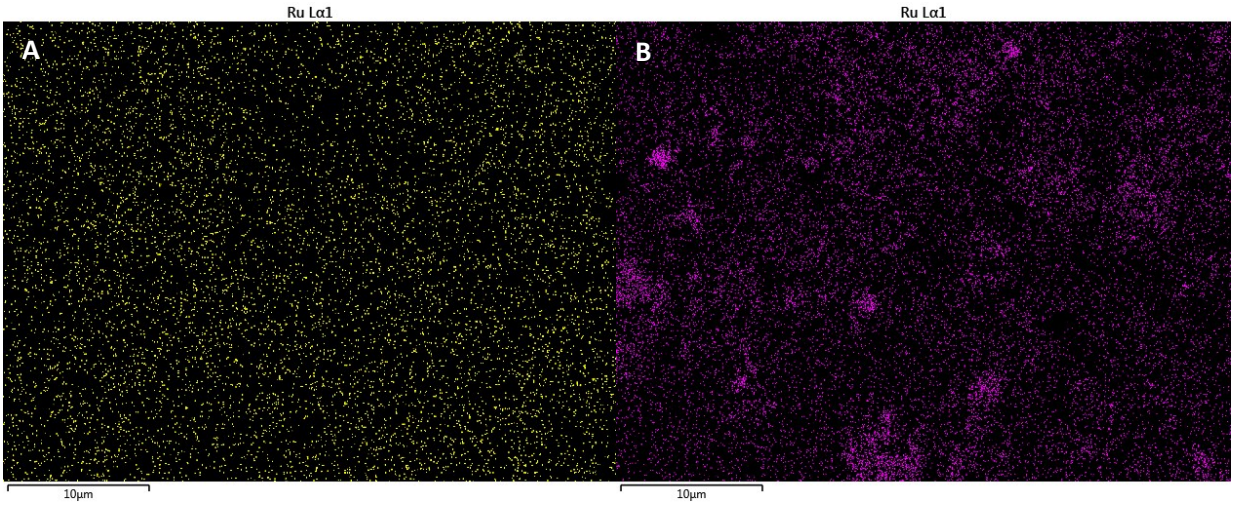

Figure S10. XEDS spectra of Ru/alumina. A) Fresh catalyst. B) Spent catalyst (sugarcane bagasse).

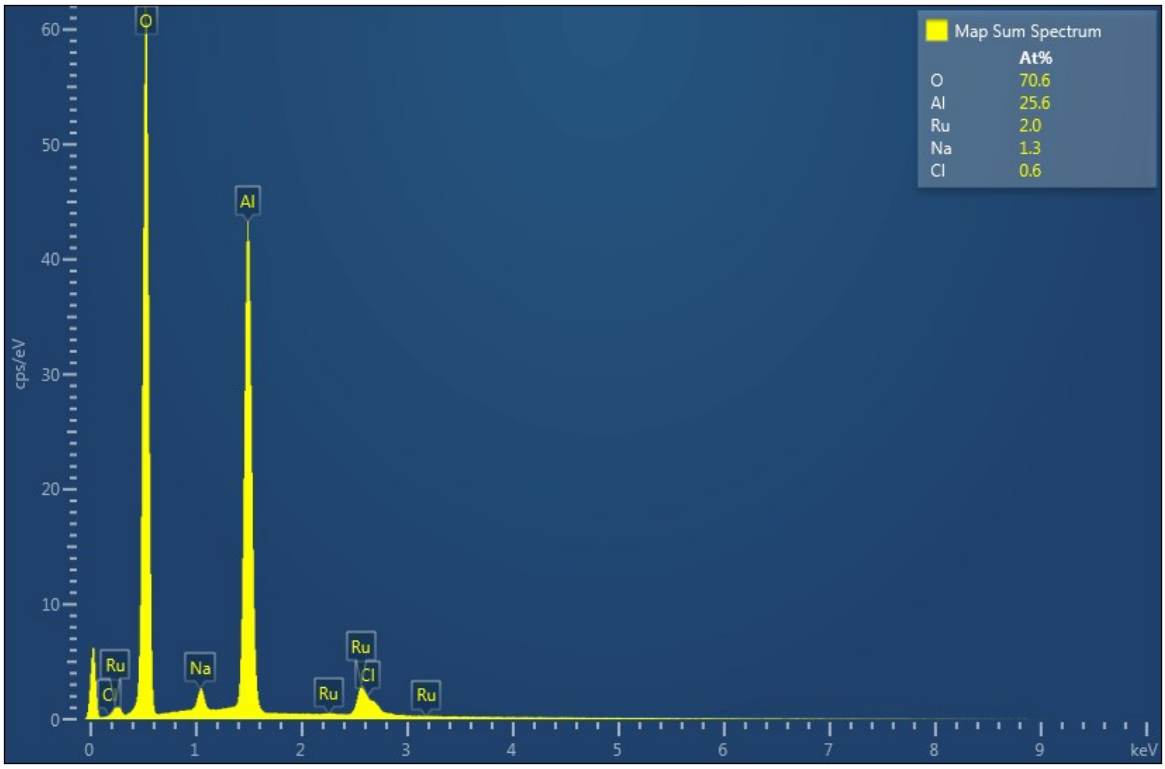

Figure S11. XEDS spectra of the fresh Ru/alumina pellets.

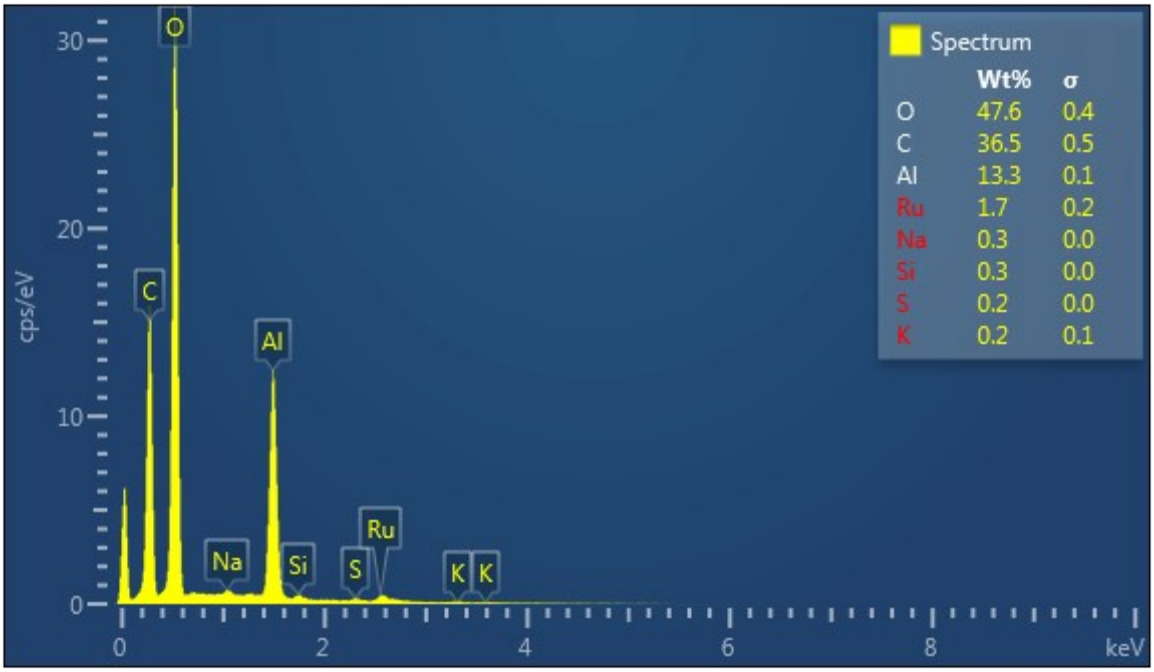

Figure S12. XEDS spectra of the spent Ru/alumina pellets (sugarcane bagasse).

Table S5. Mass balance for each feedstock.

	Corn Stover	Switchgrass	Miscanthus	Sugarcane Bagasse	Wheat Straw
Residual solid	0.34 ± 0.02	0.31 ± 0.03	0.4 ± 0.03	0.42 ± 0.01	0.26 ± 0.03
Oil yield	0.48 ± 0.02	0.51 ± 0.01	0.41 ± 0.02	0.4 ± 0.02	0.56 ± 0.01
Ash*	0.04	0.02	0.01	0.07	0.06
Mass balance	0.86 ± 0.02	0.84 ± 0.02	0.82 ± 0.03	0.89 ± 0.01	0.88 ± 0.02

*based on ash content of original biomass. Extractives, soluble sugars, and proteins make the unknown products during lignin depolymerization.

Table S6. Leached metal content in reaction solution.

Metal components (%)	Sugarcane bagasse			Corn stover			Miscanthus			Switchgrass			Wheat straw		
	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
Si	26.6	15.7	75	4.7	n.d	n.d	n.d	5.95	n.d	n.d	n.d	21.3	n.d	22.0	9.94
Ru	26.6	15.7	8.6	38	38	48	41	52.5	60.4	29.1	52	24.5	35.6	21.6	16.8
K	34.7	41.4	16.4	52	53	49	34.9	41.5	28.3	49.2	36.1	44.7	51.1	46.3	66.9
Cl	6.5	6.2	n.d	5.4	9.1	2.9	7.2	n.d	n.d	21.7	8.87	9.52	9.2	7.75	6.37
Ca	n.d	10.8	n.d	n.d	n.d	n.d	16.9	n.d	11.4	n.d	n.d	n.d	n.d	n.d	n.d
Fe	9.9	22.7	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	4.15	2.40	n.d
S	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	2.98	n.d	n.d	n.d	n.d

n.d = not detected

Table S7. Mass of recovered catalyst after each recycle.

Biomass	Recovered catalyst				
	Initial	Run 1	Run 2	Run 3	% Δ
Sugarcane Bagasse	0.2565	0.2613	0.2692	0.2803	9.2788
Miscanthus	0.2574	0.2609	0.2754	0.2811	9.2075
Corn stover	0.256	0.2596	0.2748	0.2914	13.8281
Switchgrass	0.2533	0.2625	0.2718	0.2814	11.0936
Wheat Straw	0.2493	0.2677	0.2693	0.2748	10.2286

% Δ = change in catalyst weight after run 3 from initial weight

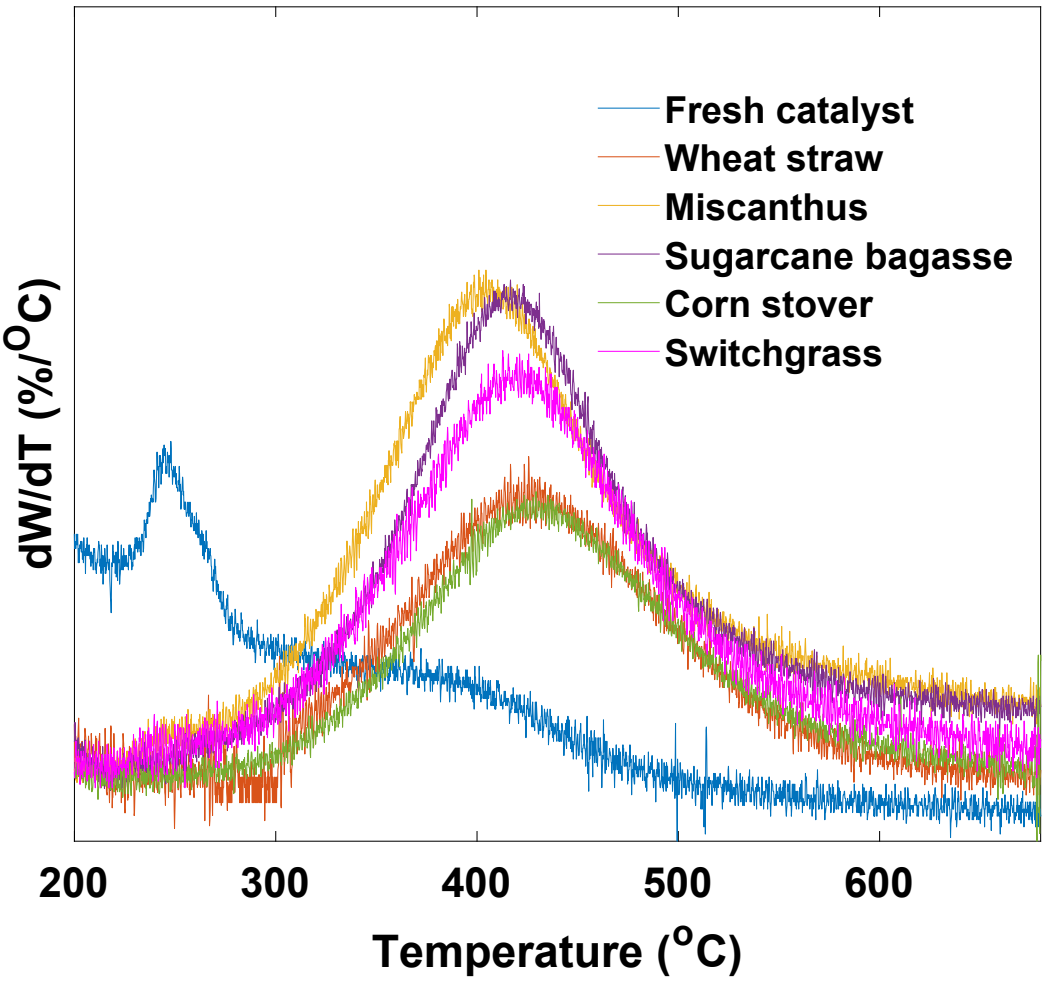

Figure S13. DTG of the fresh Ru/alumina and recovered spent catalysts from RCF of different herbaceous feedstocks.

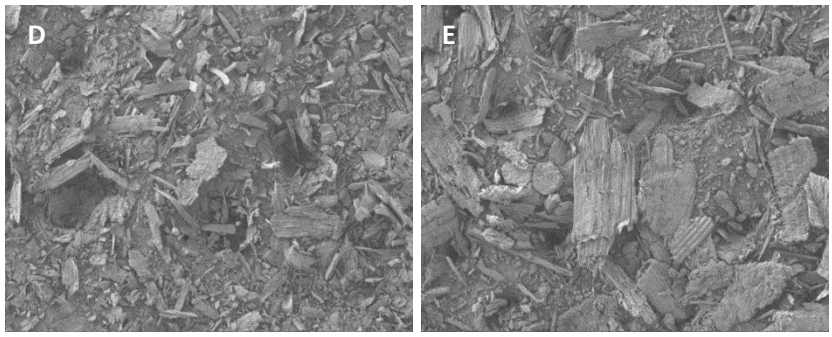

Figure 14. SEM images of residual pulps. A) Corn stover. B) Miscanthus. C) Switchgrass. D) Sugarcane bagasse. E) Wheat straw.

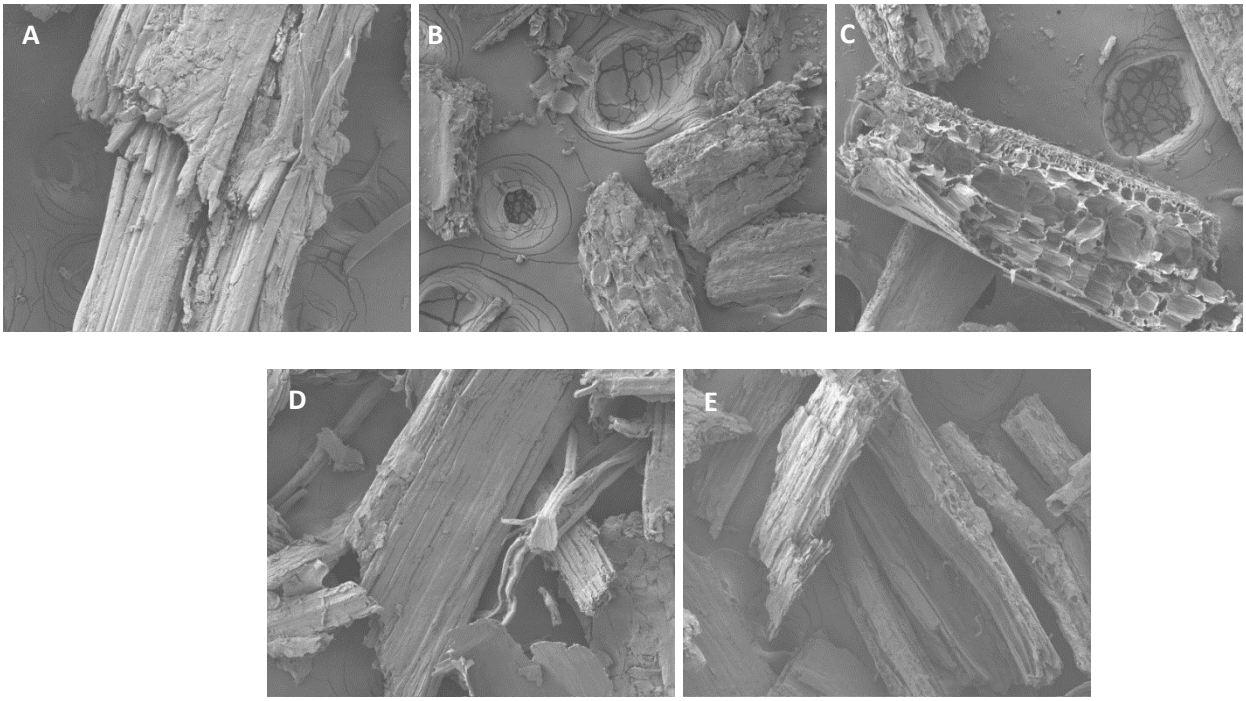

Figure S15. SEM images of herbaceous feedstocks. A) Corn stover. B) Miscanthus. C) Switchgrass. D) Sugarcane bagasse. E) Wheat straw.

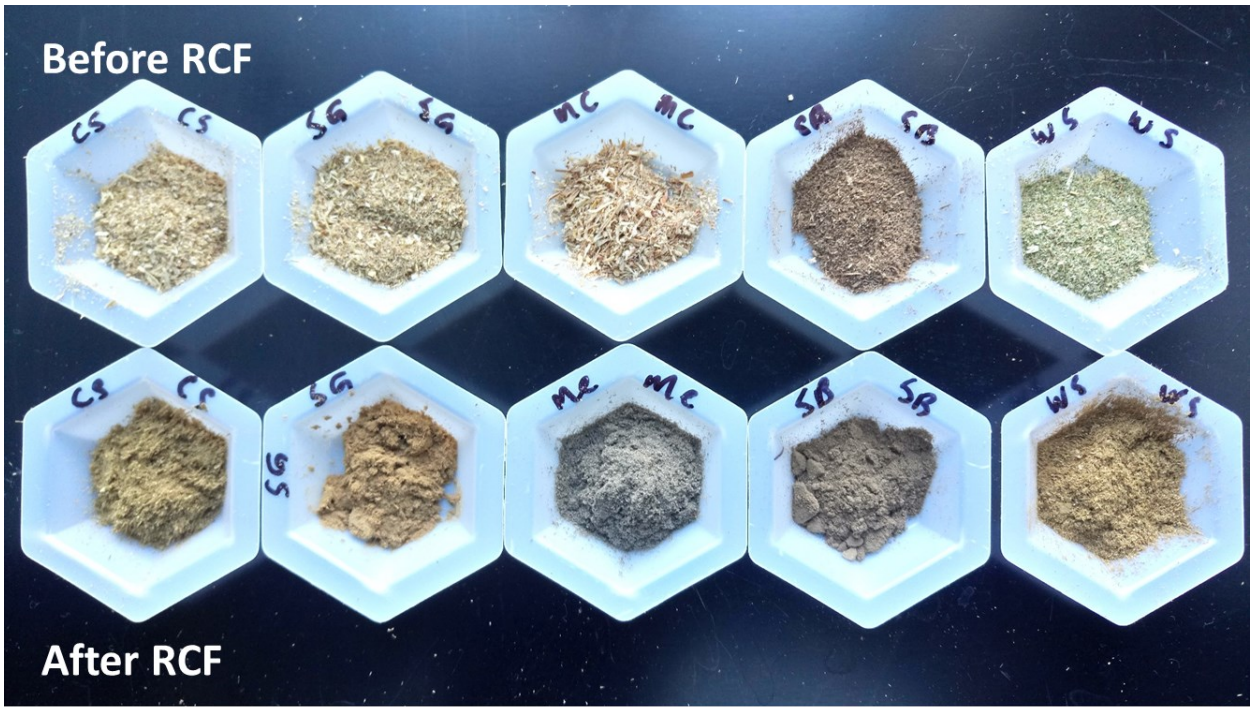

Figure S16. Residual biomass pulp recovered after reductive catalytic fractionation (RCF).

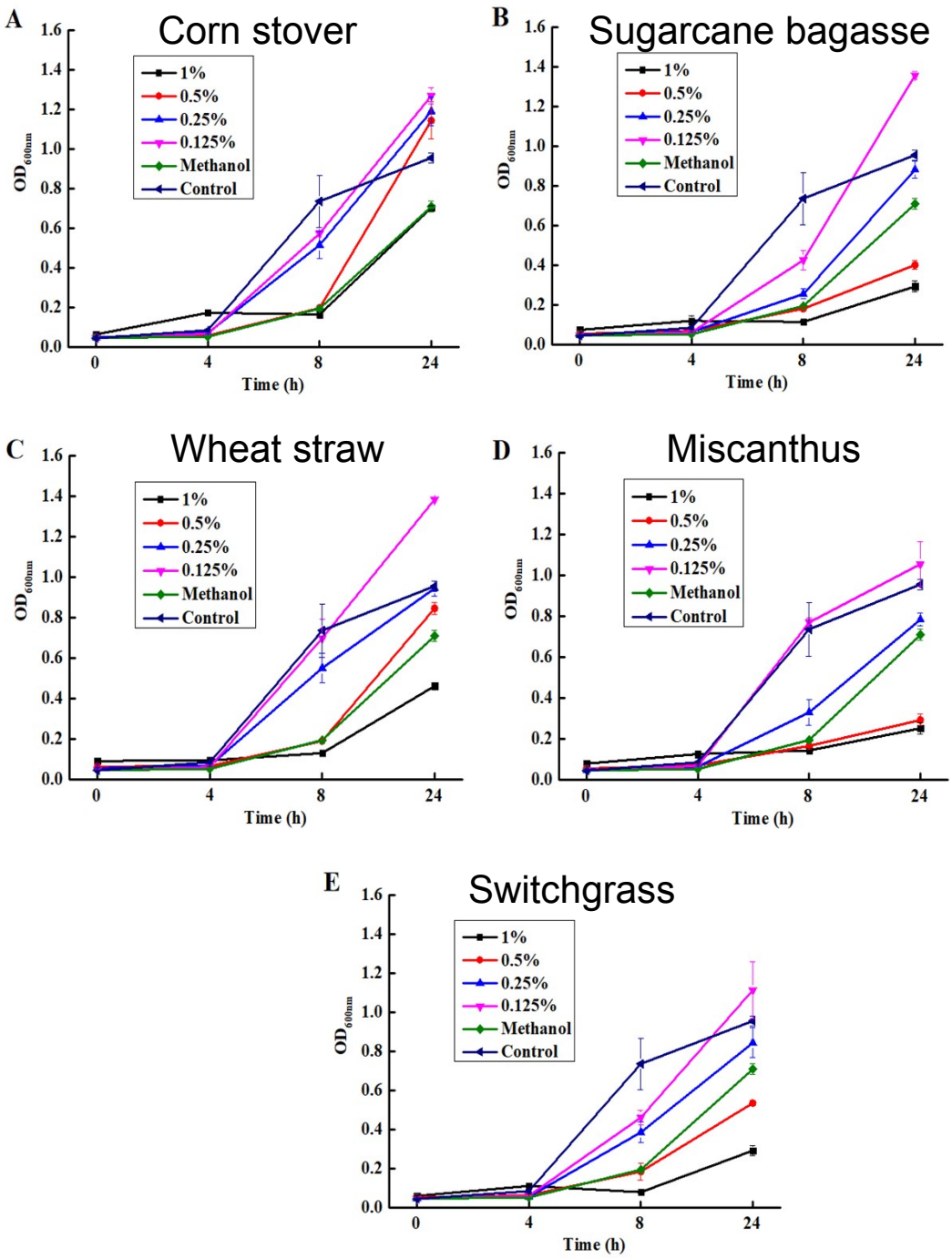

Figure S17. Optical density value of different working solution concentrations at 0, 4, 8, 24 h (A: corn stover; B: sugarcane bagasse; C: wheat straw D: miscanthus; E: switchgrass).

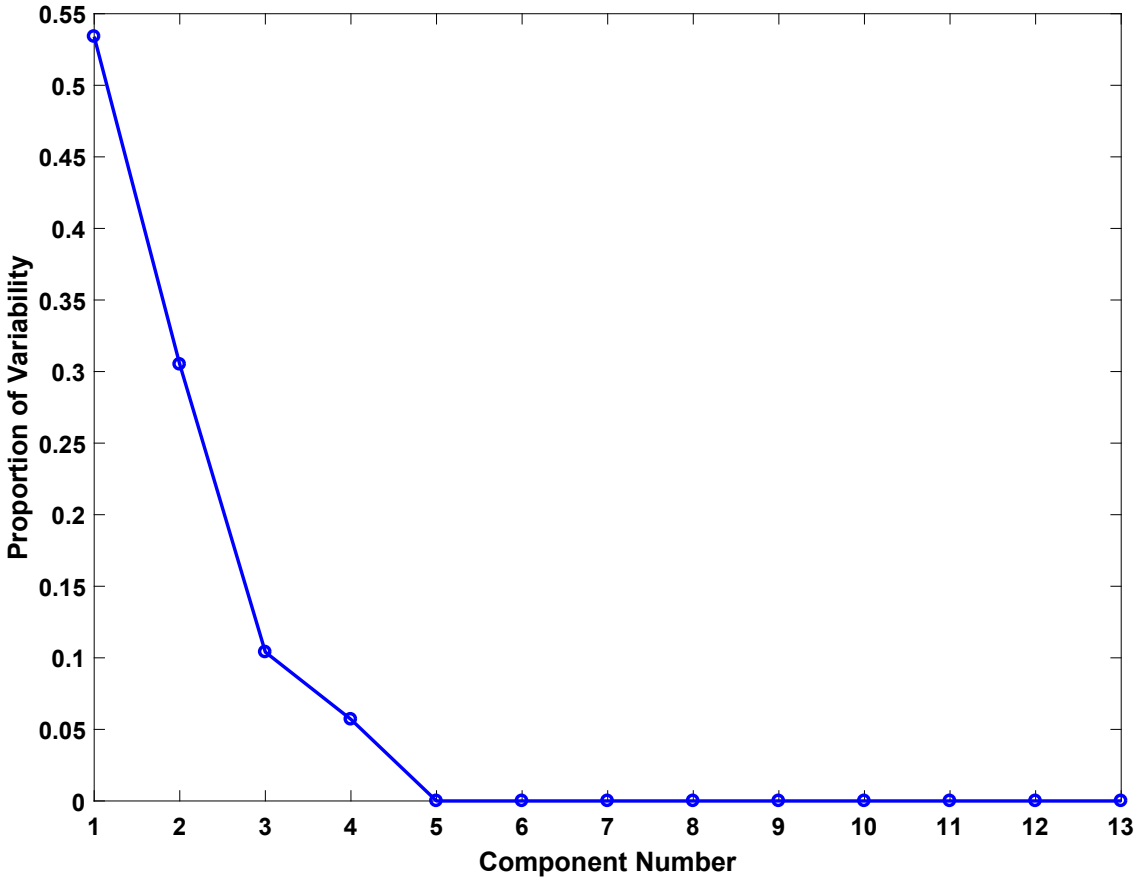

Figure S18. Scree plot showing the contributions of each principal component towards explaining the variance of the dataset.

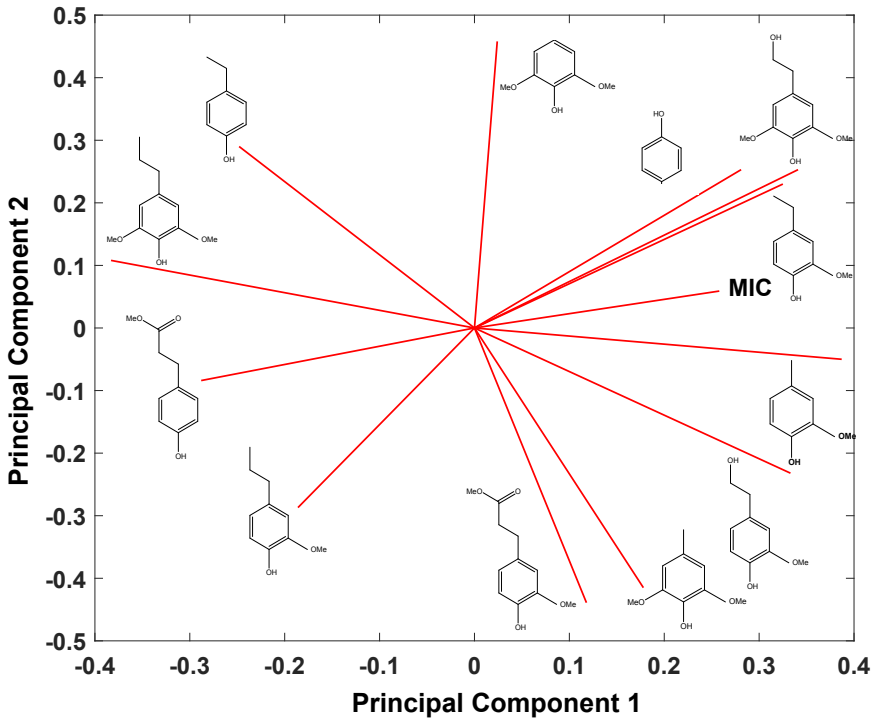

Figure S19. Principal component analysis of the amount of each lignin oil constituent on the oil antimicrobial activity.

Thioacidolysis Method (400 ul) – TMS Derivatization (Quantitative)

- Weigh 2mg of material into a small glass screw-cap vial and record weights
- Wash tube walls with acetone to collect material at the bottom and let dry on the bench overnight. Store the samples in a desiccator while in queue for analysis.
- Carefully prepare the 2.5% BF_3, 10% EtSH, 87.5% Dioxane, 0.05 mg/ml Bisphenol solution in a 100 ml volumetric flask.

  Volumes needed:

  -**10 ml** EtSH

  -**500 µl** 10mg/ml Bisphenol E stock

-2.5 ml BF_3

- Fill up the volumetric flask to **100.0 ml** with dioxane. Mix well before filling into the neck, fill up, then decant the contents into a screw cap bottle for dispensing.
- Add **1000µl** of Dioxane mix solution to each sample
- Gently purge vial headspace with nitrogen gas for **10 seconds** then cap immediately with PTFE lined cap
- Heat at **100 °C** for **4 hours**
- End reaction by cooling on ice for **5 minutes**
- Vortex Samples and allow material to settle
- Transfer **400µl** of reaction to culture tube
- Add **250ul** of [1M] Sodium Bicarbonate to neutralize BF3 (should be ~ pH 7)
- Add **100ul** of 2N HCl to acidify aqueous layer (should be ~ pH 1)
- Add **1000 µl** of Water and **800 µl** of EtOAc
- Vortex at **speed 6**
- Add **1500 µl** of Water to increase volume for electronic pipetting
- Let stand for 10 minutes to allow phases to separate
- Transfer **100 µl** of the EtOAc layer into a GC vial with insert
- In the following order, add **10 µl** pyridine, and **50 µl** N-O-bis(trymethylsilyl) acetamide
- Cap and mix vials by inverting, load onto GC, and allow 2 hours of derivatization time
- For 2 hr delay, run a solvent blank with the method "2hourDelay"
- Run Samples with GC Method "SGH – Aryl Glycerol Curve" using a 15-meter Supelco SLB-5MS column.
- Quantitate Samples with Method "SGH Quant (400ul Method) 2019 July 03"

- For GC wash solvents, use ethyl acetate in both vials
- Average BPE Signal for ion 343 should be ~ 5 million counts

Reagent Abbreviations:

EtOAc = Ethyl Acetate

BF_3 = Boron Trifuoride Diethyl Etherate

EtSH = Ethanethiol