Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting information

Biotoxicity of Degradable Carbon Dots towards Microalgae *Chlorella vulgaris*

1. Detail experiments and procedures

1.1 The fluorescent stability of CDs

CDs powder was fully dissolved in the different pH solution (pH=3-11) and different NaCl solution (NaCl concentration: 0-2.0 mol/L). The final concentration of CDs was 180 μg/mL. After 24 h, the fluorescence spectra of CDs in different pH value and different ionic strength were measured (excitation wavelength: 370 nm). The value of the emission peak located at 455 nm of these solutions were compared for assessing the fluorescent stability of CDs.

1.2 Determination of photosynthetic pigment content

The content of photosynthetic pigment (chlorophyll *a*, chlorophyll *b* and carotenoids) were detected by colorimetric method with 90% acetone. *Chlorella* suspension (4 mL) incubated for 24, 48, 72 and 96 h were collected. Then, 8 mL of 90% acetone was added for extracting pigment. The extract was centrifuged at 5000 r/min for 10 min to remove residue and the pigment solution was obtained, which was monitored the absorbance of 664, 647 and 480 nm by UV-Vis spectrophotometer. The photosynthetic pigment content could be detected by following equation:

$$Chl.\,a\ (mg/L) = 11.93 \times OD_{664} - 1.93 \times OD_{647} \qquad (1)$$

$$Chl.\ b\ (mg/L) = 20.36 \times OD_{647} - 5.50 \times OD_{664} \quad (2)$$

$$Chl.\ (mg/L) = Chl.\ a + Chl.\ b \quad (3)$$

$$Car\ (mg/L) = 4.0 \times OD_{480} \quad (4)$$

1.3 Determination of carbohydrate content

Carbohydrate content of *Chlorella* was detected by anthrone method. The detection process refer to the previous reports.[1, 2]

1.4 Effects of CDs on SOD and Rubisco activity

The Super Oxidase Dimutase (SOD) activity and Rubisco activity *in vivo* and *in vitro* were measured by SOD elisa test kit and Rubisco elisa test kit, which includes Microelisa stripplate, Standard, Sample diluent, one kinds of reagents (SOD HRP-conjugate reagent or Rubisco HRP-conjugate reagent) and four kinds of solutions (Chromogen solution A, Chromogen solution B, Stop solution and Wash solution).

1.4.1 *In vivo* effects of CDs on SOD activity

A volume of 30 mL of *Chlorella* cells (control and 70 μg/mL CDs treated group) were harvested by centrifugalization (5000 r/min for 10 min) and suspended in 10 mL 0.01 mol/L PBS. The SOD extraction of *Chlorella* suspension sample was crushed by ultrasonication in an ice bath (interval 3 s between each ultrasonic 3 s in 400 W for 30 min). Then the crushing cells solution was centrifuged by 8000 r/min for 5 min and the SOD extraction was obtained. 10 μL of SOD extraction and 40 μL of sample diluent were added in microelisa stripplate, respectively. Then 100 μL of SOD HRP-conjugate reagent was added in each sample wells and the microelisa stripplate was covered with an adhesive strip. After 60 min, the reaction mixture was discarded and the sample wells

were washed by filling each well with wash solution (350 μL) for five times. Equal chromogen solution A and chromogen solution B were added to each well (total volume: 100 μL) and the mixture was incubated at 37 °C in the dark. After 15 min, 50 μL of stop solution was added and the color in the wells changed from blue to yellow. The OD_{450} value of the mixture was recorded by a microplate reader. Finally, the Rubisco activity was calculated using the standard curve (a series of concentration of standard treated with the same procedure). Three independent experiments were performed in triplicate.

1.4.2 *In vitro* effects of CDs on SOD activity

50 mg of SOD powder was dissolved in 2 mL of 0.01 mol/L PBS and the SOD solution was obtained. Then, 5 μL CDs of 50 mg/mL was added into 20 μL above SOD solution (25 mg/mL) and diluted this solution to 50 μL with 0.01 mol/L PBS (pH = 7.0). The control (without CDs treatment) and the SOD/CDs hybrids were incubated at 4 °C for 24 h. Finally, the activity of control and SOD/CDs hybrids were tested by SOD ELISA test kit.

1.4.3 *In vivo* effects of CDs on Rubisco activity

The assessment of the effects of CDs on Rubisco activity *in vivo* was similar with SOD. The extraction of Rubisco solution was obtained by ultrasonication and centrifugalization, which parameters were the same with the extraction of SOD solution and the test process was analogous to describe above.

1.4.4 *In vitro* effects of CDs on Rubisco activity

48 mg of Rubisco powder was dissolved in 2 mL of 0.01 mol/L PBS (pH = 7.0) and the

mixture was centrifuged by 8000 r/min for 5 min to discard insoluble substance. Then 5 μL CDs of 50 mg/mL was added into 20 μL above Rubisco solution and diluted this solution to 50 μL with 0.01 mol/L PBS (pH = 7.0). The control (without CDs treatment) and the Rubisco/CDs hybrids were incubated at 4 °C for 24 h. Finally, the activity of control and Rubisco/CDs hybrids were tested by Rubisco ELISA test kit.

1.5 Scanning electron microscopy (SEM) of *Chlorella*

Chlorella suspension (5mL) was centrifuged at 5000 r/min for 10 min and washed three times with 0.01 mol/L phosphate buffer saline (PBS, pH=7.0). Then the cells were fixed using 2.5% glutaraldehyde in 25 °C for 4~6 h. After fixation, the mixture was centrifuged (5000 r/min, 10min) and washed three times with 0.01 mol/L PBS for removing redundant glutaraldehyde solution. Then, *Chlorella* cells were dyed in 1% OsO_4 for 4 h and dehydrated through a graded series of ethanol aqueous solution (30%, 50%, 70%, 85%, 95% and pure ethanol for 2 min). The *Chlorella* -pure ethanol mixture solution was dripped in silicon foil and the sample dried in 4 °C. The *Chlorella* cells-silicon foil was coated with gold for increasing conductivity.

1.6 Transmission electron microscopy (TEM) of *Chlorella*

The sample preparation method according to the previous report, which treated with fixation, dye and dehydration.[2]

1.7 ESR measurements

Electronic spin resonance (ESR) spectrum was used for demonstrating if the radicals could be generated by CDs under visible light. 5, 5-dimethyl-1-pyrroline N-oxide (DMPO), as a radical trapping agent, can capture $^{\bullet}O_2^-$ or $^{\bullet}OH$ radicals and form to

DMPO-$^{\bullet}O_2^-$ or DMPO-$^{\bullet}$OH. 10 mg of CDs was dispersed in 500 μL methanol (for $^{\bullet}O_2^-$) or deionized water (for $^{\bullet}$OH) and then 45 μL of DMPO was added. ESR spectrum was recorded under visible light.

1.8 Statistical analysis

The degradation curve, growth rate, pigments, carbohydrate and ROS content, the activity of SOD and Rubisco, and the transcription of photosynthesis genes were conducted in triplicate and this results were presented as mean ± standard deviation (SD).Statistical analysis of the experimental data utilized Independent-Samples T Test. $P < 0.05$ is indicated by a single asterisk (*), and $P < 0.01$ is indicated by a double asterisk (**)

1.9 Characterizations

Transmission electron microscopy (TEM) images were obtained using a FEI/Philips Tecnai G2 F20 transmission electron microscope. Scanning electron microscopy (SEM) images were acquired on FEI-quanta 200 scanning electron microscope with an acceleration voltage of 20 kV equipped. Fourier Transform Infrared (FT-IR) spectra of CDs were obtained with a Perkin Elmer FT-IR spectrometer. The X-ray photoelectron spectroscopy (XPS) were measured using an Axis Ultra DLD X-ray photoelectron spectroscope. Photoluminescence (PL) study was carried out with Fluromax-4 (France Jobin Yvon company). The ultraviolet-visible (UV-vis) absorption spectra were obtained with a Perkin Elmer UV–vis spectrophotometer (Lambda 750). The CDs were irradiated by a Xe-lamp (300 W, PLS-SXE 300, Beijing Trusttech Co. Ltd, China) for obtaining

the degradation curve. The Electronic spin resonance (ESR) spectra were collected on a Bruker EPR A300-10/12 spectrometer. The flow cytometry (FCM) analyses of *Chlorella* cells were performed on a BD FACSCalibur flow cytometer. The hydrodynamic diameter of CDs and enzyme/CDs hybrids were measured by using a dynamic light scattering (DLS) instrument (Zetasizer Nano ZS, ZEN 3690, Malvern). A laser-scanning confocal fluorescence micro-Q6 scope (LSM, Leica, TCS-SP5), equipped with 405, 488 and 514 nm laser for excitation. Circular dichroism (CD) spectra were recorded on a JASCO J-815 Spectropolarimeter. The Real-time polymerase chain reaction (PCR) was carried out with a Real-time PCR instrument (CFX connect, Bio-red, America).

2. Supplementary figures

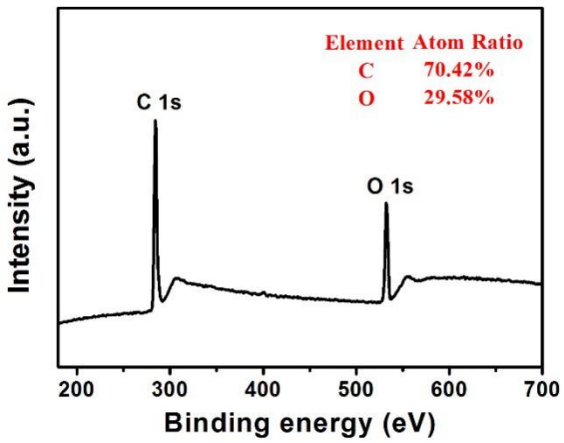

Fig. S1 Full scan XPS spectrum of CDs.

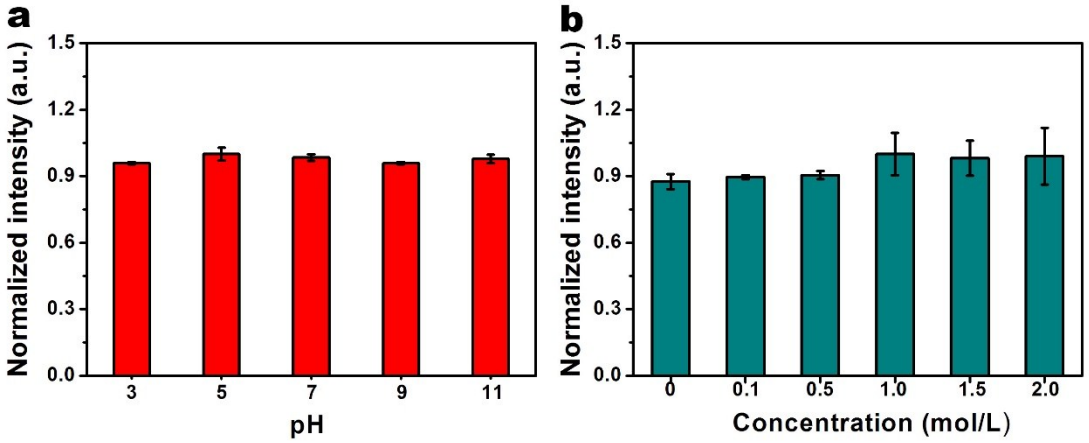

Fig. S2 The fluorescence intensity of CDs in (a) different pH values (3-11) and (b) different ionic strength (0-2.0 mol/L).

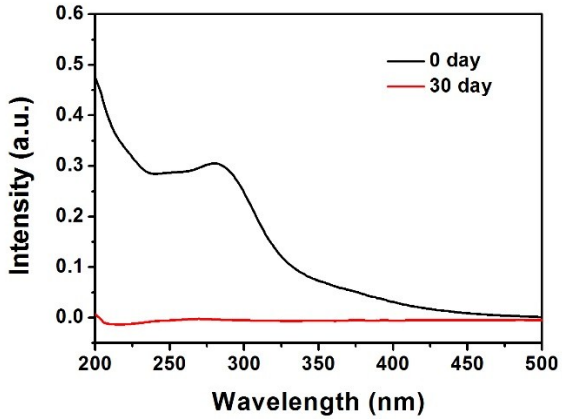

Fig. S3 The UV-vis absorption spectra of CDs solution (50 μg/mL) before and after degradation for 30 days.

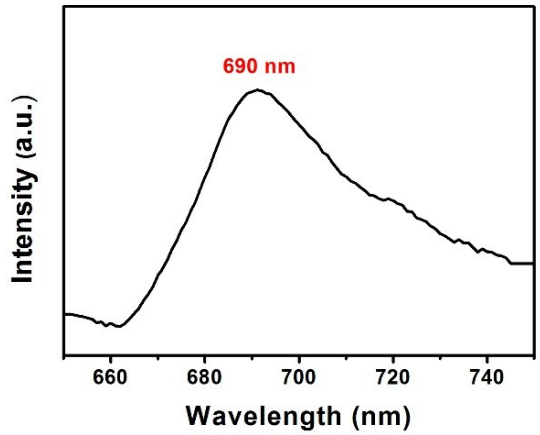

Fig. S4 UV-vis absorption spectrum of *Chlorella*.

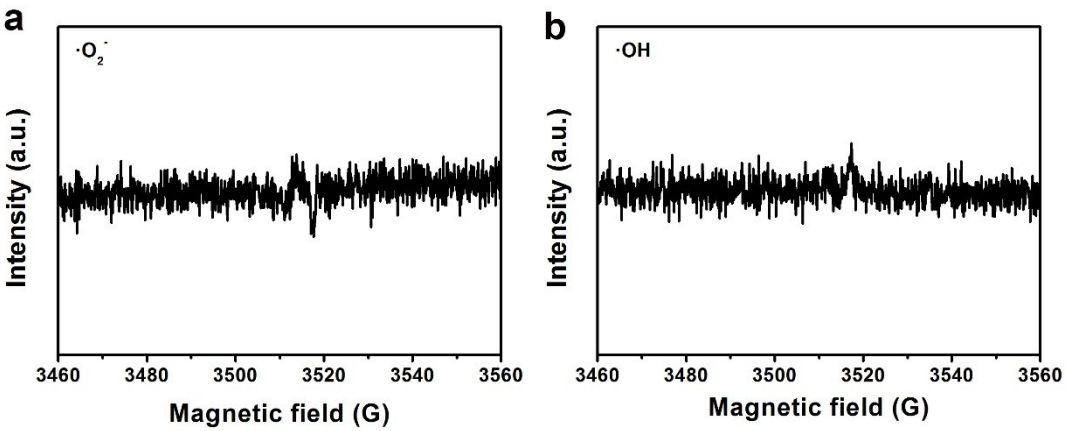

Fig. S5 The ESR spectrum of CDs dispersed in (a) methanol and (b) deionized water.

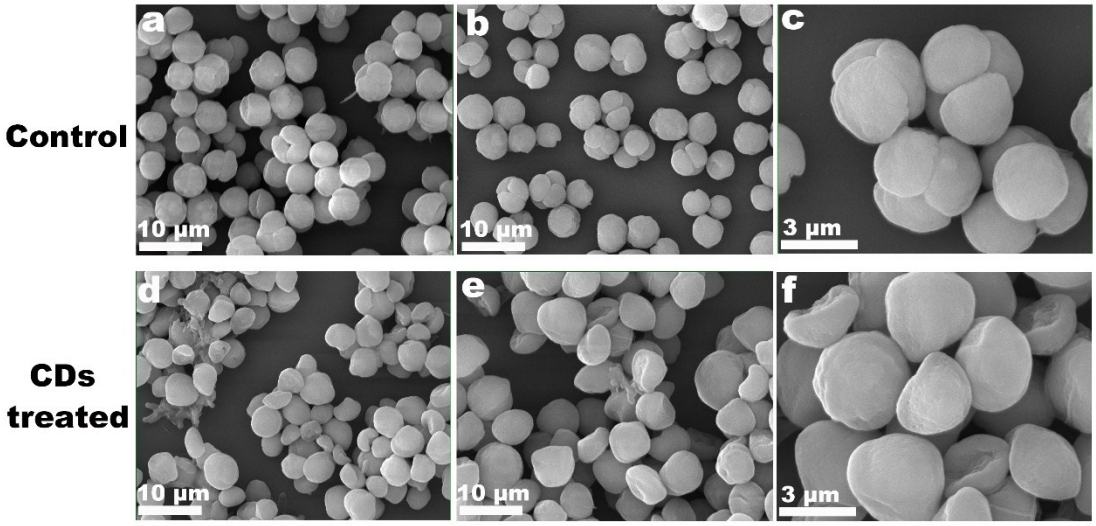

Fig. S6 The SEM images of *Chlorella* cells treated without (Control, a-c) and with CDs (CDs treated, 70 μg/mL, d-f).

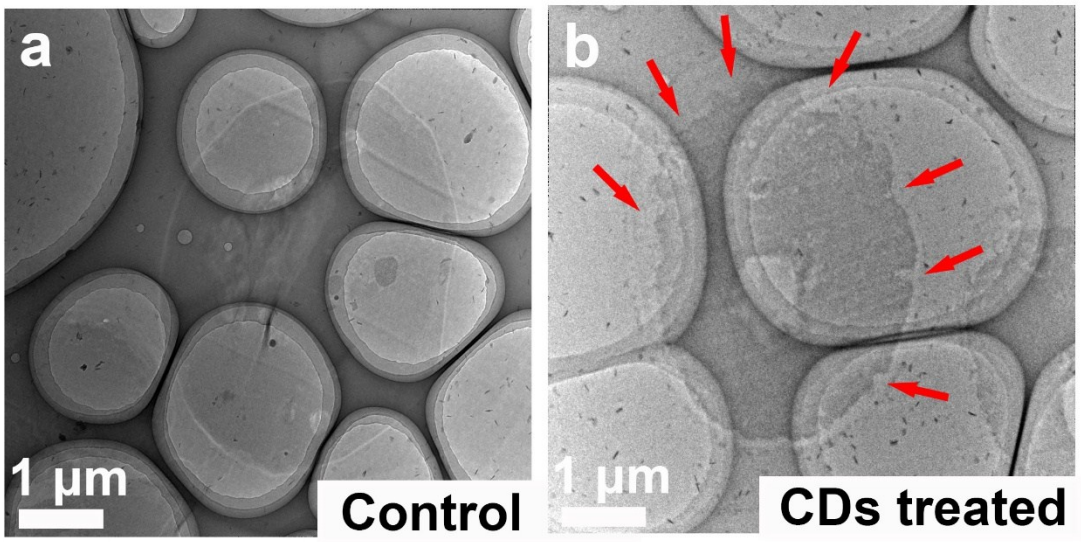

Fig. S7 The TEM images of *Chlorella* treated (a) without CDs (Control) and (b) with CDs (70 μg/mL).

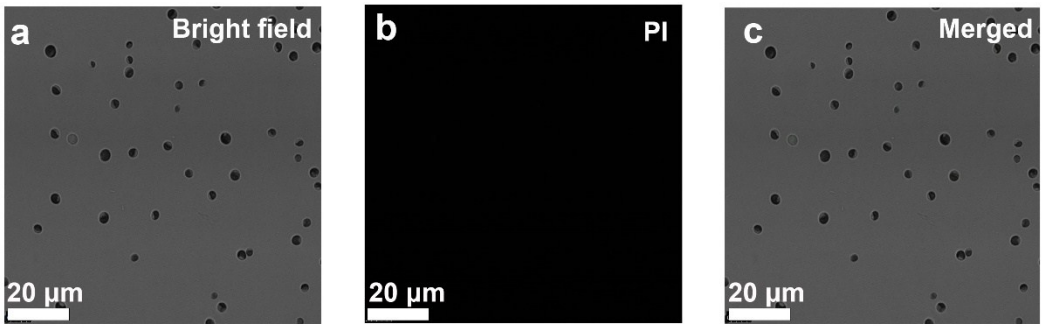

Fig. S8 LSM images of *Chlorella* incubated for 4 days and dyed with PI.

References

(1) A. Pons, P. Roca, C. Aguilo and F. J. Garcia, *J. Biochem. Biophys. Methods*, 1981, **4**, 227-231.

(2) M. Zhang, H. Wang, Y. Song, H. Huang, M. Shao, Y. Liu, H. Li and Z. Kang, *ACS Appl. Bio Mater.*, 2018, **1**, 894-902.