Aldehydes-aided lignin-first deconstruction strategy for facilitating lignin monomers and fermentable glucose production from poplar wood

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Appendix A. Supplementary material

Chemicals

All the reagents were purchased from Sigma Chemical Co. (Beijing, China), except for cellulase (Cellic@ CTec2, 100 FPU/ml), which was kindly provided from Novozymes (Beijing, China). 5% Ru on carbon catalyst (Evonik Noblyst® P3060 5% Ru), methylguaiacol (2-methoxy-4-methylphenol, >98%), ethylguaiacol (4-ethyl-2-methoxyphenol, >98%), propylguaiacol (2-methoxy-4-propylphenol, >99%), guaiac-aldehyde (4-ethoxy-3-methoxybenzaldehyde, >98%), methylvanillate (4-Hydroxy-3- methoxy-benzoic acid methyl ester, >97%), propylsyringol (2,6-dimethoxy-4-propylphenol, 95%), propionaldehydsyringol [3-(4-Hydroxy-3,5-dimethoxyphenyl)-propionaldehyde, 95%], allylsyringol (4-allyl-2,6-dimethoxyphenol, 95%), ethanonesyringol [1-(4-Hydroxy-3,5-dimethoxy-phenyl)-ethanone, 95%], 1,4-dioxane (99%), formaldehyde solution (36.5 wt % in H_2O), acetaldehyde solution (40 wt% in H_2O), sodium acetate, acetic acid, sulfuric acid and fuming hydrochloric acid (37%), all were analytical reagents. Methanol (>99%) and tetrahydrofuran (THF, >99%) were chromatographic grade reagent. Dimethylsulfoxide was deuterium reagent. All the reagents were used without further purification.

Solvents

Sodium acetate buffer: 2.1 g sodium acetate was added into 500 mL deionized water, and stirred until dissolved. The buffer was prepared by adjusting the pH to 4.8 with acetic acid.

The analysis of sugar

The filtrate of enzymatic hydrolysis was diluted with ultrapure water, and filtered through a 0.45 μm water phase needle filter (Jinteng, Tianjin; Diameter:13 mm,

Aperture pore, 0.45 μm; Texture, PES). The final filtrate was directly added into injected bottle for HPAEC detection.

HPAEC system (Dionex ICS5000) with pulsed amperometric detector and an ion exchange Carbopac PA-1 column (4×250 mm). The neutral sugars were separated in 18 mM NaOH (carbonate free and purged with nitrogen) with post column addition of 0.3 M NaOH at a rate of 0.5 mL/min. Run time was 45 min, followed by a 10 min elution with 0.2 M NaOH to wash the column and then a 15 min elution with 18 mM NaOH to re-equilibrate the column. Calibration was performed with a standard solution of L-arabinose, L-glucose, L-galactose, D-mannose, D-xylose, glucuronic acid, and galacturonic acids. Measurements were conducted with two parallels, and reproducibility of the values was found within the range of 5%. The content of sugar was calculated as follow,

$$\text{Sugar\%} = \frac{\text{C} \times \text{Dilute fold} \times \text{Volume} \times \text{Conversion fraction}}{\text{m} \times \text{Sugar\% in substrate}}$$

Where, C, the concentration of sugar calculated by HPAEC system, mg/L;

Dilute fold, dilute with ultrapure water to ensure the result of HPAEC system within 25 mg/L;

Volume, the total volume of filtrate, mL;

Conversion fraction, 0.9for hexose, 0.88 for pentose;

m, the mass of substrate, mg;

Sugar% in substrate, obtained from component analysis of substrate;

Component analysis of substrate

3 mL 72%H_2SO_4 was added into a hydrolysis of bottle with 300 mg substrate and hydrolyzed in a water bath at 30 °C for 1 h, stirred every 10 minutes to make it hydrolyzed as completely as possible. After the strong acid hydrolysis, 84 mL of deionized water was added to reduce the concentration of H_2SO_4 to 4%, and the bottle was placed in a autoclave at 121 °C for 1 h. After the reaction, the supernatant was filtered and used for the determination of sugar content with HPAEC.

Correction

The yield of lignin fractions was corrected to exclude the impact of the attached aldehydes according to the results of 2D-HSQC NMR.

Mass of the monolignols with solvent incorporation.

	No incorporation	FA	AA
S	226	240	254
G	196	210	224

Correction factors of the monolignols with different aldehydes

Correction factor	FA	AA
S	0.0619	0.1239
G	0.0714	0.1429

The correction yield of lignin during the lignin-first strategy (%)

	L_{FA}	L_{AA}	$L_{BM\text{-}FA}$	$L_{BM\text{-}AA}$
Correction yield	68.0	85.1	82.5	86.5

Based on the total lignin in the biomass

Table S1 Assignments of ^{13}C-^{1}H cross-signals in the HSQC spectra of lignin obtained from different conditions

Labels	δ_C/δ_H ($L_{Control}$)	δ_C/δ_H (L_{FA})	δ_C/δ_H (L_{AA})	Assignments
B_β	53.5/3.02	53.5/3.02	53.5/3.02	C_β-H_β in resinol substructures (B)
-OCH_3	55.5/3.69	55.5/3.69	55.5/3.69	C-H in methoxyls
A_γ	59.5/3.70 and 3.56	59.5/3.70 and 3.56	59.5/3.70 and 3.56	C_γ-H_γ in β-*O*-4 substructures (A)
A'_γ	64.1/4.47	64.1/4.47		C_γ-H_γ in γ-acylated β-*O*-4 substructures (A)
A''_γ		68.2/3.99 and 3.67	68.2/3.99 and 3.67	C_γ-H_γ in shifted β-*O*-4 substructures (A)
B_γ	71.4/4.17 and 3.86	71.4/4.17 and 3.86	71.4/4.17 and 3.86	C_γ-H_γ in resinol substructures (B)
A_α	71.7/4.85	71.7/4.85	71.7/4.85	C_α-H_α in β-*O*-4 substructures (A)
A''_α		73.4/4.24	73.4/4.24	C_α-H_α in shifted β-*O*-4 substructures (A)
A_β(G)	83.5/4.41		83.5/4.41	C_β-H_β in β-*O*-4 linked to a G/H unit (A)
A_β''(G)		81.7/4.48	81.7/4.48	C_β-H_β in shifted β-*O*-4 linked to a G unit (A)
B_α	84.9/4.62	84.9/4.62	84.9/4.62	C_α-H_α in resinol substructures (B)
A_β(S)	86.0/4.12			C_β-H_β in β-*O*-4 linked to a S unit (A)
$S_{2,6}$	103.5/6.62	103.5/6.62	103.5/6.62	$C_{2,6}$-$H_{2,6}$ in syringyl units (S)
$S'_{2,6}$	106.2/7.27			$C_{2,6}$-$H_{2,6}$ in oxidized(C=O) phenolic syringyl units (S)
G_2	110.6/6.91	110.6/6.91		C_2-H_2 in guaiacyl units (G)
G_5	114.9/6.76	114.9/6.76	114.9/6.76	C_5-H_5 in guaiacyl units (G)
G_6	118.8/6.77	118.8/6.77	118.8/6.77	C_6-H_6 in guaiacyl units (G)
$PB_{2,6}$	131.2/7.66	131.2/7.66	131.2/7.66	$C_{2,6}$-$H_{2,6}$ in *p*-hydroxybenzoate units (S)

Table S2 Detailed yields of the main aromatic products from the lignin depolymerization reaction over different conditions (based on the weight of starting lignin)

Entry[a]	**A**	**B**	**C**	**D**	**E**
Lignin	$L_{Control}$	L_{FA}	L_{AA}	L_{FA}	L_{FA}
Solvent	THF	THF [b]	THF	MeOH	Dioxane
Con/w%[c]	16.55	42.57	33.00	33.07	26.58
1	1.16[c] (7.01[d])	ND	1.30 (3.94)	ND [e]	ND
2	1.23 (7.43)	1.15 (2.70)	1.33 (4.03)	1.15 (3.48)	1.22 (4.59)
3	1.41 (8.52)	3.51 (8.25)	2.56 (7.76)	2.68 (8.10)	2.33 (8.77)
4	2.47 (14.93)	2.77 (6.51)	2.73 (8.27)	3.03 (9.16)	2.73 (10.27)
5	1.50 (9.06)	1.15 (2.70)	2.63 (7.97)	1.03 (3.12)	1.08 (4.06)
6	4.20 (25.38)	17.00 (39.93)	15.38 (46.61)	10.81 (32.69)	7.30 (27.47)
7	3.07 (18.55)	13.44 (31.57)	3.42 (10.36)	13.08 (39.56)	8.83 (33.22)
8	ND	1.92 (4.51)	1.96 (5.94)	1.29 (3.90)	1.55 (5.83)
9	1.51 (9.12)	1.63 (3.83)	1.69 (5.12)	ND	1.54 (5.79)

[a] **(A)** $L_{Control}$ degraded in THF as solvent system. **(B)** L_{FA} degraded in THF as solvent system. **(C)** L_{AA} degraded in THF as solvent system. **(D)** L_{FA} degraded in MeOH as solvent system. **(E)** L_{FA} degraded in dioxane as solvent system.

[b] THF, tetrahydrofuran

[c] The conversion ratio of lignin is based on the weight of starting lignin

[d] The selectivity of monomer is based on the total monomer yield

[c] Not detected

Table S3 The main components of the degraded products

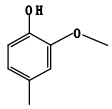
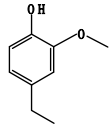
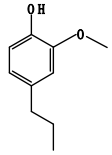
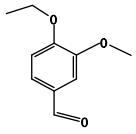
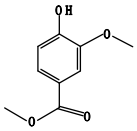
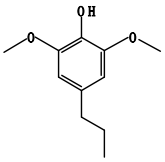
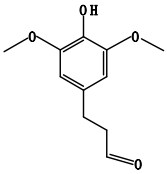
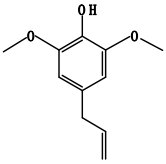
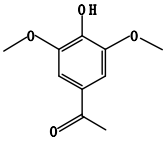
Entry	Retention time (min)	Component	Structure
1	18.455	2-Methoxy-4-methyl-phenol	
2	22.295	4-Ethyl-2-methoxy-phenol	
3	26.216	2-Methoxy-4-propyl-phenol	
4	29.493	4-Ethoxy-3-methoxy-benzaldehyde	
5	32.809	4-Hydroxy-3-methoxy-benzoic acid methyl ester	
6	34.385 (36.026)	2,6-Dimethoxy-4-propyl-phenol	
7	37.406	3-(4-Hydroxy-3,5-dimethoxy-phenyl)-propionaldehyde	
8	39.701	4-Allyl-2,6-dimethoxy-phenol	
9	42.16	1-(4-Hydroxy-3,5-dimethoxy-phenyl)-ethanone	

Table S4 The composition analysis of the control and delignified substrates under different conditions

Sample	Cellulose	Hemicelluloses	Klason lignin	Acid-soluble lignin
Raw	45.82	21.06	21.80	0.98
$R_{Control}$	78.62	6.40	3.19	3.34
R_{FA}	74.53	3.00	7.59	3.76
R_{AA}	89.19	0.68	3.09	2.63
$R_{H\text{-}FA}$	80.25	0.36	13.79	0.54
$R_{H\text{-}AA}$	84.08	0.15	3.71	0.61

Table S5 The glucose yield of the substrates after lignin extraction.

	24 h	48 h
$R_{Control}$	40.10	61.40
R_{FA}	13.28	15.40
R_{AA}	50.38	75.12
$R_{H\text{-}FA}$	28.17	30.38
$R_{H\text{-}AA}$	58.19	85.14

Figure Caption

Fig. S1. The chemical reaction occurs between aldehydes (formaldehyde and acetaldehyde) and α-and γ-OH in the side-chain of lignin.

Fig. S2. GC-MS Chromatographic of the products obtaining from the degradation of different lignin samples and solvent system. (A) $L_{Control}$ degraded in THF as solvent system. (B) L_{FA} degraded in THF as solvent system. (C) L_{AA} degraded in THF as solvent system. (D) L_{FA} degraded in MeOH as solvent system. (E) L_{FA} degraded in dioxane as solvent system.

Fig. S3. XRD spectra of the raw material and treated substrates

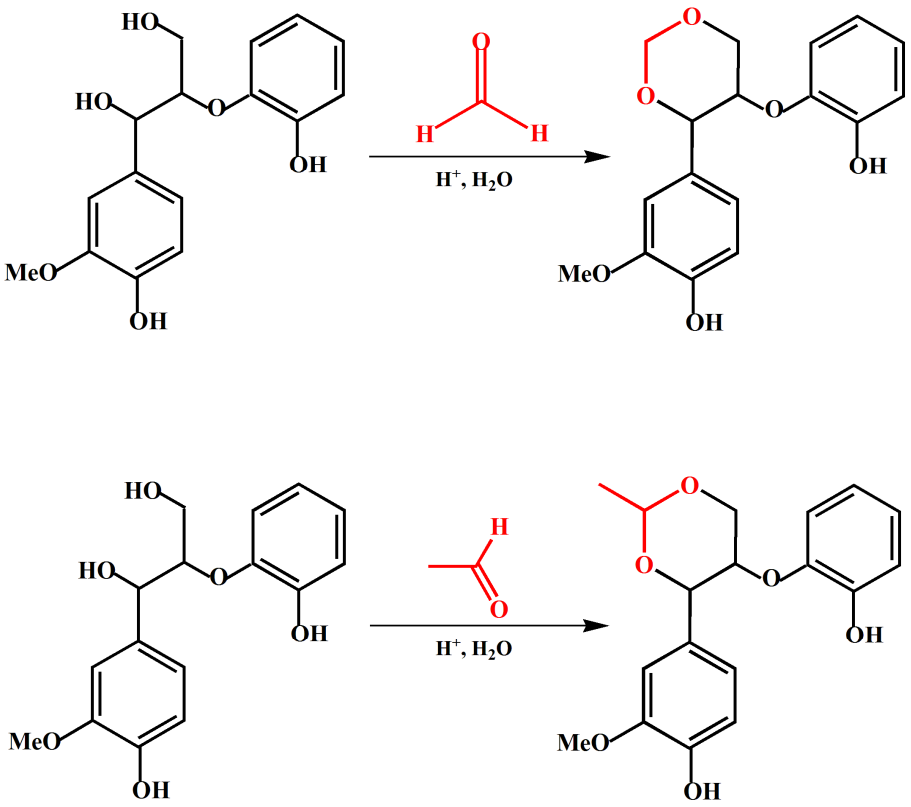

HO
HO
O
OH
MeO
OH
O
H
H
H+, H2O
O
O
O
OH
MeO
OH
HO
HO
O
OH
MeO
OH
H
O
H+, H2O
O
O
O
OH
MeO
OH

Fig. S1

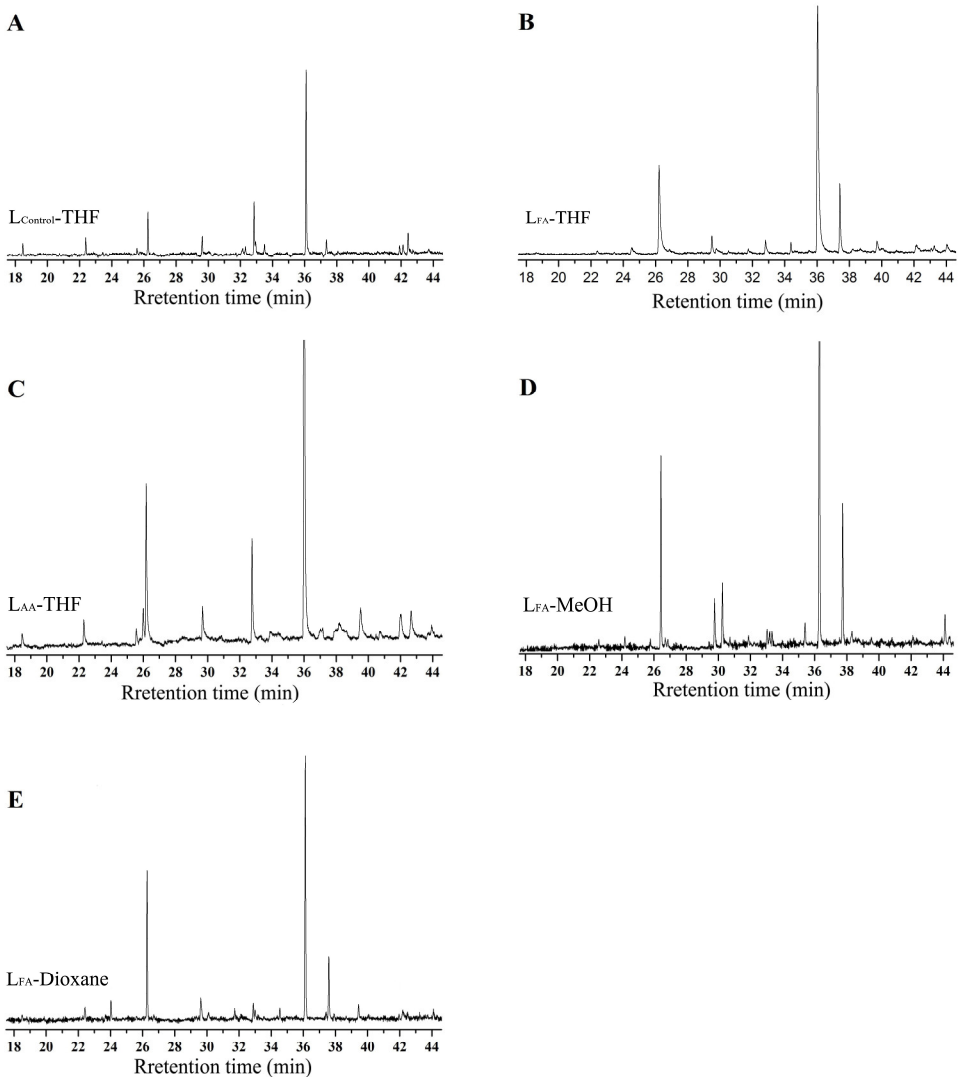

A
LControl-THF
18 20 22 24 26 28 30 32 34 36 38 40 42 44
Rretention time (min)
B
LFA-THF
18 20 22 24 26 28 30 32 34 36 38 40 42 44
Retention time (min)
C
LAA-THF
18 20 22 24 26 28 30 32 34 36 38 40 42 44
Rretention time (min)
D
LFA-MeOH
18 20 22 24 26 28 30 32 34 36 38 40 42 44
Rretention time (min)
E
LFA-Dioxane
18 20 22 24 26 28 30 32 34 36 38 40 42 44
Rretention time (min)

Fig. S2

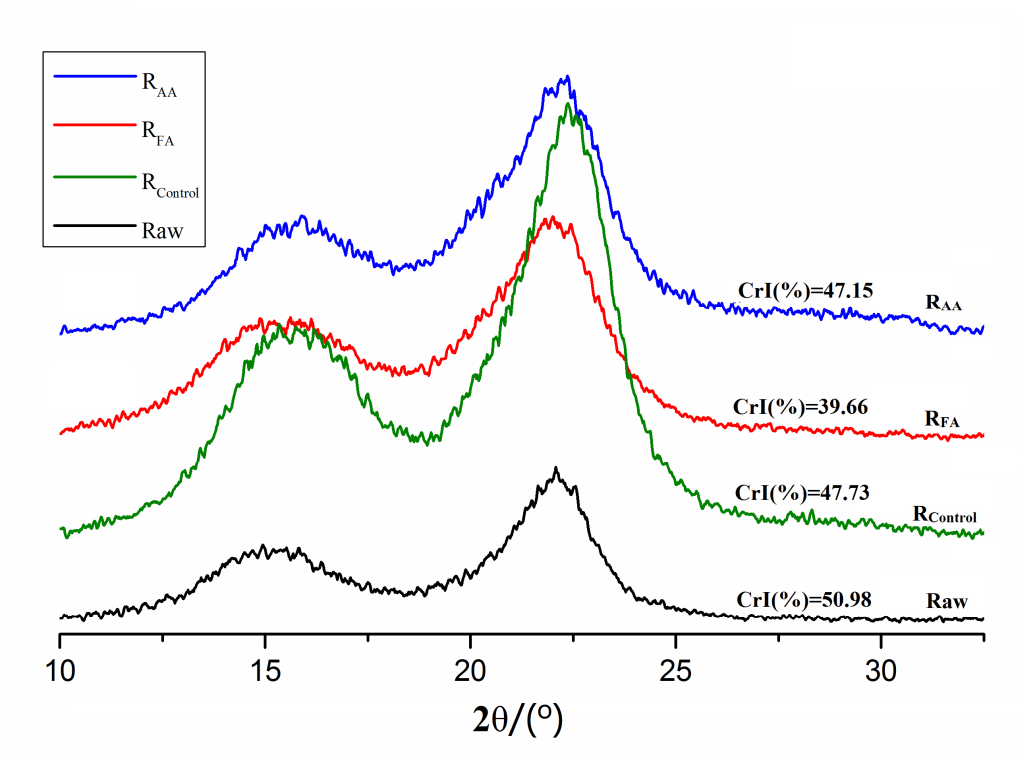

R_AA
R_FA
R_Control
Raw
CrI(%)=47.15
R_AA
CrI(%)=39.66
R_FA
CrI(%)=47.73
R_Control
CrI(%)=50.98
Raw
10
15
20
25
30
2θ/(°)

Fig. S3