Electronic Supplementary Material (ESI) for Green Chemistry.

SUPPORTING INFORMATION

Structural elucidation of whole lignin from *Eucalyptus* based on preswelling and enzymatic hydrolysis

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EXPERIMENTAL SECTION

1.Alkaline Treatment of CEL

To evaluate the effect of mild alkaline treatment (preswelling) on the the lignin structure. In this study, the obtained CEL was selected as a model. The CEL (150 mg) was treated with 4% NaOH for 24 hours at room temperature (S/L, 1g/25ml). After treatments, the solution was neutralized to pH 4.8 and precipitated into acid water (pH=2.0) to obtain alkaline treated CEL (CEL-AT). After centrifugation, purification and lyophilization, 122 mg of CEL-AT was obtained. The obtained lignin was characterized by 2D-HSQC spectrum as compared to the original CEL to investigate the structural changes of lignin during this treatment.

2. XRD and SEM

X-ray diffraction patterns were obtained with an XRD-6000 instrument (Shimadzu, Japan). Changes in morphology before and after different pretreatments were observed by SEM (S-3400N, HITACHI, Japan).

RESULTS AND DISCUSSION

1. Strcutural changes of lignin during alkaline preswelling treatment

As can be seen from Fig S5, the spectra of CEL and CEL-AT were very similar. The detailed assignments of structural units and linkages have been discussed in the text. After quantification of different linkages (Table S1), it was found that the content of β-*O*-4′ and β-β′ is slightly increased, while the content of β-5′ is slightly decreased. In addition, S/G ratio of CEL-AT is increased as compared to that of CEL. The increased S/G ratio is probably because that minor substructure mainly formed by G-type lignin units (β-5′ linkage) were lost during the alkaline treatment, as can be seen from the yield of CEL-AT. Moreover, the higher S/G ratio is also in line with the higher content of β-*O*-4′ linkage. To sum up, mild alkaline treatment has slight effects on the lignin structures.

Table S1. Quantification of the lignins by quantitative 2D-HSQC method: results expressed per 100 Ar.

Sample	**Yield (Mass)**	**β-*O*-4′**	**β-β′**	**β-5′**	**S/G**
CEL	100.0% (150 mg)	55.3	12.0	4.0	1.42
CEL-AT	81.0% (122 mg)	56.5	13.3	3.0	1.52

Table S2. Identification and relative abundance (%) of the lignin derived compounds released after the Py-GC/GS of the lignin polymers

Label	R. T (min)	Formula	Compound	Origin	SREL	CEL	AL
1	7.19	C_6H_6O	Phenol	H	1.24	0.81	1.31
2	8.39	C_7H_8O	Phenol, 2-Methyl	G	0.90	1.04	0.86
3	8.75	C_7H_8O	Phenol, 2-Methyl	G	1.38	0.85	0.85
4	8.93	$C_9H_{10}O_3$	Phenol,4-Methoxy-, Acetate	H	5.63	3.62	6.34
5	10.52	$C_8H_{10}O_2$	Phenol,2-Methoxy-4-Methyl	G	3.86	4.26	0.65
6	10.61	$C_6H_6O_2$	1,2-Benzenediol	G	3.30	3.16	2.95
7	11.50	$C_7H_8O_3$	1,2-Benzenediol, 3-Methoxy	S	5.30	5.48	5.22
8	11.73	$C_9H_{12}O_2$	Phenol, 4-Ethyl-2-Methoxy	G	1.07	1.24	0.69
9	11.94	$C_7H_8O_2$	1,2-Benzenediol,4-Methyl	G	2.46	3.42	1.99
10	12.26	$C_9H_{10}O_2$	2-Methoxy-4-Vinyl-phenol	G	5.88	4.43	4.39
11	12.36	$C_8H_{10}O_2$	3-Methoxy-5-Methyl-phenol	S	0.45	0.32	0.22
12	12.75	$C_8H_{10}O_3$	Phenol,2,6-Dimethoxy-	S	9.40	5.02	11.07
13	12.82	$C_{10}H_{12}O_2$	Eugenol	G	1.56	1.82	0.95
14	12.87	$C_8H_{10}O_3$	Phenol,2,6-Dimethoxy	S	1.35	2.04	1.04
15	13.20	$C_8H_{10}O_2$	4-Ethylcatechol	G	0.58	1.83	0.65
16	13.43	$C_8H_8O_3$	Vanillin	G	3.61	4.33	5.17
17	13.51	$C_{10}H_{12}O_2$	Phenol,2-Methoxy-4-(1-Propenyl)	G	1.18	1.19	1.02
18	13.98	$C_9H_{12}O_3$	1,2,4-Trimethoxy-Benzene	G	5.33	6.49	3.34
19	14.07	$C_{10}H_{12}O_2$	Phenol,2-Methoxy-4-(1-Propenyl)	G	4.00	4.09	3.00
20	14.45	$C_{11}H_{12}O_3$	Ethyl ester-hydroxycinnamic acid	H	0.82	0.74	1.03
21	14.52	$C_9H_{10}O_3$	Ethanone,1-(4-Hydroxy-3-Methoxyphenyl)	G	2.37	3.09	3.07
22	14.59	$C_9H_{10}O_3$	Ethanone,1-(4-Hydroxy-3-Methoxyphenyl)	G	1.31	0.90	0.99
23	14.94	$C_{10}H_{14}O_3$	Benzene, 1,2,3-Trimethoxy-5-Methyl	S	0.91	0.93	0.65
24	15.02	$C_{10}H_{12}O_3$	Phenol,2-Methoxy-4-Propyl	G	1.19	0.92	1.39
25	15.44	$C_{10}H_{12}O_3$	3',5'-Dimethoxy-Aceto-Phenone	S	6.86	5.42	6.47
26	15.84	$C_{11}H_{14}O_3$	Phenol, 2,6-Dimethoxy-4-(2-Propenyl)	S	2.12	2.00	1.50
27	16.25	$C_{10}H_{10}O_3$	2-Propenoic acid, 3-(2-Methoxyphenyl)	G	0.94	1.36	1.09

28	16.43	$C_{11}H_{14}O_3$	Phenol, 2,6-Dimethoxy-4-(2-Propenyl)	S	2.45	2.12	1.40
29	16.56	$C_9H_{10}O_4$	Benzaldehyde,4-Hydroxy-3,5-Dimethoxy	S	4.92	6.78	6.71
30	16.70	$C_{10}H_{12}O_3$	Phenol, 4-(3-Hydroxy-1-Propenyl)-2-Methoxy	G	1.00	1.02	1.61
31	16.80	$C_{11}H_{12}O_3$	3-Buten-2-One, 4-(4-Hydroxy-3-Methoxyphenyl)	G	0.90	1.09	1.21
32	16.86	$C_{11}H_{12}O_3$	3-Buten-2-One, 4-(4-Hydroxy-3-Methoxyphenyl)	G	0.45	0.58	0.63
33	17.02	$C_{11}H_{14}O_3$	Phenol, 2,6-Dimethoxy-4-(2-Propenyl)	S	6.35	7.76	5.83
34	17.36	$C_{10}H_{12}O_4$	Ethanone, 1-(4-Hydroxy-3,5-Dimethoxyphenyl)	S	1.84	2.64	2.26
35	17.43	$C_{10}H_{10}O_3$	2-Propenal,3-(4-Hydroxy-3-Methoxyphenyl)	G	1.14	2.31	2.47
36	17.47	$C_{10}H_{12}O_3$	Phenol, 4-(3-Hydroxy-1-Propenyl)-2-Methoxy-	G	3.80	2.91	6.04
37	17.76	$C_{10}H_{12}O_4$	Ethanone,1-(4-Hydroxy-3,5-Dimethoxyphenyl)	S	0.34	0.17	0.59
38	18.03	$C_{12}H_{16}O_3$	1,2-Dimethoxy-4-(3-Methoxy-1-Propenyl)-Benzene	G	0.25	0.36	0.42
39	18.37	$C_{11}H_{12}O_4$	3,5-Dimethoxy-4-Hydroxy-cinnamaldehyde	S	0.65	0.65	0.60
40	19.93	$C_{11}H_{12}O_4$	3,5-Dimethoxy-4-Hydroxy-cinnamaldehyde	S	0.87	0.75	2.21
				H%	7.69	5.17	8.68
				G%	48.50	52.75	45.53
				S%	43.81	45.43	45.89
				S/G	0.90	0.86	1.01

Figure Captions

Fig. S1. The overall scheme for the isolation of swollen residual enzyme lignin (SREL)

Fig. S2. XRD patterns of ball-milled wood and preswelled ball-milled wood

Fig. S3. SEM images of ball-milled wood (A) and preswelled ball-milled wood (B)

Fig. S4. The dissolving experiment of different residues after enzymatic hydrolysis of preswelled and ball-milled wood (photograph)

Fig. S5. 2D HSQC NMR spectra of the CEL and CEL-AT

Fig. S6. 2D HSQC NMR spectra of the SREL, CEL, and AL (Amplifying side-chain)

Fig. S7. 2D HSQC NMR spectra of the SREL and cellulase

Fig. S8. Main classical substructures, involving different side-chain linkages, and aromatic units identified by 2D HSQC NMR of the lignins. (A) β-O-4′ aryl ether linkages with a free -OH at the γ-carbon; (B) resinol substructures formed by β-β′, α-O-γ′, and γ-O-α′ linkages; (C) phenylcoumarane substructures formed by β-5′ and α-O-4′ linkages; (D) spirodienone structures formed by β-1′ linkages; (I) *p*-hydroxycinnamyl alcohol end groups; (S) syringyl units; (S′) oxidized syringyl units with a Cα ketone; (G) guaiacyl units; (H) *p*-hydroxyphenyl units.

Fig. S9. Py-GC/MS chromatogram of the lignin samples. The identifies and relative abundances of the released compounds are listed in Table S2.

Fig. S10. Different aromatic compound structures of H-, G-, and S-type lignin units.

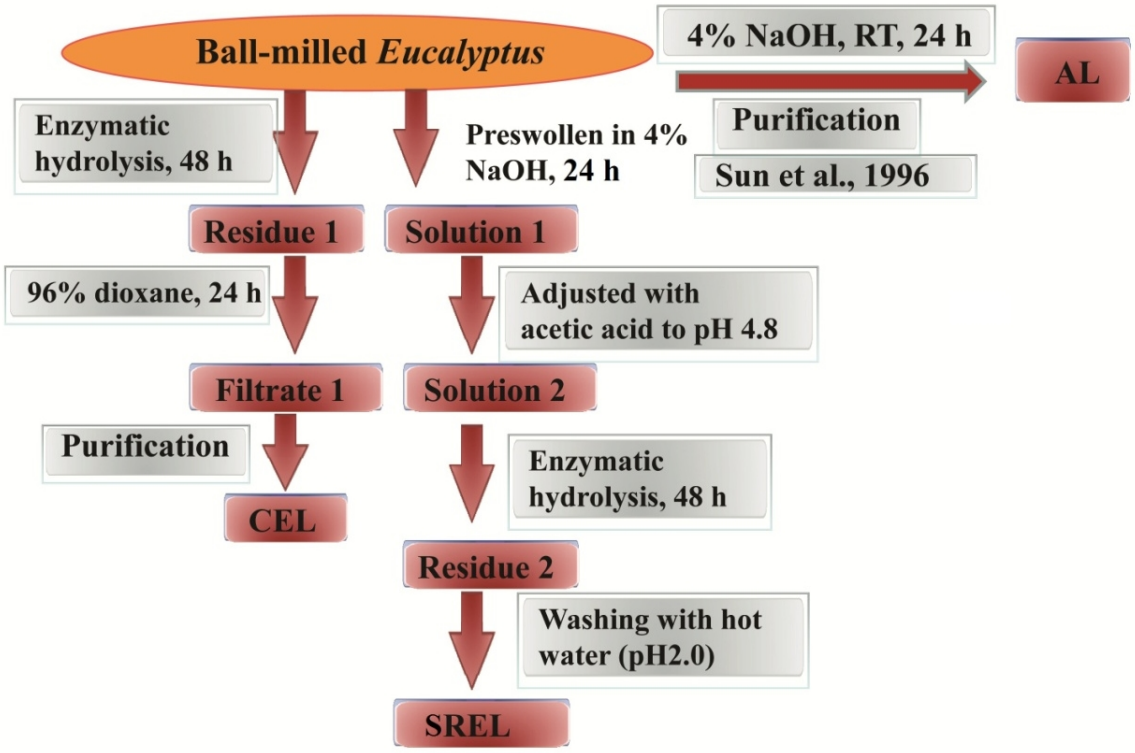

Ball-milled *Eucalyptus*
4% NaOH, RT, 24 h
AL
Purification
Sun et al., 1996
Enzymatic hydrolysis, 48 h
Preswollen in 4% NaOH, 24 h
Residue 1
Solution 1
96% dioxane, 24 h
Adjusted with acetic acid to pH 4.8
Filtrate 1
Solution 2
Purification
CEL
Enzymatic hydrolysis, 48 h
Residue 2
Washing with hot water (pH2.0)
SREL

Fig. S1.

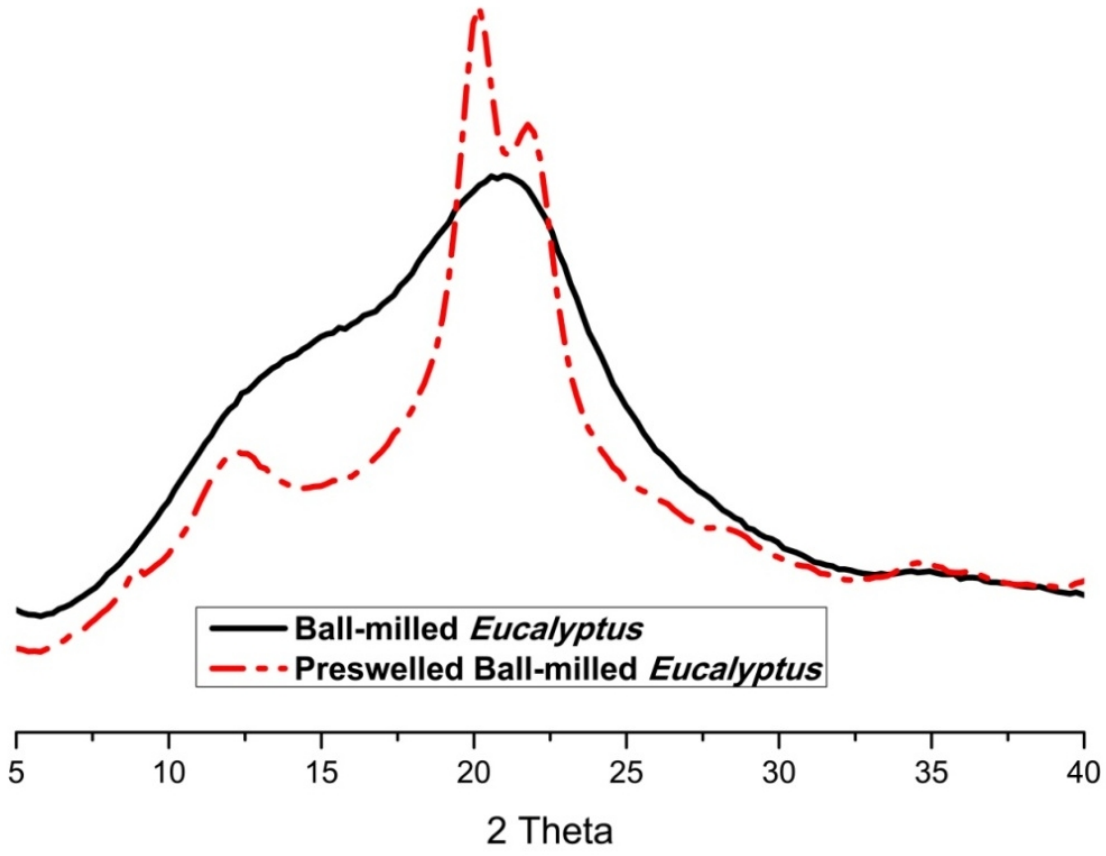

Ball-milled *Eucalyptus*
Preswelled Ball-milled *Eucalyptus*
5
10
15
20
25
30
35
40
2 Theta

Fig. S2.

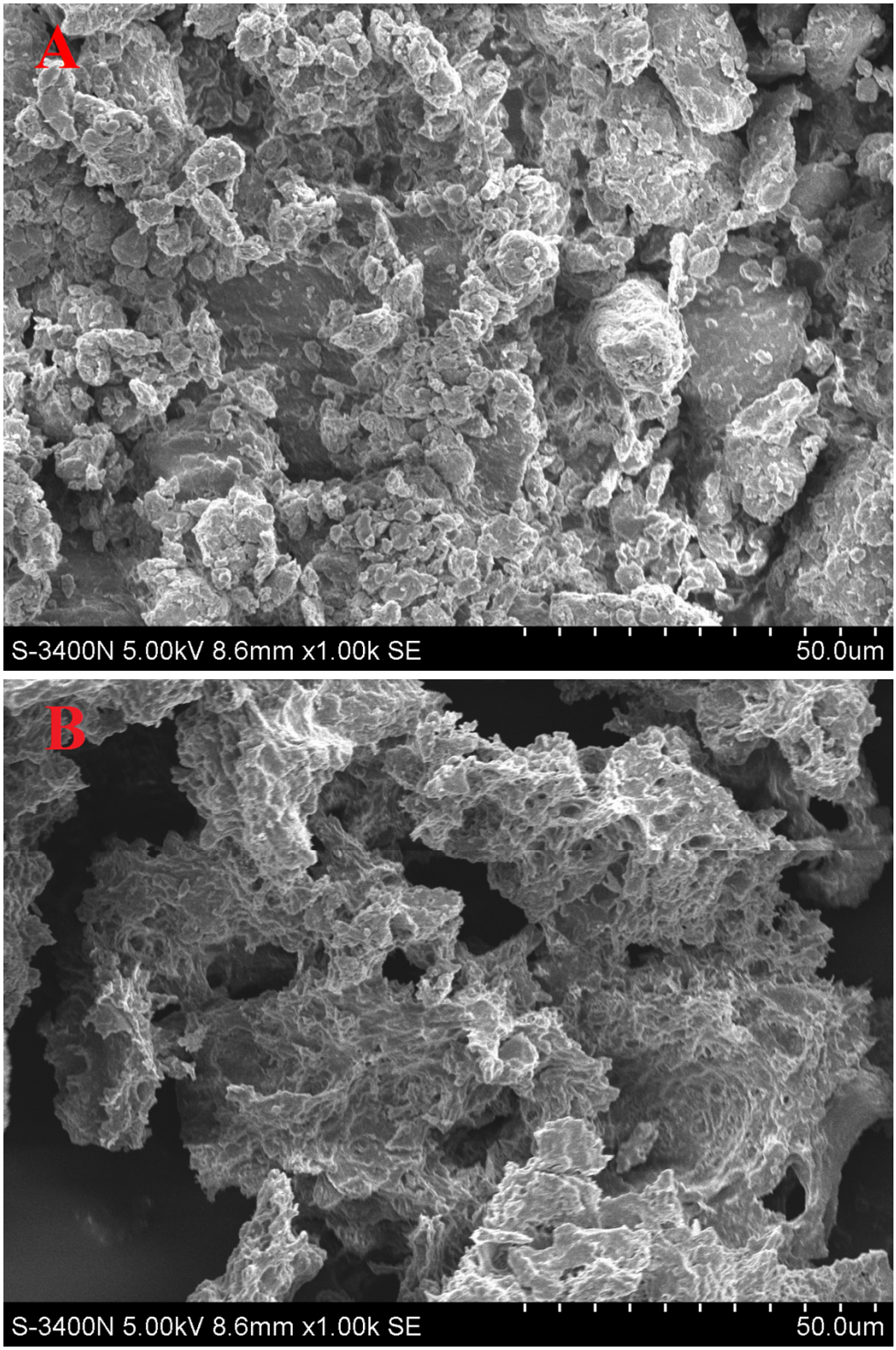

A
S-3400N 5.00kV 8.6mm x1.00k SE
50.0um
B
S-3400N 5.00kV 8.6mm x1.00k SE
50.0um

Fig. S3.

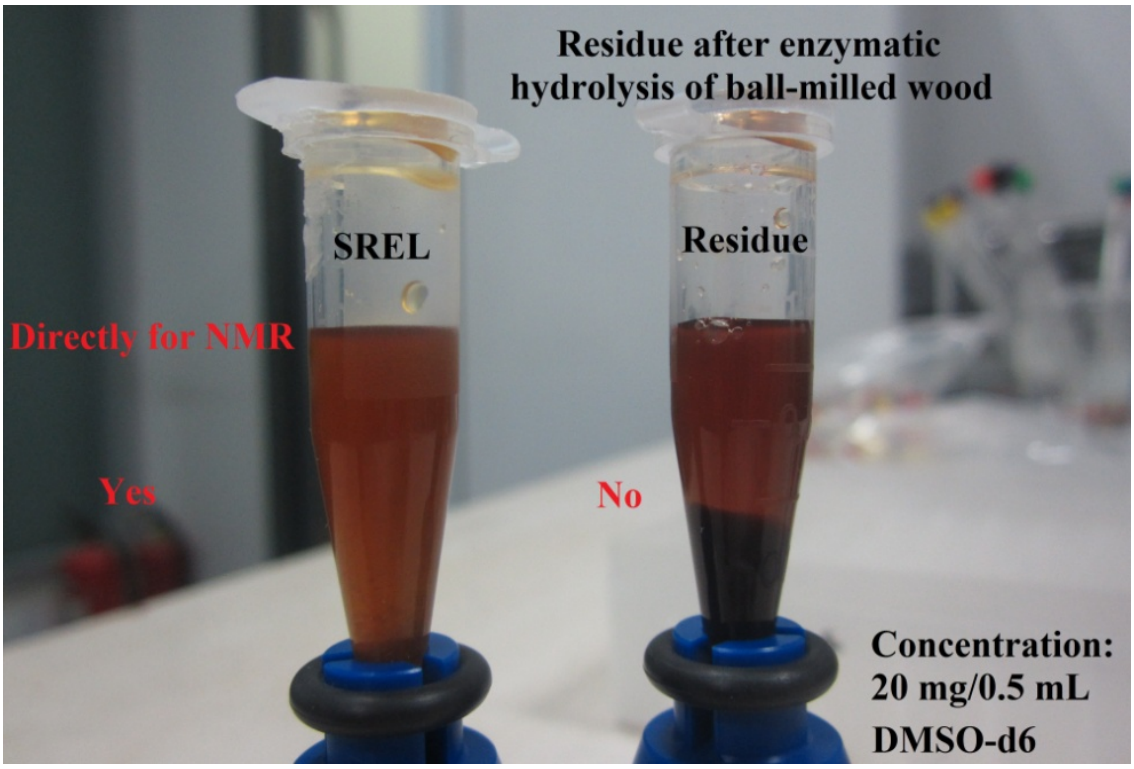
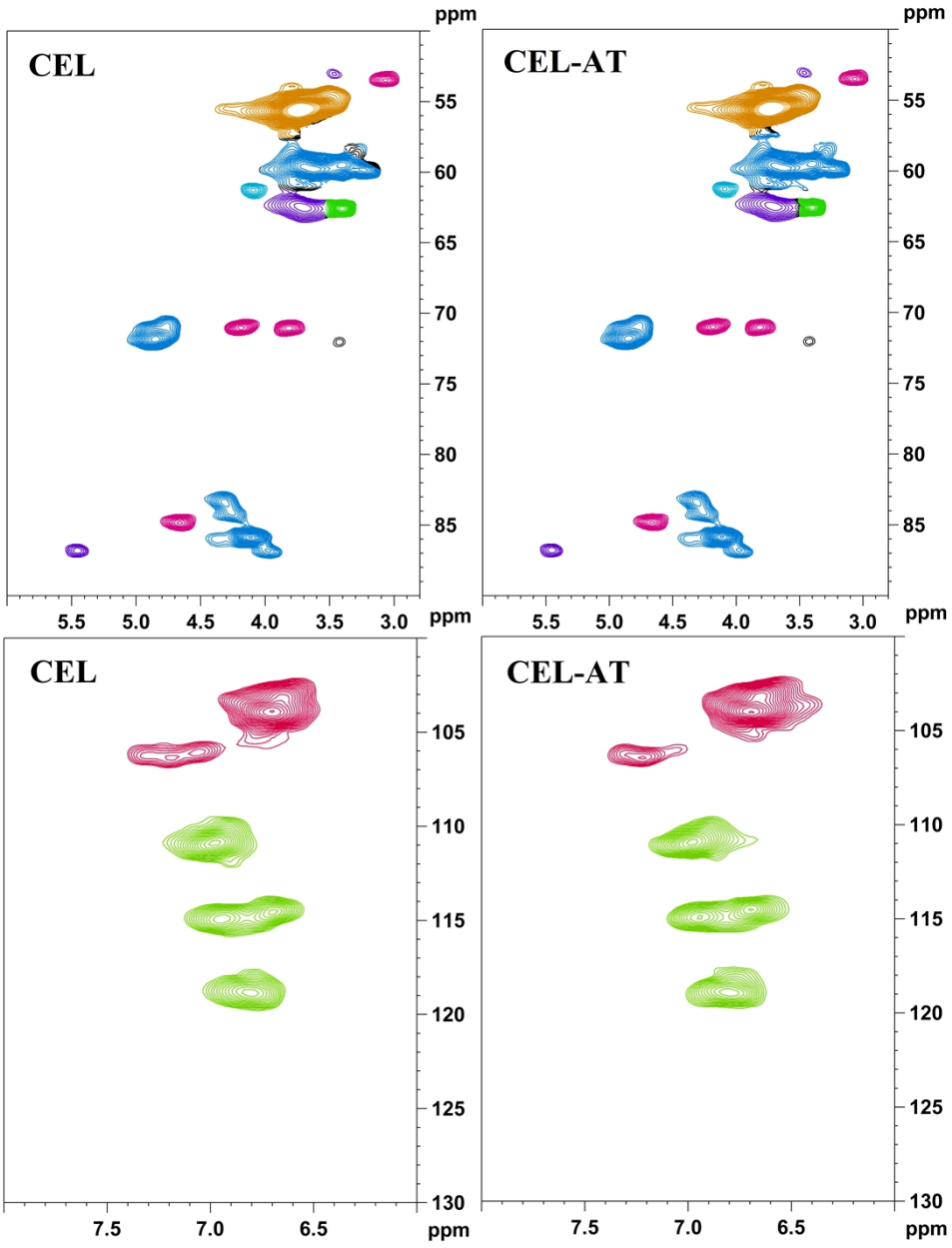

Residue after enzymatic
hydrolysis of ball-milled wood
SREL
Residue
Directly for NMR
Yes
No
Concentration:
20 mg/0.5 mL
DMSO-d6

Fig. S4.


CEL
CEL-AT
ppm
55
60
65
70
75
80
85
5.5
5.0
4.5
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105
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115
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125
130
7.5
7.0
6.5

Fig. S5.

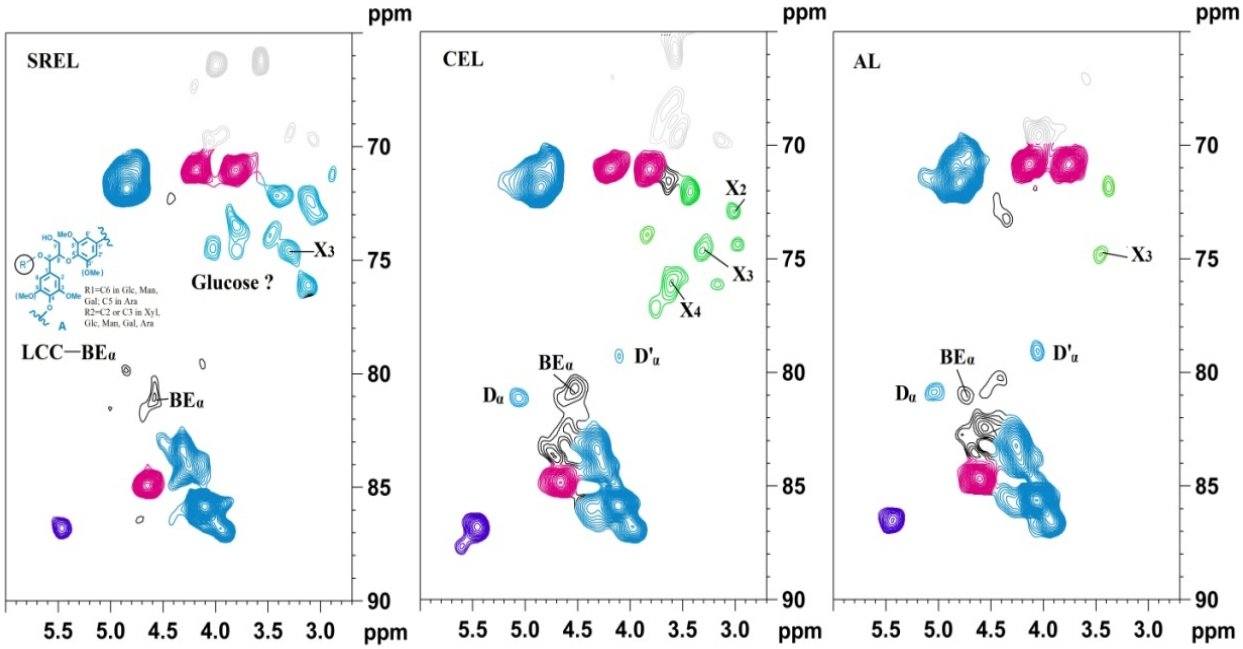
SREL
CEL
AL
ppm
70
75
80
85
90
5.5
5.0
4.5
4.0
3.5
3.0
X3
Glucose ?
LCC—BEα
BEα
X2
X4
Dα
D'α
R1=C6 in Glc, Man, Gal; C5 in Ara
R2=C2 or C3 in Xyl, Glc, Man, Gal, Ara

Fig. S6.

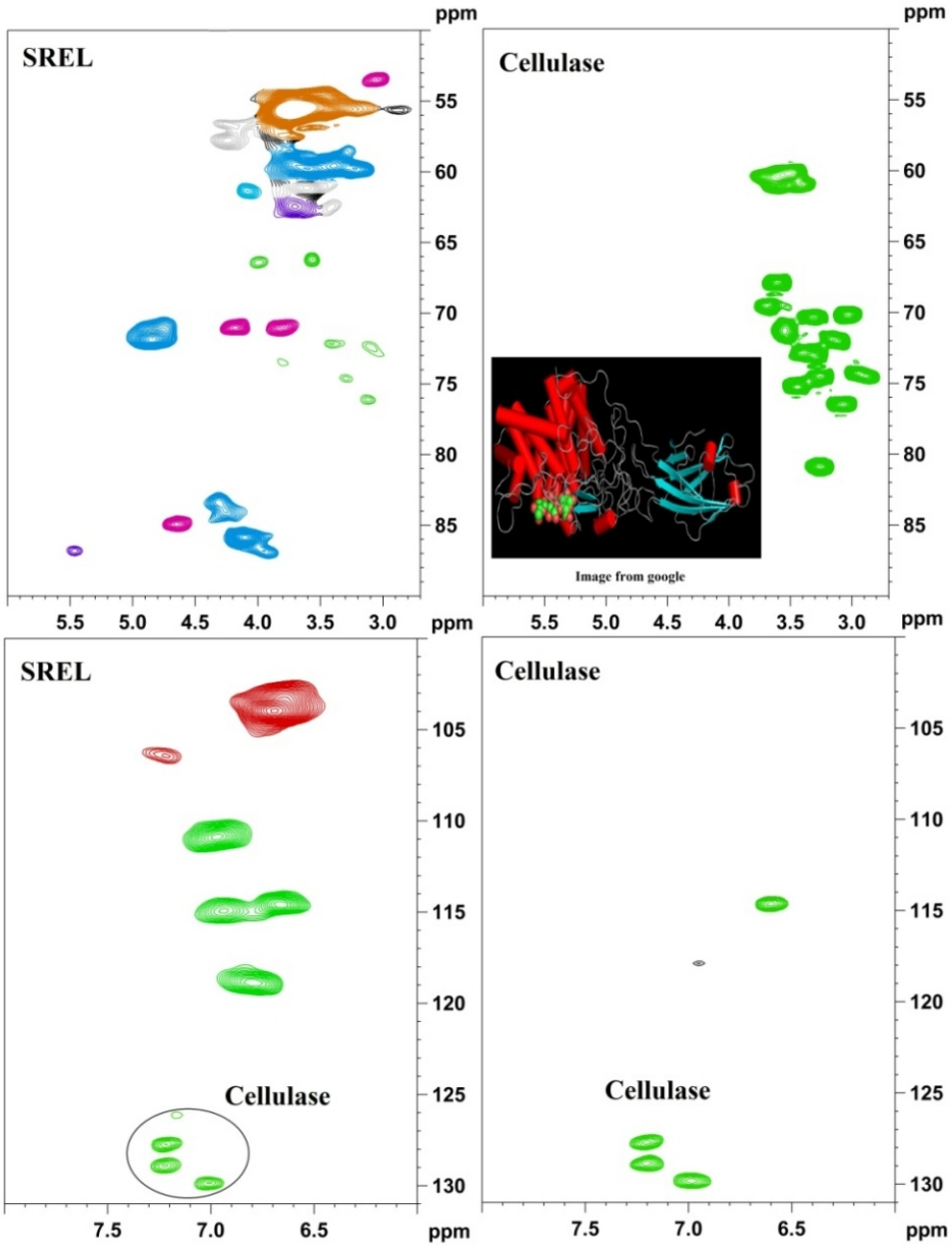
SREL
Cellulase
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Image from google
SREL
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Fig. S7.

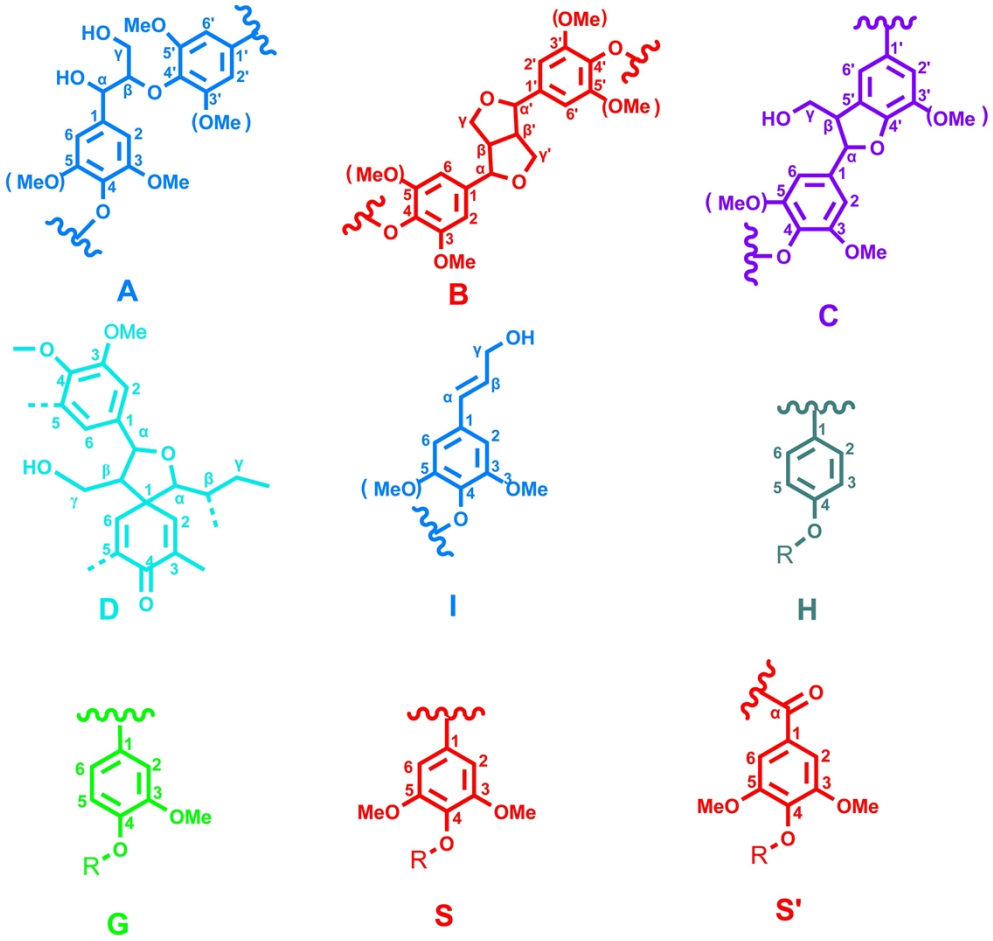

Fig. S8.

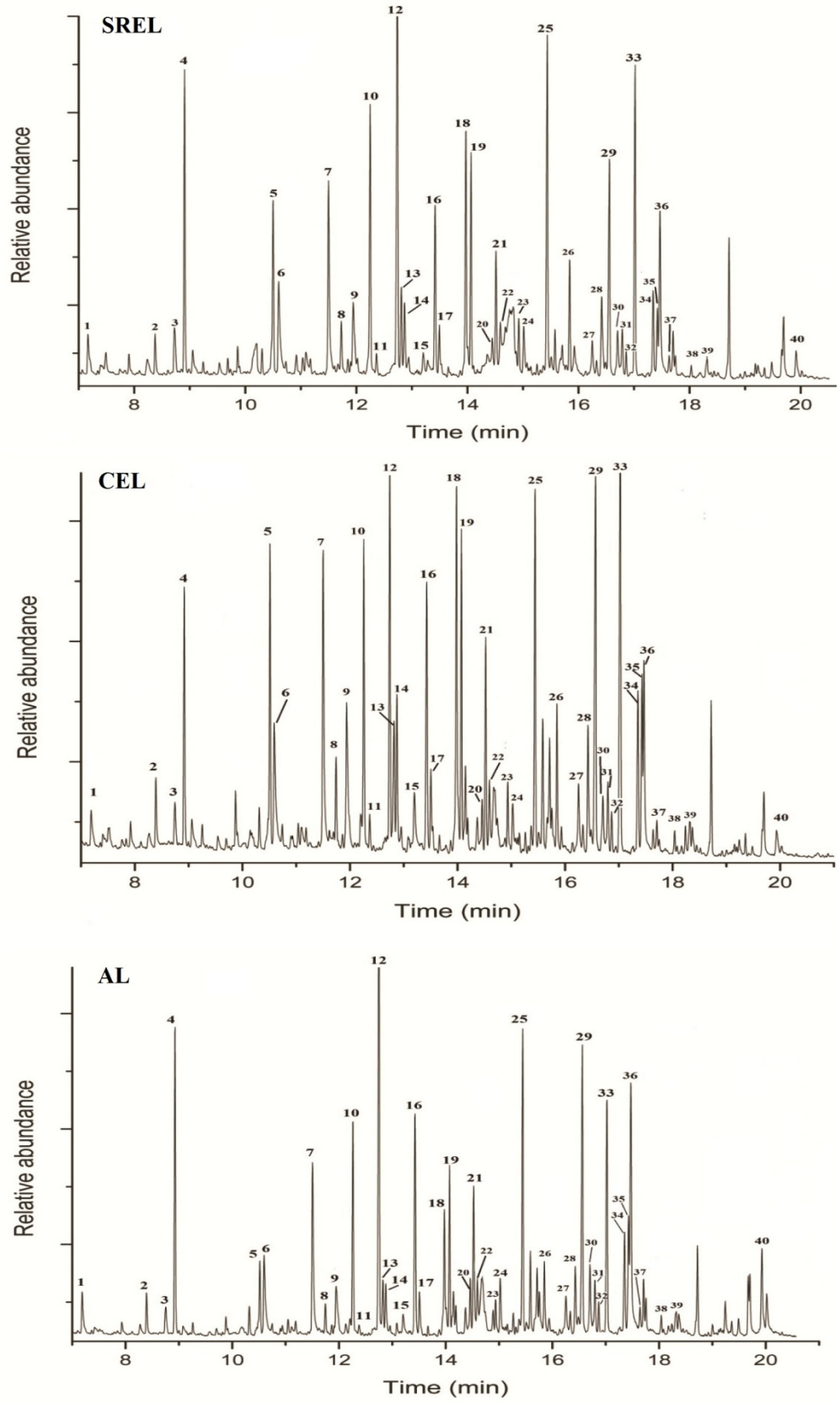

Fig. S9.

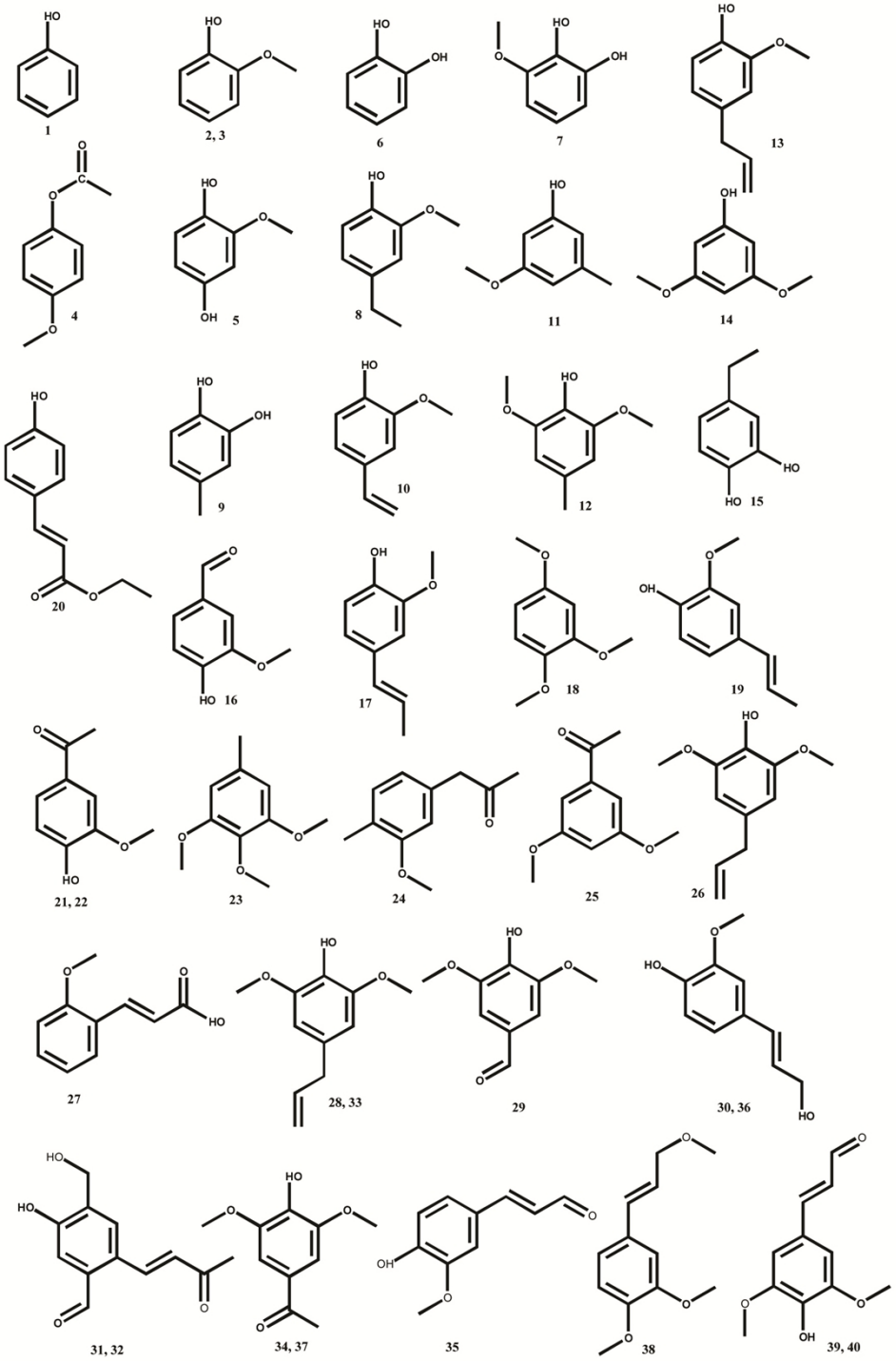
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Fig. S10.