Supplementary information

Effect of lignin molecular weight on the formation and properties of carbon quantum dots

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Contents

1. Materials and methods

1.1 Materials

Alkali lignin (AL) was purchased from Sigma-Aldrich (St. Louis, MI, USA) and AL was not purified before use. Acetone (AR, 98%), methanol (AR, 98%), n-hexane (AR, 98%), and dimethyl sulfoxide (DMSO) were provided by Macklin Corporation (Shanghai, China). All chemicals were purchased at analytical grade. Fetal bovine serum (FBS), trypsin-EDTA, phosphate buffered saline (PSB), MEM, 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di- phenytetrazoliumromide (MTT), and Luria-Bertani (LB) broth medium were purchased from Biological Industries Co., Ltd. (Israel). All reagents were used as received. Ultrapure water was prepared using a Milli-Q ultrapure water purification system (Merck KGaA, Germany).

1.2 Fractionation of lignin

The method of lignin fractionation was modified based on the study of Wang et al.,[1] and the schematic diagram of the lignin fractionation process is shown in Supplemental Fig. S1†: 20 g of AL was added to 200 mL of acetone/methanol (7:3, v/v) co-solvent and stirred at 60 rpm for 30 min at 25 °C. Then the mixture was centrifuged (8000 rpm, 4 °C), and precipitate 1 and supernatant 1 were collected. precipitate 1 was labeled as lignin fraction F1. Subsequently, 20 mL of n-hexane was added to the supernatant 1 and the mixture was stirred with a glass rod for 1 min. After which the mixture was centrifuged (8000 rpm, 4 °C), and precipitate 2 and supernatant 2 were collected. The precipitate 2 was labeled as lignin fraction F2. Next, 40 mL of n-hexane was added to the collected supernatant 2, and the stirring and centrifugation operation of the previous

step was repeated to collect the precipitate 3 and supernatant 3. The precipitate 3 was labeled as lignin fraction F3. Similarly, 60 mL of hexane was added to the obtained supernatant 3, and the stirring and centrifugation operation of the previous step was repeated to obtain supernatant 4 and precipitate 4. The precipitate 4 was labeled as lignin fraction F4. Finally, the supernatant 4 was concentrated at 40 °C, and the residue lignin fraction F5 was obtained after solvent evaporation.

1.3 Preparation of L-CQDs

AL (0.1 g) and 25 mL of deionized water were added to a 50 mL Polytetrafluoroethylene-lined reactor, which was then heated to 180 °C in an oven and maintained at this temperature for 12 hours. The solid particles were then removed by filtration through a 0.22 μm microporous membrane, and then the filtrate was dialyzed in ultrapure water with a dialysis bag (MWCO = 1000 Da) for 48 hours to remove incomplete small molecules. And the solid product was obtained by freeze-drying and named L-CQDs-0. The L-CQDs prepared using above five lignin fractions (F1, F2, F3, F4, and F5) under the same experimental conditions described above were named L-CQDs-1, L-CQDs-2, L-CQDs-3, L-CQDs-4, and L-CQDs-5, respectively.

1.4 Characterization of lignin fractions and L-CQDs

The relative molecular weight and polydispersity (PDI) of lignin fractions were measured using gel permeation chromatography (GPC; Agilent PL-GPC50, USA) with DMSO as the mobile phase. ^{31} P NMR and 2D-HSQC nuclear magnetic resonance spectrometers (NMR, AVANCE IIITM HD 500, Bruker, Germany) were used to determine the linkage structure as well as the phenolic hydroxyl content of lignin

fractions. The UV-Vis absorption spectra of lignin solutions were determined in the 200-600 nm range using a UV-Vis spectrophotometer (Hitachi U-4100 UV-Vis, Japan). A Fourier transform infrared spectrometer (FTIR, TENSOR II, Bruker, Germany) in attenuated total reflection (ATR) mode was used to determine the chemical structures of lignin samples and L-CQDs. An x-ray photoelectron spectroscopy (XPS, Thermo Fisher Scientific K-alpha+, USA) was used for the determination of the carbon, oxygen, and nitrogen contents and functional group compositions of lignin samples and L-CQDs surfaces. The microstructure of L-CQDs was observed using a transmission electron microscope (TEM, FEI TECNAI G2 F30, USA). The fluorescence spectra (FLs) of L-CQDs were measured using a fluorescence spectrometer (FS5, Edinburgh), and the slit widths of both excitation and emission light were 1.5 nm for FLs measurements, and the ordinate-valued PL spectra were used to quantify the fluorescence intensity. The compositions of the hydrothermal fluids at different hydrothermal times were tested using gas chromatography-mass spectrometry (GC-MS; Agilent 7890A GC-5975C MS, USA) with a HP-5MS column. The hydrothermal fluids (5mg/mL) tested by GC-MS. The GC-MS conditions were as follows: the split ratio was 1:1, the helium flow rate was 1.8 mL/min, the injection volume was set at 2 μL, the injection temperature was set at 280 °C, and the column temperature was maintained at 60 °C for 2 min, then heated to 250 °C at a heating rate of 10 °C/min, and maintained for another 5 min.

1.5 Measurement of quantum yield of L-CQDs

The quantum yields (QYs) of the L-CQDs were calculated using quinine sulfate (dissolved in 0.05 mol/L sulfuric acid; QY = 54%) as a reference.[2] The QY of the L-CQDs were calculated at the optimal excitation wavelength for each sample using the

equation :

$$QY = QY_{qs} \cdot \frac{S}{S_{qs}} \cdot \frac{A_{qs}}{A} \cdot \frac{\eta^2}{\eta^2_{qs}}$$

where QY is the quantum yield of the L-CQDs, qs denotes the quinine sulfate reference solution, S means the integral area of the fluorescence peak in the photoluminescence (PL) spectra of the L-CQDs, A is the absorbance of the L-CQDs at the excitation wavelength, and η represents the refractive index of the solution.

1.6 Biocompatibility

The toxicity of L-CQDs-3 on L02 cells was evaluated by MTT method. L02 cells in logarithmic growth phase were diluted with complete medium to a cell density of 1×10 4 cells/mL, and then inoculated into 96-well plates with 100 μL per well. 96-well plates were cultured at 37 ℃ in a 5% CO_2 thermostatic cell culture incubator. After 24 hours of incubation, the original medium was aspirated and different concentrations of L-CQDs-3 (0.1, 0.25, 0.5, 1, 2, 0.5, 1, 2 mg/mL) were added. Then 150 μL of DMSO was added to each well, and after sufficient shaking, the absorbance value of each well was measured at 570 nm using an enzyme marker (EPOCH2, Biotek), and then cell viability was calculated:

$$Cell\ relative\ viability(\%) = \frac{A\ sample}{A\ control} \cdot 100\%$$

where A denotes the absorbance value at 570 nm.

1.7 Cell imaging

L02, HepG2 and *E. coli* cells in logarithmic growth phase were collected and counted, and the cells were counted and seeded in laser confocal dishes at 4×10^3

cells/well containing different media (1640 medium with 20% FBS for L02 cells; MEM medium with 10% FBS for HepG2 cells; LB broth medium for *E. coli*). L-CQDs-3 was dissolved in all three media at a concentration of 0.5 mg/mL, respectively. Cells were then incubated at 37 °C and 5% CO_2 in a constant temperature incubator for 24 h. The medium was removed and each well was washed three times with PBS. Images of the three cells were captured using a laser scanning confocal microscopy (LSCM) at excitation wavelengths of 405 nm, 488 nm, and 559 nm, respectively.

2. Figure S1 to S6

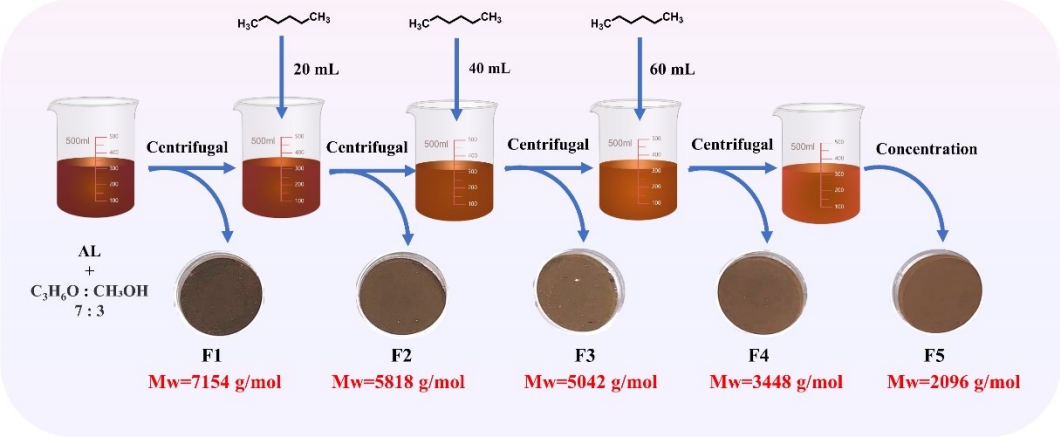


Figure S1. Schematic diagram of the lignin grading process.

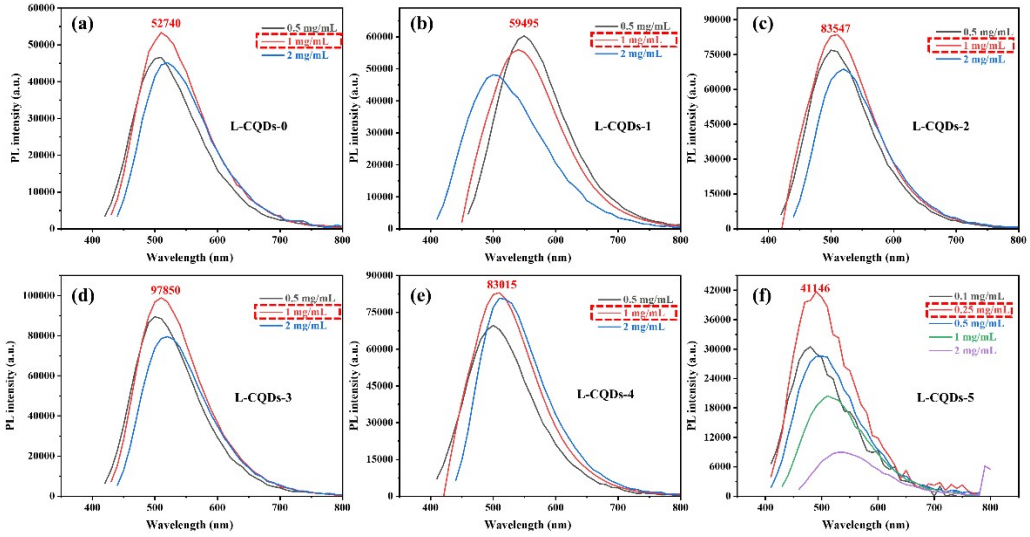


Figure S2. Emission spectra of (a) L-CQDs-0, (b) L-CQDs-1, (c) L-CQDs-2, (d) L-CQDs-3, (e) L-CQDs-4 and (f) L-CQDs-5 at optimal excitation wavelengths at different concentrations.

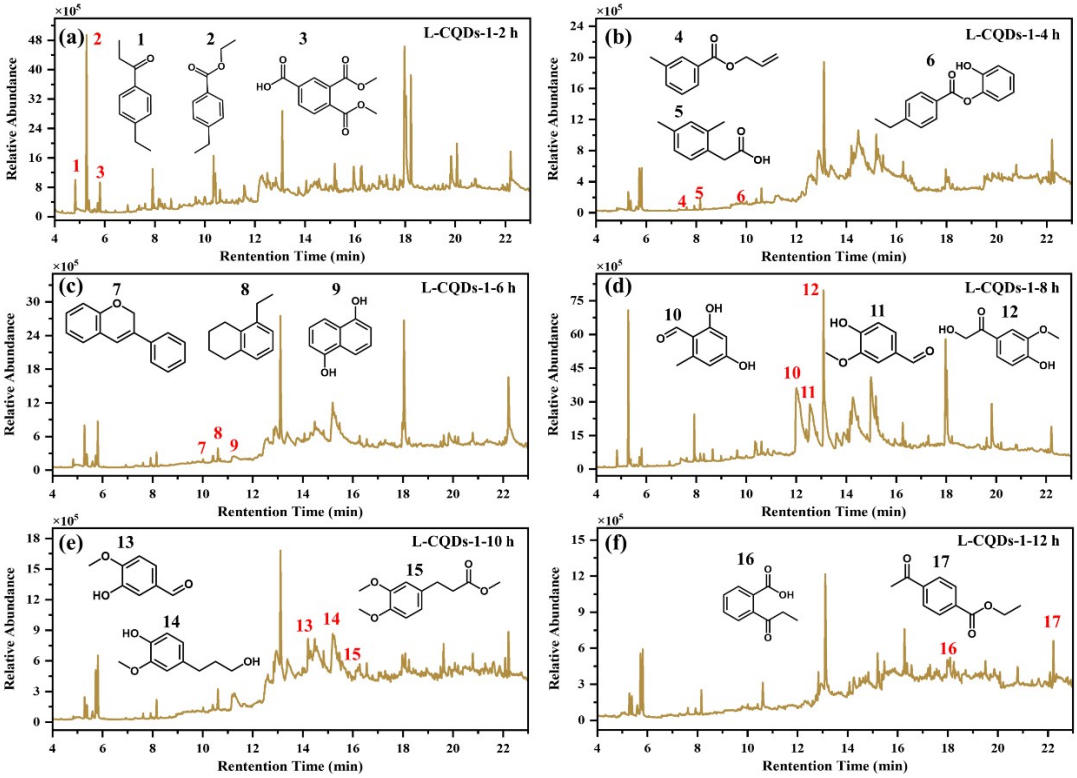


Figure S3. TIC spectra of L-CQDs-1 from F1 with different hydrothermal times: (a) 2 h, (b) 4 h, (c) 6 h, (d) 8 h, (e) 10 h, (f) 12 h.

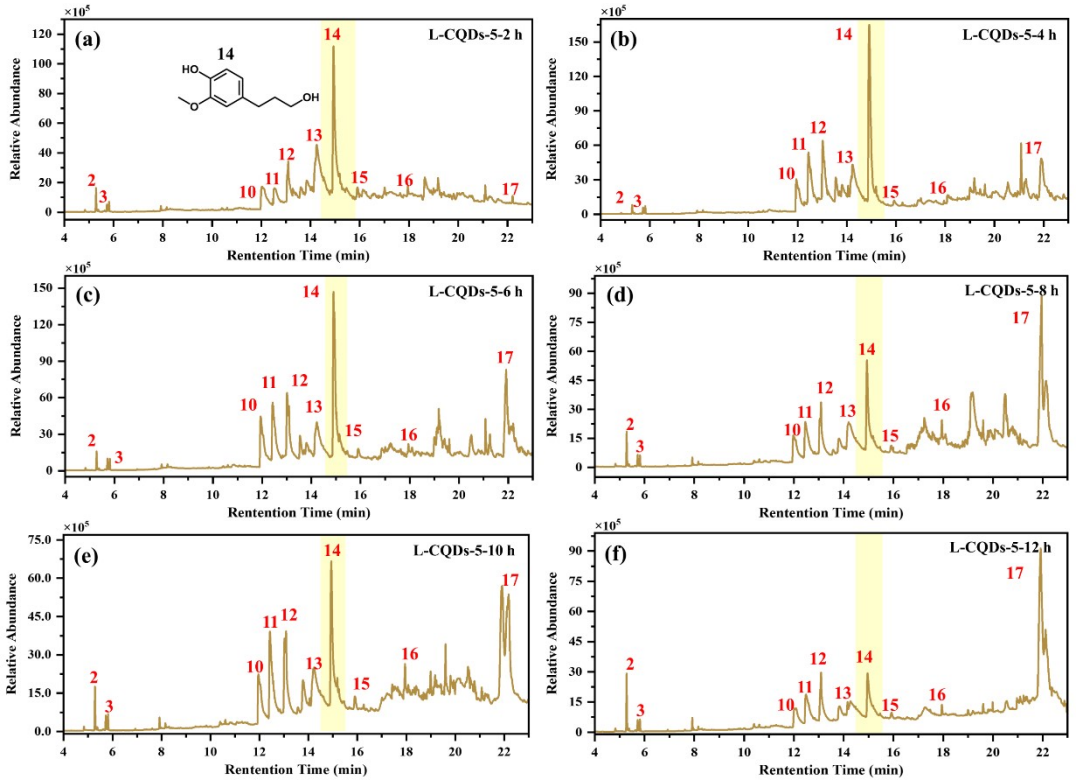


Figure S4. TIC spectra of L-CQDs-5 from F5 with different hydrothermal times: (a) 2 h, (b) 4 h, (c) 6 h, (d) 8 h, (e) 10 h, (f) 12 h.

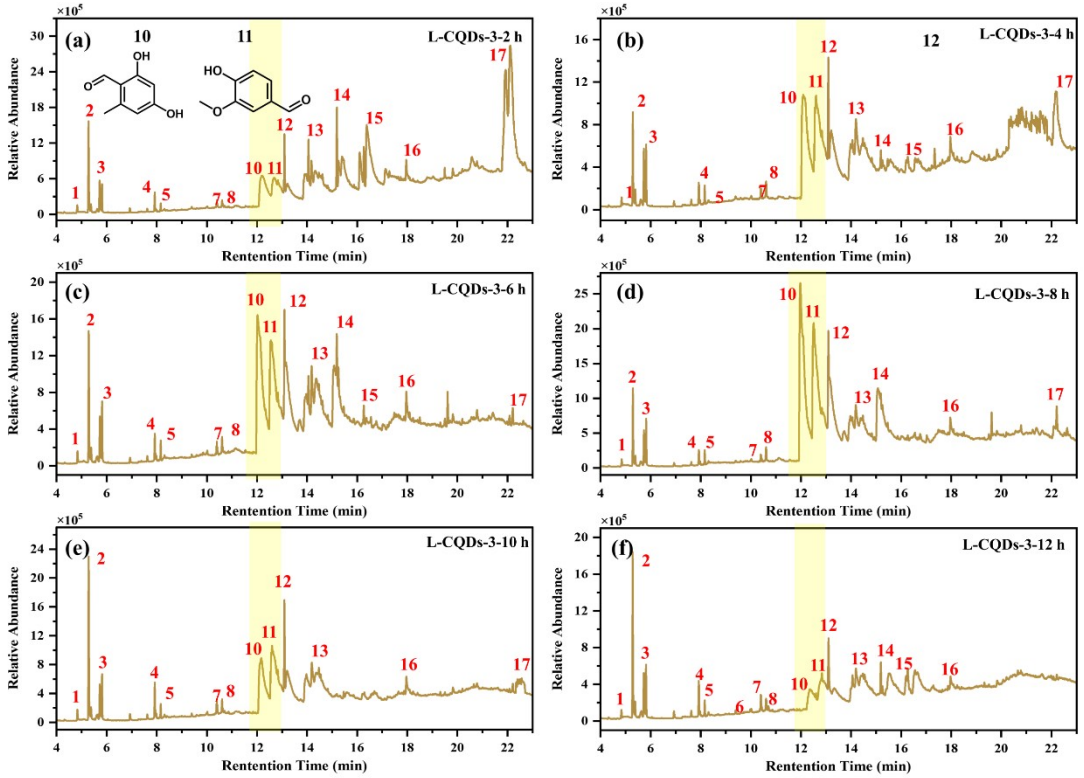


Figure S5. TIC spectra of L-CQDs-3 from F3 with different hydrothermal times: (a) 2 h, (b) 4 h, (c) 6 h, (d) 8 h, (e) 10 h, (f) 12 h.

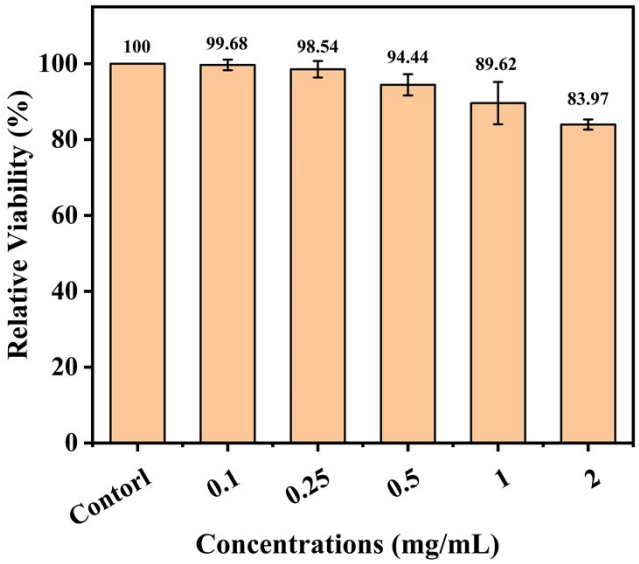


Figure S6. Effects of different concentrations of L-CQDs-3 on the activity of L02 cells.

3. Table S1 to S5

Table S1 Molecular weight statistics of lignin samples

Samples	Yields (%)	Mw (g/mol)	Mn (g/mol)	PDI
AL		3864	562	6.9
F1	32.8	7154	2527	2.8
F2	16.1	5818	2634	2.2
F3	17.8	5042	2374	2.1
F4	9.4	3448	2031	1.7
F5	23.5	2096	1007	2.1

Table S2. Statistical table of 31P NMR of lignin samples

Samples	δ (ppm)	AL	F1	F2	F3	F4	F5
Aliphatic OH	148.91–144.97	1.79	2.07	2.17	2.01	1.86	1.14
C_5–substituted phenolic OH	143.99–141.17	1.15	0.66	0.97	1.11	1.28	0.10
Guaiacyl phenolic OH	141.14–137.98	1.49	0.71	1.10	1.24	1.61	3.01
p–Hydroxylphenyl phenolic OH	137.88–136.79	0.10	0.05	0.08	0.06	0.12	0.16
Carboxylic acid OH	135.29–133.04	0.14	0.06	0.07	0.08	0.09	0.21
Total OH		4.67	3.55	4.39	4.50	4.96	5.52
Total phenolic OH		2.74	1.42	2.15	2.41	3.01	4.16

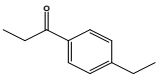
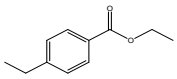
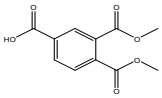
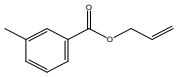
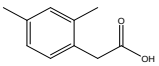
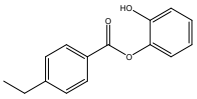
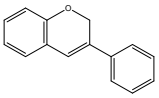
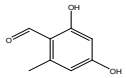
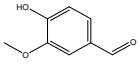
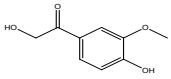
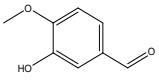
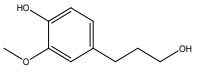
Table S3. 2D-HSQC NMR statistics of lignin samples

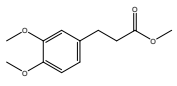
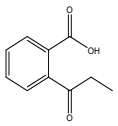
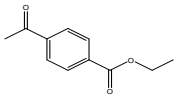
Lable	δC/δH (ppm)	Assignments
–OCH_3	55.5/3.70	C–H in methoxyls
Aγ	59.9/3.55	Cγ–Hγ in *β*–O–4’ substructures (A)
Fγ	61.6/4.01	Cγ–Hγ in p-hydroxycinnamyl alcohol end groups (F)
Cγ	63.0/3.63	Cγ–Hγ in phenylcoumaran substructures (C)
Bγ	71.0/3.65—4.06	Cγ–Hγ in *β*–*β*’ resinol substructures (B)
Aα	71.16/4.70	Cα–Hα in *β*–O–4’ substructures (A)
A*β*(G/H)	84.2/4.20	C*β*–H*β* in *β*–O–4’ substru ctures linked to G/H units (A)
Bα	85.17/4.54	Cα–Hα in *β*–*β*’ resinol substructures (B)
G_2	110.54/6.82	C_2–H_2 in guaiacyl units (G)
FA_2	112.46/7.41	C_2–H_2 in ferulate (FA)
$PCE_{3,5}$	115.5/6.73	$C_{3,5}$–$H_{3,5}$ in p-coumarate (PCE)
G_6	119.3/6.81	C_6–H_6 in guaiacyl units (G)
FA_6	120.2/7.15	C_6–H_6 in ferulate (FA)
F*β*’	125.74/6.88	C*β*–H*β* in the terminal group structure of hydroxycinnamaldehyde (F’)
$H_{2,6}$	128.48/7.13	$C_{2,6}$–$H_{2,6}$ in p-hydroxyphenyl units (H)

Table S4. Optical properties of L-CQDs.

Samples	Optimal excitation wavelength (nm)	Optimal emission wavelength (nm)	Fluorescence intensity	Quantum yield (%)
L-CQDs-0	410	510	52740	1.31
L-CQDs-1	400	500	59495	2.26
L-CQDs-2	420	510	83548	3.34
L-CQDs-3	420	510	97850	3.86
L-CQDs-4	410	510	83015	3.28
L-CQDs-5	410	510	41147	1.04

Table S5. GC-MS results of hydrothermal liquid products of lignin at different times.

Numbers	Retention time (min)	Compounds	Matching rate (%)	Structural formula
1	4.85	4'-Ethylpropiophenone	38	
2	5.28	4-Ethylbenzoic acid, ethyl ester	38	
3	5.81	1,2,4-Benzenetricarboxylic acid, 1,2-dimethyl ester	43	
4	7.89	m-Toluic acid, allyl ester	38	
5	8.17 8.65	Acetic acid, (2,4-xylyl)-	27	
6	9.64	1,2-Benzenediol, o-(4-ethylbenzoyl)-	50	
7	10.37	3-Phenyl-2H-chromene	11	
8	10.62	Naphthalene, 5-ethyl-1,2,3,4-tetrahydro-	15	
9	11.29	1,5-Naphthalenediol	11	
10	12.26	Benzaldehyde, 2,4-dihydroxy-6-methyl-	90	
11	12.50	Vanillin	95	
12	13.11	2,4'-Dihydroxy-3'-methoxyacetophenone	35	
13	14.56	Benzaldehyde, 3-hydroxy-4-methoxy-	42	
14	15.20	Benzenepropanol, 4-hydroxy-3-methoxy-	60	

15	16.03	Benzenepropanoic acid, 3,4-dimethoxy-, methyl ester	42	
16	18.01	Phenol, 4-ethyl-2-methoxy-	87	
17	22.22	Ethyl 4-acetylbenzoate	27	

4.References

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2. S. Zhao, G. Yue, X. Liu, S. Qin, B. Wang, P. Zhao, A. J. Ragauskas, M. Wu and X. Song, *Adv. Compos. Hybrid Mater.*, 2023, **6**, 73-85.