

Electronic Supplementary Information (ESI) for  
A spectroscopic method for quantitating lignin in lignocellulosic biomass based on  
LiCl/DMSO completely dissolved solution

*Haonan Zhang, Hui Zhao, Yan Yang, Hao Ren\* and Huamin Zhai \**

Jiangsu Provincial Key Lab of Pulp and Paper Science and Technology, NanJing Forestry  
University, NanJing, Jiangsu Province, 210037, China.

\*To whom the correspondence should be addressed. Email: [renhao@njfu.edu.cn](mailto:renhao@njfu.edu.cn);

[hzhai@njfu.edu.cn](mailto:hzhai@njfu.edu.cn)

<b>Table of Contents</b>	<b>page</b>
1. The feasibility validation of LiCl/DMSO solvent system as reference solution for UV quantification .....	2
2. Determination of UV characteristic absorption wavelengths of lignin in lignocellulosic biomass LiCl/DMSO CDS.....	2
3. Study on UV spectral characteristics of biomass samples with trace amounts of lignin (oxygen delignification samples) in LiCl/DMSO CDS .....	3
4. Table S1. Effect of ball milling time on the dissolution of lignocellulosic biomass in LiCl/DMSO (turbidity value) .....	4
5. Table S2. Lignin contents measured by the Klason method of a series of wheat straw samples ..	4
6. Table S3. Lignin contents measured by the Klason method of a series of black poplar samples .	5
7. Table S4. Lignin contents measured by the Klason method of a series of redwood samples .....	5
8. Table S5. Lignin contents measured by the Klason method of a series of eucalyptus bark samples.....	6
9. Table S6. Lignin contents measured by the Klason method of a series of redwood bark samples .....	6
10. Table S7. Lignin content of chemical treated lignocellulosic biomass samples for turbidity measurement .....	6
11. Table S8. Effect of chemical treatment on the dissolution of lignocellulosic biomass samples in LiCl/DMSO (turbidity value) .....	6
12. Table S9. Assignments of <sup>1</sup> H- <sup>13</sup> C overlapped signals in 2D-NMR spectra of wheat straw and regenerated wheat straw.....	7
13. Figure S2. 2D-NMR side chain spectra of ball milled wheat straw and LiCl/DMSO dissolved/regenerated wheat straw at a lower contour level.....	9
14. Figure S3. The UV - Vis spectra of LiCl/DMSO solution .....	10

15. Figure S4. Effects of milling time on UV-Vis spectra of (a) wheat straw, (b) populus deltoides, (c) redwood and (d) eucalyptus bark in 8% LiCl/DMSO completely dissolved solution .....	10
16. Figure S5. UV-Vis spectra of oxygen delignification samples of (a) wheat straw, (b) populus deltoides, (c) eucalyptus bark .....	10
17. Recommended protocol for lignin quantitation in lignocellulosic biomass by LiCl/DMSO CDS-UV method.....	11

## 1. The feasibility validation of LiCl/DMSO solvent system as reference solution for UV quantification

To determine the UV absorbance of the solution, a reference solution should be selected to adjust the transmittance to 100% (absorbance to 0) first, so as to eliminate the measurement error caused by the reflection and absorption of light by other components and the absorbance pool. In order to examine the feasibility of choosing the LiCl/DMSO solvent as the reference solution, deionized water was used as the blank, and the UV-Vis absorption spectra of 8% LiCl aqueous solution, DMSO pure solution and 4%, 6% and 8% LiCl/DMSO solvent systems were scanned in the wavelength range of 200-800 nm (Fig.S3). The scanning interval is 1 nm, the scanning speed is medium speed, the slit width is 1 nm, and the measurement method is Abs. It was found that LiCl aqueous solution shows practically no absorption after 230 nm. The UV absorption spectra of DMSO pure solvent and LiCl/DMSO solvent systems with different LiCl concentrations are not significantly different, indicating that the UV absorption of LiCl/DMSO solvent system mainly comes from DMSO. DMSO molecule has S=O bond, which can occur  $\pi$ - $\pi^*$  electron transition, and can be conjugated to different degrees with methyl and lone pair electrons on oxygen atom, so it has characteristic absorption in near ultraviolet region. The UV absorption strength of LiCl/DMSO decreases rapidly in the range of more than 260 nm, and the absorbance of LiCl/DMSO is less than 0.3 at 280 nm, which meets the requirements of being reference liquid. Therefore, if lignin in lignocellulosic biomass LiCl/DMSO CDS has stable characteristic absorption peaks in the range of UV spectrum greater than 260 nm, LiCl/DMSO solvent can be selected as reference solution.

## 2. Determination of UV characteristic absorption wavelengths of lignin in lignocellulosic biomass LiCl/DMSO CDS

According to the UV spectra of lignocellulosic biomass samples after different ball milling time treatment and different chemical delignification treatment (Fig. S4, Fig. 2), the characteristic absorption peaks of various samples that can be used for subsequent UV quantification of lignin were determined. The previous researches on UV quantification of lignin showed that typical herbaceous lignin has strong absorption peaks near 205 nm and 280 nm, and weak absorption peaks near 230 nm and 310-350 nm. In this study, the three dissolution systems of wheat straw (Fig. 2a) have strong absorption peaks at 282 nm and 321 nm, and a sharp peak at 253 nm. The absorption band near 282 nm is the characteristic absorption region of lignin aromatic ring. Due to the different composition of lignin structural units, the position of this peak is slightly different. Due to the influence of *p*-coumaric acid ester and ferulate, wheat straw lignin should have a wide shoulder (or peak) near 315 nm. Therefore, it is inferred that the peak at 321 nm in this study is caused by these

two acid esters. At the same time, it also shows that ball milling does not completely destroy the ester bonds of lignin. In addition, the absorption peak at 253 nm is the absorption of  $\alpha$  or  $\alpha$ - $\beta$  unsaturated functional groups. At 282 nm and 321 nm, the absorption peaks of wheat straw samples are strong and flat, and the accuracy and sensitivity would be relatively high if these two peaks are selected as the determination wavelength. After various chemical treatments, the characteristic absorption wavelength of wheat straw samples does not shift at 282 nm since the benzene ring structure has not been damaged and the number of oxygen-containing substituents of remaining lignin has not changed during the chemical treatments. However, the characteristic absorption peak of wheat straw at 321 nm gradually moved to shorter wavelength and weakened with the increase strength of alkali treatment, which is due to the saponification of *p*-coumaric acid and ferulate in dilute alkali environment. Therefore, 282 nm can be selected for the quantitative determination of lignin in wheat straw LiCl/DMSO CDS. For black poplar samples (Fig. 2b), since hardwood lignin contains more S groups, the absorption peak at 280 nm is "blue shifted" with maximum absorption values at 275-277 nm. It can be seen from the figure that there is no difference in the position of the maximum absorption peak of black poplar LiCl/DMSO CDS with different ball milling times and chemical delignification treatment, both at 276 nm, which is consistent with the UV absorption characteristics of typical hardwood lignin. Therefore, 276 nm can be selected as the characteristic absorption wavelength for hardwood lignin content determination in LiCl/DMSO CDS. The UV spectrum of redwood LiCl/DMSO CDS (Fig. 2c) showed a strong absorption peak at 282 nm, which is characteristic absorption peak of lignin aromatic rings, and a sharp absorption peak at 254 nm, which is the absorption peaks of  $\alpha$  or  $\alpha$ - $\beta$  unsaturated functional groups. After chemical delignification treatment, the absorption peak at 254 nm shifted to shorter wavelength, and the position of the maximum absorption peak at 282 nm did not change, therefore, 282 nm was selected as the characteristic absorption wavelength for redwood lignin content determination. Eucalyptus bark LiCl/DMSO CDS showed a distinct characteristic absorption peak at 278 nm, (Fig. 2d) and the absorption peak at 278 nm was not changed after chemical delignification, therefore, 278 nm could be selected as the UV characteristic absorption peak for eucalyptus bark lignin. In this experiment, 1 h ball milled eucalyptus bark could not be completely dissolved in 8% LiCl/DMSO solvent, and UV spectroscopic determination of this incompletely dissolved system found that the absorption values of eucalyptus bark of the same mass were lower than those of the completely dissolved solutions, but the shape of absorption peaks were consistent with those of the completely dissolved solutions. This result further illustrates that ball milling intensity has no influence on the characteristic absorption peak position of the biomass LiCl/DMSO CDS, but the extent to which the sample dissolves in the LiCl/DMSO solvent system can affect the quantitative accuracy. A small amount of redwood bark was also selected to determine the UV spectra after similarly delignified by NaOH/Na<sub>2</sub>S treatments. The characteristic absorption peak of redwood bark is at 280 nm and does not change with the degree of chemical treatment. In summary, this study confirmed the characteristic absorption wavelengths that can be used for quantitative analysis of lignin by LiCl/DMSO CDS-UV method, which are 282 nm, 276 nm, 282 nm, 278 nm and 280 nm for wheat straw, black poplar, redwood, eucalyptus bark and redwood bark, respectively.

### **3. Study on UV spectral characteristics of biomass samples with trace amounts of lignin (oxygen delignification samples) in LiCl/DMSO CDS**

In order to test the feasibility of LiCl/DMSO-UV method for the determination of samples with

trace amounts of lignin, oxygen delignification was carried out on wheat straw, black poplar and eucalyptus bark samples after chemical cooking. After ball milling the samples for 3 h, the UV spectrum was determined according to the above method (Fig. S5). The UV absorption peaks of the samples treated by AP, KP and AP-AQ methods were significantly weakened at 282 nm, and the oxygen delignification degree of ASP method was low, but the characteristic absorption peak shifted to the shorter wavelength. Figure S5b shows the UV spectrum of the black poplar kraft pulp after different degrees of oxygen delignification. In the figure, 12<sup>#</sup> and 13<sup>#</sup> are oxygen delignified samples. The UV characteristic absorption peak intensity of the two samples decreased significantly, and the position of the characteristic absorption peak became not obvious. As a reference, 10<sup>#</sup> and 11<sup>#</sup> are NaOH/Na<sub>2</sub>S treated samples without oxygen delignification. Although the lignin content is very low, the characteristic absorption peak at 276 nm still exists obviously. Similar to black poplar, the UV absorption peak of oxygen delignified eucalyptus bark samples (10<sup>#</sup> and 11<sup>#</sup>) at 278 nm becomes not obvious even if its absorption value is higher than that of samples without oxygen delignification (6<sup>#</sup>). All the above illustrate that oxygen delignification treatment affected the structure of lignin in lignocellulosic biomass, thus affecting its UV characteristic absorption. The chemical reactions of oxygen delignification mainly utilize unpaired electrons of oxygen molecular, which able to generate a series of derived groups and oxidize organic compounds. Under the condition of alkali activation, O<sub>2</sub> reacts with phenolic lignin structure and benzene ring conjugated carbonyl of lignin to produce epoxy compounds and mucofuroic acid derivatives, resulting in the breaking of C<sub>α</sub>-C<sub>β</sub> bond and destruction of lignin benzene ring structure. Therefore, the characteristic absorption peak of lignin near 280 nm is no longer obvious.

**Table S1.** Effect of ball milling time on the dissolution of lignocellulosic biomass in LiCl/DMSO (turbidity value)

Ball milling time	DMSO	LiCl/DMSO	1 h	2 h	3 h	4 h
Wheat straw turbidity /NTU	0.24	3.44	98.2	33.6	3.70	3.62
Black poplar turbidity /NTU	0.24	3.64	126	47.3	3.74	3.66
Redwood turbidity /NTU	0.24	5.43	168	77.2	5.92	5.51
Eucalyptus bark turbidity /NTU	0.24	5.95	176	109	6.02	5.99

**Table S2.** Lignin contents measured by the Klason method of a series of wheat straw samples

Delignification methods	Samples	Klason lignin <sup>a</sup> , %	Acid soluble lignin <sup>b</sup> , %	Total lignin, %
Raw material	1	23.0±0.4	2.30±0.05	25.3±0.4
	1	1.3±0.0	0.43±0.01	1.7±0.0
	AP-AQ	2	0.3±0.0	0.44±0.01
	3	0.2±0.0	0.42±0.02	0.6±0.0
KP	1	1.5±0.0	0.42±0.01	1.9±0.0
	2	0.4±0.0	0.38±0.01	0.8±0.0
	3	0.3±0.0	0.35±0.01	0.7±0.0
ASP	1	2.8±0.1	1.68±0.03	4.5±0.1
	2	1.9±0.1	1.17±0.02	3.1±0.1
	3	1.6±0.1	0.86±0.07	2.5±0.1

Formacell	1	6.5±0.0	0.42±0.00	6.9±0.0
	2	5.0±0.0	0.31±0.01	5.3±0.0
	3	3.4±0.1	0.26±0.02	3.7±0.1
AP	1	20.3±0.2	1.51±0.02	21.8±0.2
	2	18.6±0.1	1.54±0.03	20.1±0.1
	3	14.6±0.1	1.46±0.03	16.0±0.1
	4	12.3±0.2	1.30±0.04	13.6±0.2
	5	13.6±0.1	1.28±0.02	14.9±0.1
	6	11.1±0.1	1.29±0.08	12.4±0.1
	7	5.0±0.1	0.99±0.03	6.0±0.1
	8	0.7±0.0	0.50±0.01	1.2±0.0

a, b: Lignin contents based on biomass samples.

Notes: For samples with low lignin content, 4 times of the sample mass was used for lignin determination to ensure data accuracy.

**Table S3.** Lignin contents measured by the Klason method of a series of black poplar samples

Delignification methods	Samples	Klason lignin <sup>a</sup> , %	Acid soluble lignin <sup>b</sup> , %	Total lignin, %	
Raw material	1	22.9±0.1	2.34±0.03	25.2±0.1	
	2	22.1±0.1	2.24±0.05	24.3±0.1	
	3	24.2±0.2	2.10±0.03	26.3±0.2	
	4	23.5±0.2	2.11±0.04	25.6±0.2	
	5	19.3±0.3	2.03±0.02	21.3±0.3	
	KP	6	14.1±0.1	1.58±0.01	15.7±0.1
		7	8.6±0.3	1.27±0.01	9.9±0.3
		8	16.4±0.1	1.67±0.00	18.1±0.1
		9	1.1±0.0	5.39±0.06	6.5±0.1
		10	1.9±0.0	0.81±0.03	2.7±0.0
		11	1.2±0.1	0.75±0.03	2.0±0.1
Oxygen delignification	12	0.5±0.1	0.65±0.05	1.2±0.1	
	13	0.4±0.0	0.92±0.03	1.3±0.0	

a, b: Lignin contents based on biomass samples.

Notes: For samples with low lignin content, 4 times of the sample mass was used for lignin determination to ensure data accuracy.

**Table S4.** Lignin contents measured by the Klason method of a series of redwood samples

Delignification methods	Samples	Klason lignin <sup>a</sup> , %	Acid soluble lignin <sup>b</sup> , %	Total lignin, %
Raw material	1	35.5±0.6	0.32±0.04	35.8±0.6
	2	35.6±0.2	0.33±0.06	35.9±0.2
KP	3	30.8±0.1	0.40±0.01	31.2±0.1
	4	17.1±0.1	0.62±0.07	17.7±0.1
	5	9.7±0.1	0.46±0.02	10.2±0.1

6	5.5±0.1	0.33±0.01	5.8±0.1
7	3.5±0.2	0.41±0.03	3.9±0.2

a, b: Lignin contents based on biomass samples.

Notes: For samples with low lignin content, 4 times of the sample mass was used for lignin determination to ensure data accuracy.

**Table S5.** Lignin contents measured by the Klason method of a series of eucalyptus bark samples

Delignification methods	Samples	Klason lignin <sup>a</sup> , %	Acid soluble lignin <sup>b</sup> , %	Total lignin, %	
Raw material	1	21.5±0.3	3.20±0.11	24.7±0.3	
	2	18.1±0.3	2.56±0.21	20.7±0.4	
	3	15.0±0.2	2.46±0.10	17.5±0.2	
	4	11.2±0.1	2.04±0.00	13.2±0.1	
	KP	5	6.8±0.2	1.45±0.06	8.3±0.2
		6	4.2±0.0	0.98±0.01	5.2±0.0
		7	9.4±0.1	1.66±0.02	11.1±0.1
		8	2.0±0.0	1.23±0.04	3.2±0.00
		9	2.1±0.2	1.30±0.04	3.4±0.2
Oxygen delignification	10	1.4±0.0	1.09±0.10	2.5±0.0	
	11	0.6±0.1	0.96±0.03	1.6±0.1	

a, b: Lignin contents based on biomass samples.

Notes: For samples with low lignin content, 4 times of the sample mass was used for lignin determination to ensure data accuracy.

**Table S6.** Lignin contents measured by the Klason method of a series of redwood bark samples

Delignification methods	Samples	Klason lignin <sup>a</sup> , %	Acid soluble lignin <sup>b</sup> , %	Total lignin, %
KP	1	46.0±1.2	0.78±0.04	46.8±1.2
	2	40.9±0.8	0.62±0.01	41.5±0.8
	3	32.0±0.1	0.61±0.03	32.6±0.1
	4	28.5±0.3	0.61±0.03	29.1±0.3

a, b: Lignin contents based on biomass samples.

**Table S7.** Lignin content of chemical treated lignocellulosic biomass samples for turbidity measurement

Samples	1	2	3	4	5
AP wheat straw, %	21.8±0.2	16.0±0.1	14.9±0.1	12.4±0.1	6.0±0.1
KP black poplar, %	26.3±0.2	21.3±0.3	15.7±0.1	9.9±0.3	6.5±0.1
KP redwood, %	35.9±0.2	17.7±0.1	10.2±0.1	5.8±0.1	3.9±0.2
KP eucalyptus bark, %	20.7±0.4	17.5±0.2	11.1±0.1	5.2±0.0	3.4±0.2

**Table S8.** Effect of chemical treatment on the dissolution of lignocellulosic biomass samples in LiCl/DMSO (turbidity value)

Samples	DMSO	LiCl/DMSO	1	2	3	4	5
Turbidity of AP wheat straw/NTU (25 °C)	0.24	3.44	173	160	167	163	178
Turbidity of AP wheat straw/NTU (60 °C)	0.24	3.44	3.62	3.70	3.55	3.61	3.66
Turbidity of KP black poplar/NTU (25 °C)	0.24	3.64	183	170	177	181	178
Turbidity of KP black poplar/NTU (60 °C)	0.24	3.64	3.70	3.71	3.68	3.74	3.77
Turbidity of KP redwood/NTU (25 °C)	0.24	4.36	172	169	158	163	167
Turbidity of KP redwood/NTU (60 °C)	0.24	4.36	4.62	4.45	4.58	4.61	4.41
Turbidity of KP eucalyptus bark /NTU (25 °C)	0.24	5.95	166	183	157	171	168
Turbidity of KP eucalyptus bark /NTU (60 °C)	0.24	5.95	6.30	6.97	6.33	6.52	6.30

**Table S9.** Assignments of <sup>1</sup>H-<sup>13</sup>C overlapped signals in 2D-NMR spectra of wheat straw and regenerated wheat straw

Lable	$\delta_C/\delta_H$ (ppm) <sup>a</sup>	$\delta_C/\delta_H$ (ppm) <sup>b</sup>	Assignments
B <sub>β</sub>	55.25/3.12	54.85/3.11	C <sub>β</sub> -H <sub>β</sub> in β-β (resinol) (B)
OMe	55.53/3.71	55.61/3.70	C-H in methoxyls (OMe)
F <sub>γ</sub>	61.40/4.13	61.28/4.15	C <sub>γ</sub> -H <sub>γ</sub> in <i>p</i> -hydroxycinnamyl alcohol (F)
A <sub>α</sub>	71.75/4.96	71.79/4.94	C <sub>α</sub> -H <sub>α</sub> in β-O-4 substructures (A)
A <sub>β</sub> (G)	82.97/4.35	83.33/4.38	C <sub>β</sub> -H <sub>β</sub> in β-O-4 substructures linked to G unit (A)
A <sub>β</sub> (S)	86.25/4.10	86.25/4.18	C <sub>β</sub> -H <sub>β</sub> in β-O-4 substructures linked to S unit (A)
A <sub>β</sub> '	82.77/4.96	82.61/5.01	C <sub>β</sub> -H <sub>β</sub> in α oxidized β-O-4 substructures linked to G unit (A)
B <sub>α</sub>	84.29/4.65	83.73/4.69	C <sub>α</sub> -H <sub>α</sub> in β-β resinol (B)
C <sub>α</sub>	87.24/5.52	87.17/5.57	C <sub>α</sub> -H <sub>α</sub> in phenylcoumaran substructures (C)
T <sub>6</sub>	98.93/6.31	99.55/6.29	C <sub>6</sub> -H <sub>6</sub> in triclin
T <sub>8</sub>	94.31/6.63	ND	C <sub>8</sub> -H <sub>8</sub> in triclin
T' <sub>2,6</sub>	104.03/7.31	103.95/7.30	C' <sub>2,6</sub> -H' <sub>2,6</sub> in triclin
S <sub>2,6</sub>	103.87/6.74	103.91/6.75	C <sub>2,6</sub> -H <sub>2,6</sub> in syringyl units (S)
G <sub>2</sub>	111.23/7.04	111.07/7.03	C <sub>2</sub> -H <sub>2</sub> in guaiacyl units (G)
G <sub>5</sub> +G <sub>6</sub>	115.38/6.83	115.34/6.82	C <sub>5</sub> -H <sub>5</sub> in guaiacyl units (G)
	118.90/6.82	119.09/6.84	C <sub>6</sub> -H <sub>6</sub> in guaiacyl units (G)
H <sub>2,6</sub>	128.06/7.22	128.69/7.28	C <sub>2,6</sub> -H <sub>2,6</sub> in <i>p</i> -hydroxyphenyl units (H)
<i>p</i> CA <sub>2,6</sub>	129.99/7.46	129.86/7.48	C <sub>2,6</sub> -H <sub>2,6</sub> in <i>p</i> -coumarate
<i>p</i> CA <sub>α</sub>	135.85/7.73	135.81/7.72	C <sub>α</sub> -H <sub>α</sub> in <i>p</i> -coumarate
<i>p</i> CA <sub>β</sub> +FA <sub>β</sub>	114.08/6.53	114.25/6.54	C <sub>β</sub> -H <sub>β</sub> in <i>p</i> -coumarate and ferulate
FA <sub>2</sub>	111.19/7.33	111.15/7.32	C <sub>2</sub> -H <sub>2</sub> in ferulate
FA <sub>6</sub>	123.17/7.12	123.21/7.15	C <sub>6</sub> -H <sub>6</sub> in ferulate
C <sub>2</sub>	73.09/3.01	73.09/3.03	C <sub>2</sub> -H <sub>2</sub> in cellulose

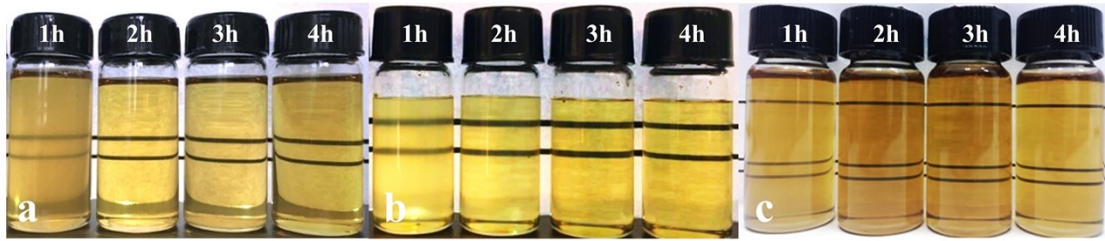
C <sub>3</sub>	73.32/3.58	73.24/3.61	C <sub>3</sub> -H <sub>3</sub> in cellulose
C <sub>4</sub>	80.02/3.45	80.10/3.46	C <sub>4</sub> -H <sub>4</sub> in cellulose
C <sub>5</sub>	76.27/3.22	76.95/3.24	C <sub>5</sub> -H <sub>5</sub> in cellulose
C <sub>6</sub>	60.06/3.69	60.33/3.70	C <sub>6</sub> -H <sub>6</sub> in cellulose
X <sub>2</sub>	72.75/3.16	72.82/3.17	C <sub>2</sub> -H <sub>2</sub> in β-D-xylopyranoside (X)
X <sub>3</sub>	74.04/3.36	74.00/3.37	C <sub>3</sub> -H <sub>3</sub> in β-D-xylopyranoside (X)
X <sub>4</sub>	75.47/3.59	75.40/3.60	C <sub>4</sub> -H <sub>4</sub> in β-D-xylopyranoside (X)
X <sub>5</sub>	63.17/3.23-3.95	63.17/3.23-3.95	C <sub>5</sub> -H <sub>5</sub> in β-D-xylopyranoside (X)
X <sub>NR5</sub>	65.82/3.12-3.76	66.58/3.09-3.78	C <sub>5</sub> -H <sub>5</sub> in β-D-xylopyranoside (X) (NR)
2- <i>O</i> -Ac-β-D-Xylp (2)	73.41/4.61	73.37/4.61	2- <i>O</i> -acetylated β-D-xylopyranosyl
3- <i>O</i> -Ac-β-D-Xylp (3)	74.87/4.91	75.05/4.91	3- <i>O</i> -acetylated β-D-xylopyranosyl
2,3- <i>O</i> -Ac-β-D-Xylp (2)	71.33/4.84	71.55/4.86	2,3- <i>O</i> -acetylated β-D-xylopyranosyl

$\delta_C/\delta_H$  (ppm)<sup>a</sup>, Assignments of wheat straw sample

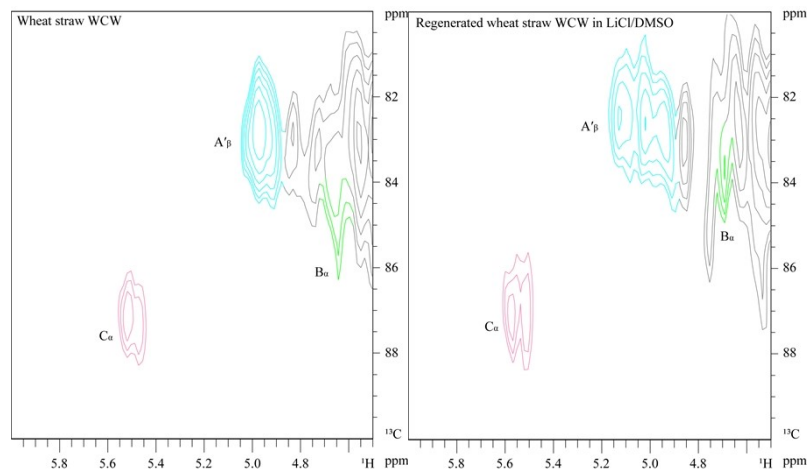
$\delta_C/\delta_H$  (ppm)<sup>b</sup>, Assignments of regenerated wheat straw sample

ND, Not detected

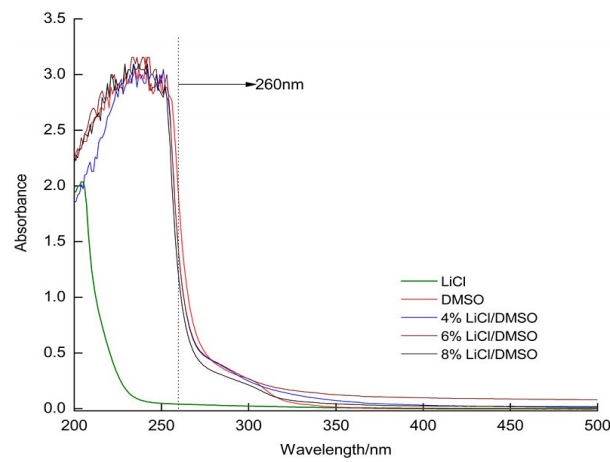




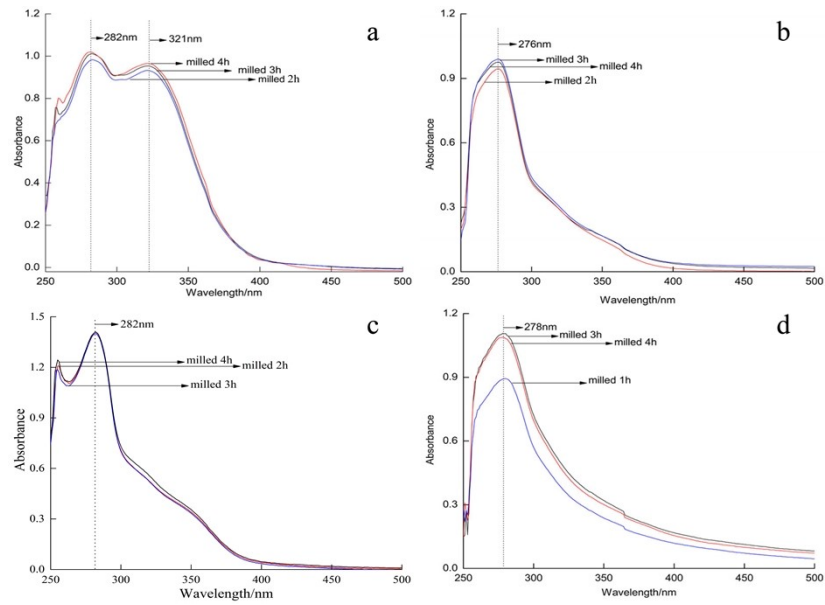
**Figure S1.** Effect of ball milling time on the dissolution of (a) wheat straw, (b) populus deltoides (c) redwood (clarity)



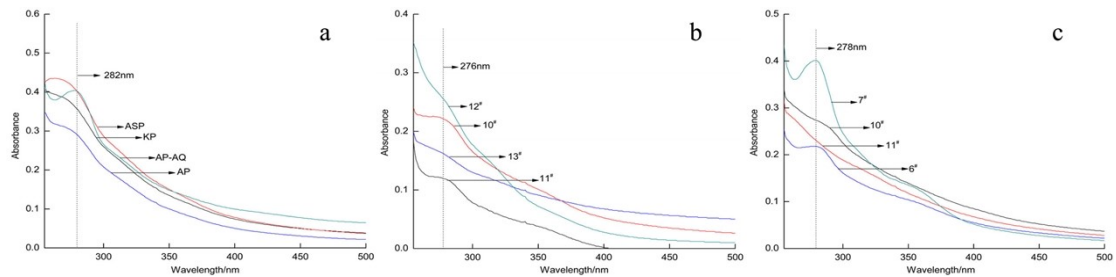
**Figure S2.** 2D-NMR side chain spectra of ball milled wheat straw and LiCl/DMSO dissolved/regenerated wheat straw at a lower contour level



**Figure S3.** The UV - Vis spectra of LiCl/DMSO solution



**Figure S4.** Effects of milling time on UV-Vis spectra of (a) wheat straw, (b) populus deltoides, (c) redwood and (d) eucalyptus bark in 8% LiCl/DMSO completely dissolved solution



**Figure S5.** UV-Vis spectra of oxygen delignification samples of (a) wheat straw, (b) populus deltoides, (c) eucalyptus bark

## **Recommended protocol for lignin quantitation in lignocellulosic biomass by LiCl/DMSO CDS-UV method**

### **1. Scope**

This procedure is for the accurate and green quantitation of lignin content of lignocellulosic biomass.

### **2. Apparatus**

- 2.1 Wiley mill
- 2.2 Soxhlet extraction setup
- 2.3 Analytical balance with 0.1 mg readability
- 2.4 Drying oven for moisture determination at temperature of  $105 \pm 2$  °C
- 2.5 Oil bath heated by hot plate with magnetic stirrer and temperature controller
- 2.6 Desiccator with fresh desiccant
- 2.7 Magnetic stirrer
- 2.8 Glass vial (25 mL)
- 2.9 Volumetric flask (50 mL, 100 mL)
- 2.10 Micropipette (0.1–1.0 mL)
- 2.11 UV-Visible spectrophotometer with quartz cuvettes (1cm path-length)

### **3. Procedure**

- 3.1 Sample preparation
  - 3.1.1 Biomass grounding: Grind chipped or chopped air-dry biomass using a Wiley mill to pass a 2-mm screen. Sieve the ground biomass and collect the fraction between 20 and 100 mesh for analysis.
  - 3.1.2 Removal of extractives: Extract ground biomass with benzene/alcohol (v/v 2:1) using Soxhlet extractor for 24 h.
- 3.2 Dissolution of the biomass sample
  - 3.2.1 Preparation of LiCl/DMSO solvent: 8 g oven dry lithium chloride (LiCl) was dissolved in 92 g dimethyl sulfoxide (DMSO) which was dried by molecular sieve, stir the system by magnetic stirring until it was completely dissolved, and then the 8% LiCl/DMSO solvent system was obtained.
  - 3.2.2 Ball milling: In order to improve the solubility of the samples in LiCl/DMSO solvent system, the samples need to be pretreated by ball milling. All biomass samples were vacuum dried for 12h, and then the samples were ball milled by planetary micro mill (pulverisette 7, Fritsch, Germany). 4 g of vacuum-dried extracted wood (grass) powder was placed in a zirconia tank with a volume of 80 ml. 25 zirconia balls with an inner diameter

of 1 cm were loaded into the tank, Ball milling the samples for 1-4 h at room temperature at 600 rpm (with a transmission ratio of 1: 2 relative to the main disk). Stop for 10 min after every 5 min of operation.

3.2.3 Dissolution of lignocellulosic biomass in LiCl/DMSO solvent: Weigh 0.1 g (accurate to 0.0002g) of ball milled wood (grass) powder in 20 ml 8% LiCl/DMSO, seal, and magnetic stirring for 24 h at room temperature to obtain the completely dissolved solution. For the chemical delignified samples, the completely dissolved solution was obtained by further raising the temperature to 60 °C and stirring for 2 h.

3.3 Spectrophotometric evaluation of lignin content in LiCl/DMSO completely dissolved solution

3.3.1 Acquire a baseline (background spectrum) using the 8% LiCl/DMSO as blank solution on a UV-Visible spectrophotometer.

3.3.2 Dilute the lignocellulosic biomass LiCl/DMSO CDS 5-25 times in a volumetric flask to control the absorbance value in the range of 0.2~0.8. Then scan the LiCl/DMSO CDS in the wavelength range of 200-800 nm to determine the absorbance value of the sample at the characteristic absorption wavelength (herbage 282 nm, hardwood 276 nm, softwood 282 nm, bark 278-280 nm). The scanning interval is 1 nm, the scanning speed is medium speed, the slit width is 1 nm, and the measurement method is Abs.

#### 4. Calculation for lignin content of the biomass sample

$$C (\%) = \frac{Abs' \times V \times n}{\varepsilon \times m_0 \times L} \times 100 \quad (1)$$

Where  $C$  is the lignin content (%) of the biomass sample;  $Abs'$  is the corrected UV absorbance of the diluted solution:  $Abs'$  for herbaceous lignin (wheat straw) is  $Abs'_{282nm} - 0.0056$ ,  $Abs'$  for hardwood lignin (black poplar) is  $Abs'_{276nm} - 0.0817$ ,  $Abs'$  for softwood lignin (redwood) is  $Abs'_{282nm} - 0.018$ ,  $Abs'$  for eucalyptus bark lignin is  $Abs'_{278nm} - 0.0842$ ,  $Abs'$  for redwood bark lignin is  $Abs'_{280nm} - 0.0224$ ;  $V$  is the total volume of the diluted solution (L);  $n$  is the dilution multiple of the diluted solution;  $\varepsilon$  ( $L \cdot g^{-1} \cdot cm^{-1}$ ) is the absorptivity of lignin at the appropriate wavelength: 18.599 for herbaceous lignin (wheat straw) at 282 nm, 13.235 for hardwood lignin (Poplar) at 276 nm, 21.848 for softwood lignin (redwood) at 282 nm, 14.049 for eucalyptus bark lignin at 278 nm, 34.978 for redwood bark lignin at 280 nm;  $m_0$  (g) is the dry mass weight of the solvent-extracted lignocellulosic sample;  $L$  is the light path length, 1 cm for here.