

Supplementary Information

**Lignin-stabilizing fractionation enables production of
uncondensed lignin and digestible carbohydrates**

Shi-Chang Liu^{a b c d}, Zi-Jing He^{a b c}, Guo-Qiang Kang^{a b c}, Kai Chen^{a b c},

Zhi-Hua Liu^{a b c}, Bing-Zhi Li^{a b c *}

^a State Key Laboratory of Synthetic Biology, Tianjin University, Tianjin, China

^b Frontiers Science Center for Synthetic Biology (Ministry of Education), Tianjin
University, Tianjin, China

^c School of Synthetic Biology and Biomanufacturing, Tianjin University, Tianjin, China

^d Division of Biotechnology, Dalian Institute of Chemical Physics, Chinese Academy of
Sciences, Dalian 116082, China

Table S1. Elemental analysis of the lignin samples from easy-recycled ethylenediamine (EDA) pretreatment process

Lignin	C (%)	H (%)	N (%)	N/C	O/C
CEL	63.13	7.06	1.10	0.02	0.45
AL-180	55.09	5.80	2.00	0.04	0.67
A/NA-L-180	67.54	5.50	0.68	0.01	0.39
A/PG-L-180	55.65	6.12	1.91	0.03	0.65
A/MEA-L-180	54.36	5.92	2.51	0.03	0.68
LHW/NA-EDA-L	44.54	6.66	15.04	0.34	0.64
EDA-L	50.30	7.53	20.10	0.40	0.44

CEL represents cellulolytic enzyme lignin; AL represents the obtained lignin after alkaline fractionation. A/NA-L-180, A/PG-L-180, and A/MEA-L-180 represent the obtained lignin after alkaline fractionation with 2-naphthol, 1,3-propanediol, and ethanolamine, respectively. LHW/NA-EL represents the lignin obtained by pretreating corn stover with liquid hot water coupled with 2-naphthol, followed by ethylenediamine (EDA) fractionation with on-site EDA recovery at a heating temperature of 105 °C. EL represents the obtained lignin after EDA pretreatment with on-site EDA recovery at a heating temperature of 105 °C.

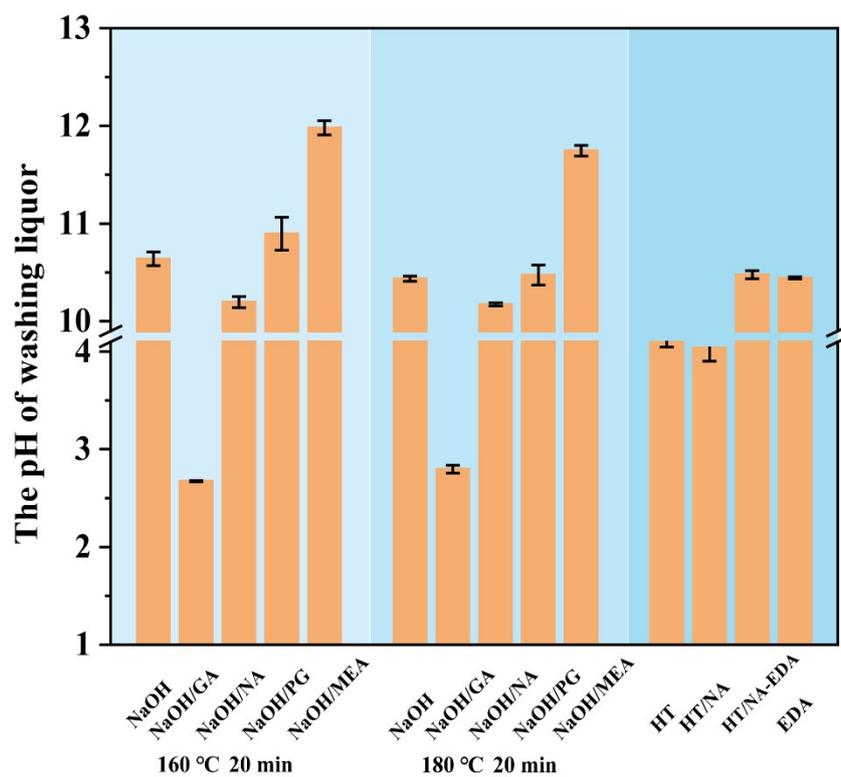


Fig. S1 The pH of washing liquor.

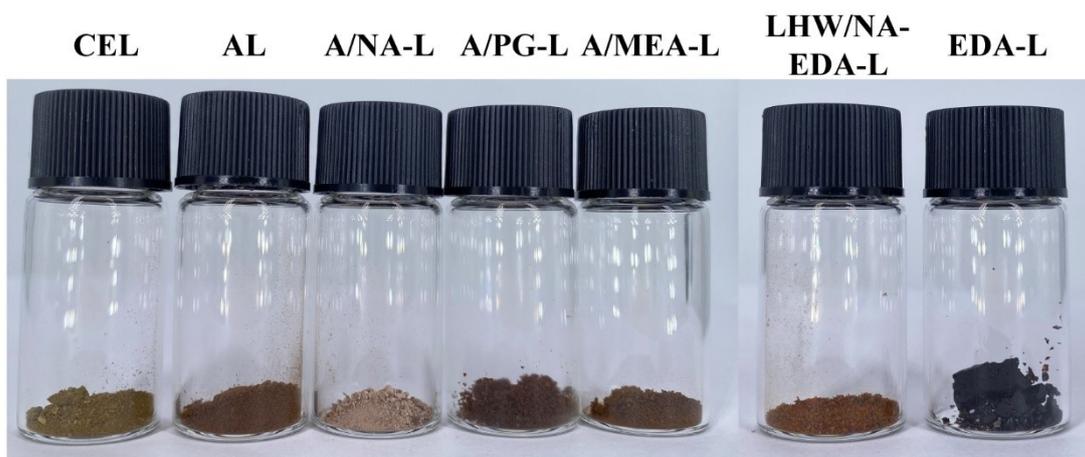


Fig. S2 Digital photographs of lignin isolated from alkaline fractionation with stabilization strategies.

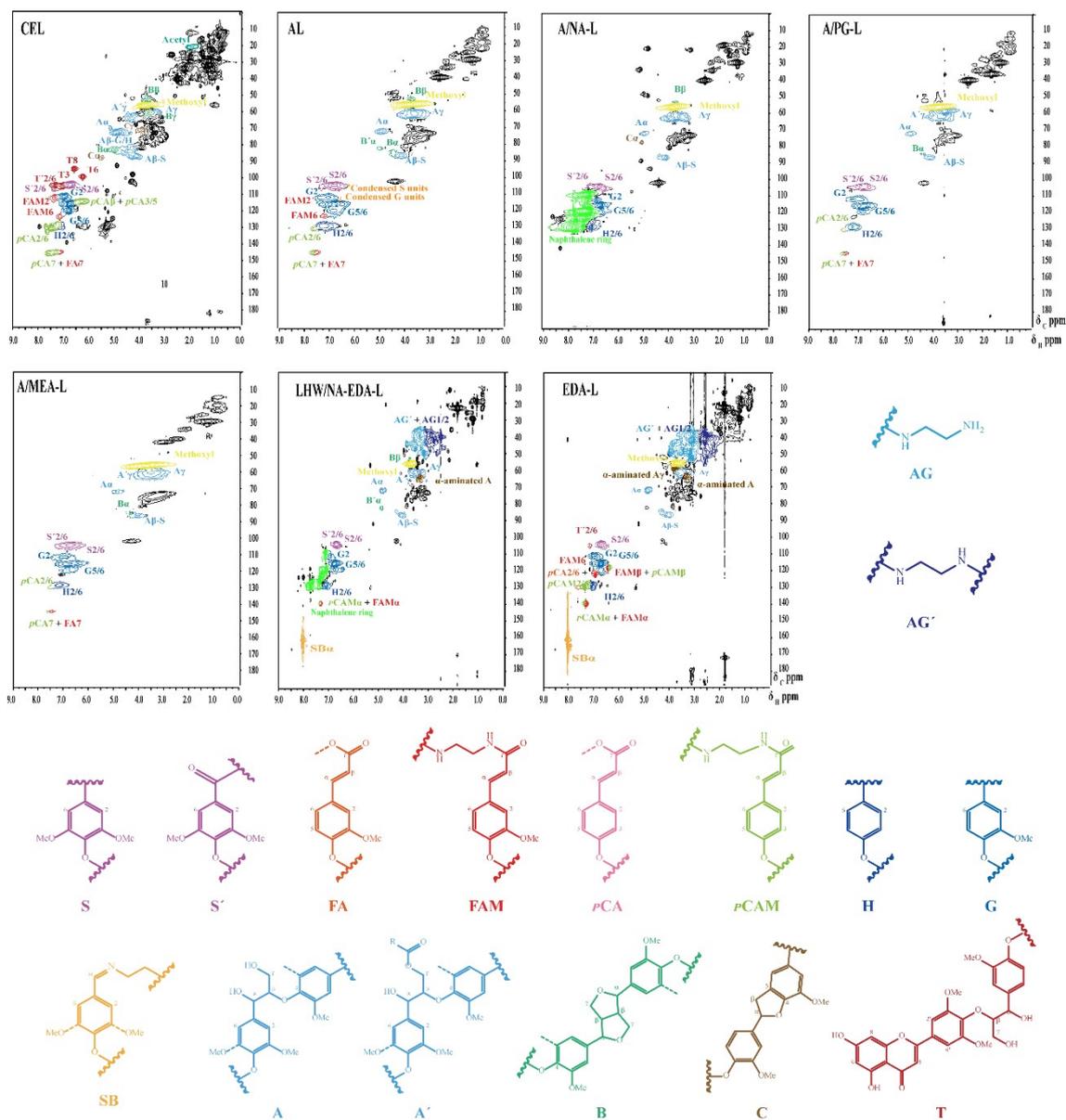


Fig. S3 2D HSQC NMR spectrum of the lignin isolated from the raw corn stover and alkali pretreated substrate with or without stabilization strategies.

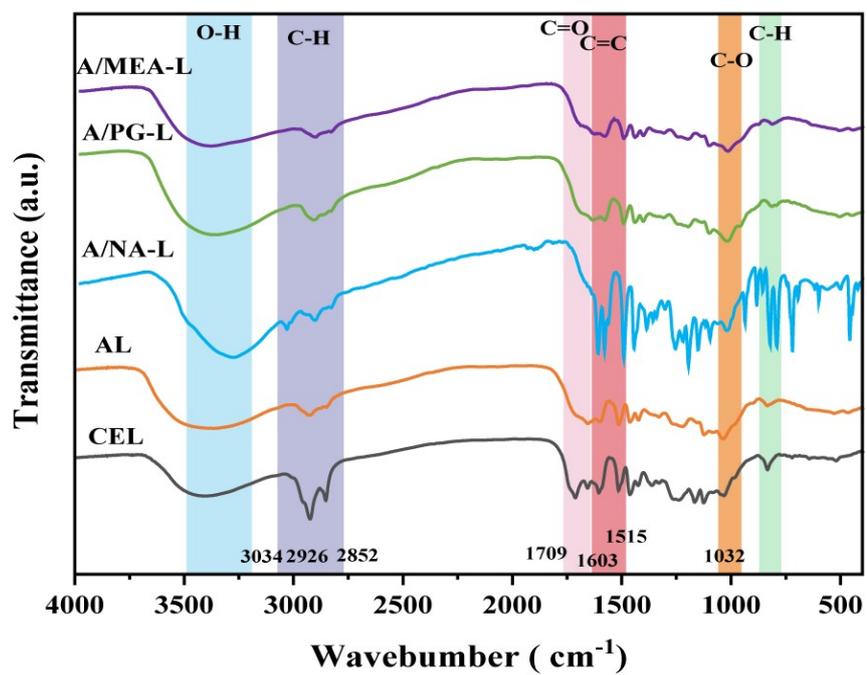


Fig. S4 The Fourier transformation infrared spectroscopy (FTIR) analysis of the obtained lignin from alkali fractionation with stabilization strategies.

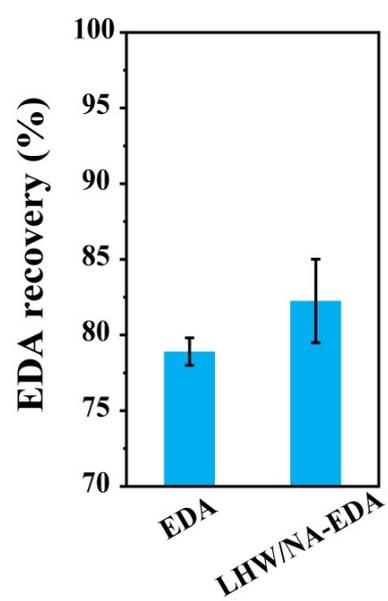


Fig. S5 The ethylenediamine (EDA) recovery.

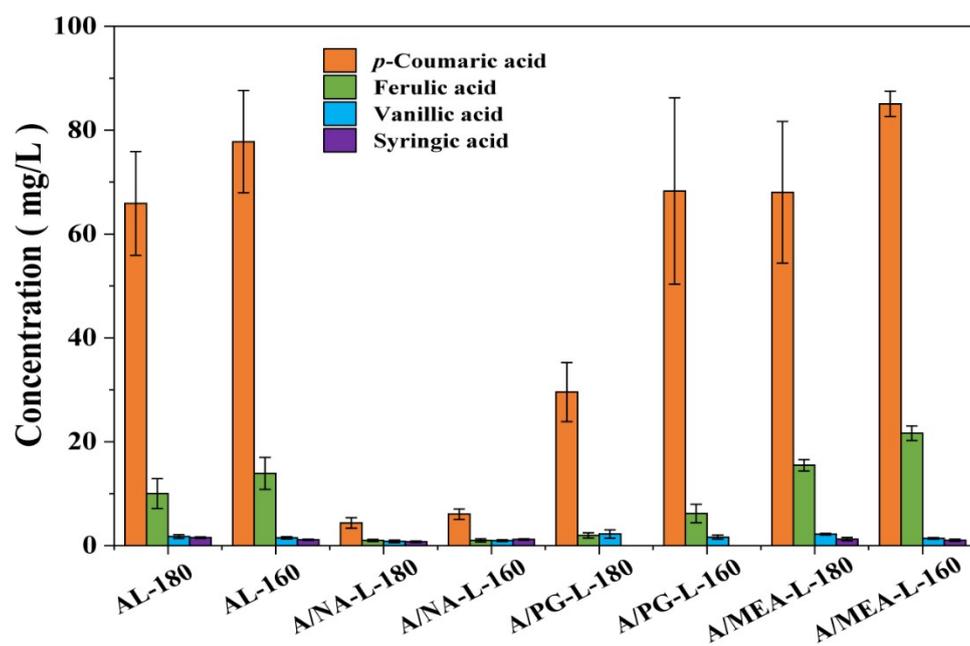


Fig. S6 Distribution of major aromatic monomers in washing liquid fraction.

Glucan (35.8 ± 0.9 g)	NaOH pretreatment	Glucan (28.2 ± 0.6 g)	Solid (52.2 ± 0.7 g)	Enzymatic hydrolysis	
		Xylan (14.4 ± 1.2 g)		4.66 FPU/g glucan, 96 h	14 FPU/g glucan, 36 h
Xylan (27.5 ± 0.4 g)	NaOH/GA pretreatment	Glucan (30.7 ± 0.4 g)	Solid (61.9 ± 1.1 g)	Enzymatic hydrolysis	
		Xylan (11.9 ± 1.4 g)		4.66 FPU/g glucan, 96 h	14 FPU/g glucan, 36 h
Lignin (19.2 ± 0.1 g)	NaOH/NA pretreatment	Glucan (28.7 ± 1.2 g)	Solid (57.4 ± 1.9 g)	Enzymatic hydrolysis	
		Xylan (13.6 ± 0.4 g)		4.66 FPU/g glucan, 96 h	14 FPU/g glucan, 36 h
Others (17.5 ± 1.2 g)	NaOH/PG pretreatment	Glucan (23.7 ± 0.6 g)	Solid (47.2 ± 0.9 g)	Enzymatic hydrolysis	
		Xylan (13.0 ± 0.6 g)		4.66 FPU/g glucan, 96 h	14 FPU/g glucan, 36 h
CS 100 g	NaOH/MEA pretreatment	Glucan (21.5 ± 1.8 g)	Solid (42.1 ± 0.5 g)	Enzymatic hydrolysis	
		Xylan (11.6 ± 0.6 g)		4.66 FPU/g glucan, 96 h	14 FPU/g glucan, 36 h
		Lignin (5.1 ± 1.2 g)		Glucose (28.5 ± 0.6 g)	Glucose (30.4 ± 0.7 g)
		Lignin (11.9 ± 0.2 g)		Xylose (10.8 ± 0.9 g)	Xylose (12.4 ± 1.0 g)
		Glucan (0.7 ± 0.0 g)	Liquid		
		Xylan (10.9 ± 0.1 g)			
		Lignin (13.2 ± 0.3 g)			
		Glucan (2.1 ± 0.1 g)	Liquid		
		Xylan (10.4 ± 1.5 g)			
		Lignin (3.0 ± 0.2 g)			
		Glucan (0.8 ± 0.0 g)	Liquid		
		Xylan (11.6 ± 0.2 g)			
		Lignin (10.0 ± 0.4 g)			
		Glucan (26.3 ± 0.6 g)		Glucose (26.3 ± 0.6 g)	Glucose (26.3 ± 0.6 g)
		Xylan (9.5 ± 0.3 g)	Liquid		
		Lignin (13.3 ± 0.2 g)			
		Glucan (21.5 ± 1.8 g)		Glucose (23.9 ± 2.0 g)	Glucose (23.9 ± 2.0 g)
		Xylan (11.6 ± 0.6 g)		Xylose (8.6 ± 0.4 g)	Xylose (10.6 ± 0.5 g)
		Lignin (5.6 ± 1.2 g)			
		Glucan (0.7 ± 0.0 g)	Liquid		
		Xylan (11.0 ± 0.2 g)			
		Lignin (11.6 ± 1.1 g)			

Fig. S7 Mass balance of corn stover by alkali fractionation with lignin stabilization and subsequent enzymatic hydrolysis.