Supplementary Information (SI) for Nanoscale.


# Supporting Information

## A Diazotrophs-Lettuce Symbiosis Platform based on the Carbon Dots-Microalgae Hybrid System

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## Experimental

### Cultivation of microalgae

*Nostoc commune Vauch* microalgae was purified by culturing in nitrogen free BG11 medium for 5 generations. The nitrogen free BG11 medium includes 1.5 g/L $K_2HPO_4$, 40 mg/L $MgSO_4 \cdot 7H_2O$, 36 mg/L $CaCl_2 \cdot 2H_2O$, 6 mg/L citric acid, 6 mg/L ammonium ferric citrate, 1 mg/L $EDTANa_2$, 20 mg/L $Na_2CO_3$, 2.86 mg/L $H_3BO_3$, 1.86 mg/L $MnCl_2 \cdot 4H_2O$, 0.22 mg/L $ZnSO_4 \cdot 7H_2O$, 0.08 mg/L $CuSO_4 \cdot 5H_2O$, 0.39 mg/L $Na_2MoO_4 \cdot 2H_2O$, and 0.05 mg/L $CoCl_2 \cdot 6H_2O$.

### Ethylene gas chromatograph

1 mL medical needle was used to extract 1mL gas sample from air-tight reactor for ethylene content determination on an instrument of GC-2014C chromatograph (Shimadzu, Japan), with 3-5 repetitions. The working conditions: SPL-2014 split injection unit is adopted; the chromatographic column is a 2 m*0.3 mm activated alumina filed column; helium as carrier gas, while hydrogen as carries gas; the carriers gas flow rate is 30 mL/min; the column temperature is 80℃, the injection pot temperature is 140℃; the FID-2014 hydrogen flame ionization detector is manually ignited with temperature of 150℃. The ethylene produce rate is calculated by follows[1]:

$$Ethylene\ content\ (\mu L/L \cdot min^{-1}) = (0.00009 \times S - 0.0174)/t \qquad (1)$$

*S:* Peak area that assigned to ethylene;

*t:* reaction time under irradiation.

### Microalgae cells preparation for SEM

The microalgae cells were collected by centrifuging at 6000 rpm for 10 min. Firstly, the collected cells were fixed by 2.5% glutaraldehyde overnight. Then, removing the glutaraldehyde by centrifuging, use diluted concentration of ethanol (30, 50, 70, 90, 95 and 99.5%) to dehydrate and wash the cells for 10 min in each grade of dilution. Finally, the cells were dried by a vacuum free dryer.

### Microalgae cells preparation for TEM

The microalgae cells were collected by centrifuging at 6000 rpm for 10 min. Firstly, the collected

cells were fixed by 2.5% glutaraldehyde overnight. Then, removing the glutaraldehyde by centrifuging, wash the cells with 0.1 M phosphate buffer solution (pH=7.0) three times (15 min for each time). Colleted the cells and fixed them with 1% osmic acid solution for 1-2h. Removing the osmic acid solution, wash the cells with 0.1 M phosphate buffer solution (pH=7.0) three times (15 mins for each time). Subsequently, use diluted concentration of ethanol (30, 50, 70, 90, 95 and 99.5%) to dehydrate and wash the cells for 20 min in each grade of dilution. Replacing 99.5% ethonal with acetone to treat the cells for 20 min. Then, treat the cells with mixture of acetone and embedding agent (Va:Ve=1:1) for 1h. Use mixture of acetone and embedding agent (Va:Ve=1:3) to treat the cells for another 3h. Finally, the sample was soaked in pure embedding agent overnight. The embedded samples can be obtained by heating them overnight at 70℃.

The slices at 70-90 nm was obtained by an ultra-thin slicer (EM UC7, Leica Microsystems, Germany). Then the slices were stained with lead citrate solution and uranyl acetate 50% ethanol saturated solution respectively for 5-10 min, and finally dried for observation using TEM.

**Photosynthetic pigment measurement**

0.5 g of lettuce leave was added into 25 mL mixture of EtOH and acetone (1:1). 48 h. later, recorded the absorptance of supernatant at $OD_{470}$, $OD_{649}$, and $OD_{665}$. The photosynthetic pigments' content was calculated as follows[2]:

$$Chl\ a\ concentration\ (mg/L)\text{: } Ca = 13.95 \times OD_{665} - 6.88 \times OD_{649} \quad (2)$$

$$Chl\ b\ concentration\ (mg/L)\text{: } Cb = 24.96 \times OD_{649} - 7.32 \times OD_{665} \quad (3)$$

$$Caro\ concentration\ (mg/L)\text{: } Cxc = (1000 \times OD_{470} - 2.05 \times Ca - 114.8 \times Cb)/245 \quad (4)$$

$$Total\ chl\ \ concentration\ (mg/L)\text{: } Ct = 8.02 \times OD_{663} + 20.20 \times OD_{649} \quad (5)$$

$$Pigment\ \ content\ (mg/L)\text{: } = (C \times V)/(W \times 10^3) \quad (6)$$

C: Pigment's concentration;

V: The volume of mixture of EtOH and acetone;

W: the weight of lettuce leave.

**Soluble sugar content measurement**

Frozen fresh lettuce powder of 1g was added into 8 mL of 80% EtOH for water bath 1 h. After cooling to room temperature, filter and add water to wash the residue to a 25 mL volumetric flask. 0.5 mL of solution from the volumetric flack was mixed with 1.5 mL water and subsequently mixed with 0.5 mL anthrone ethyl acetate and 5 mL concentrated sulfuric acid. Boiling water bath for 1 min, recorded the absorptance at $OD_{630}$. The soluble sugar content of the samples was calculated as follows[3]:

$$Soluble\ sugar\ content(mg/g) = (C \times V_1)/(10^6 \times V_2 \times W) \qquad (7)$$

C: Value calculated from standard curves;

$V_1$: The volume of the fixed volume of the lettuce filtrate;

$V_2$: The volume of sample supernatant added when recording the absorptance.

W: the weight of sample.

**Soluble protein content measurement**

Frozen fresh lettuce powder of 0.5 g was added into 2 mL of 0.1 mol/L phosphate buffer solution for 30 min at room temperature. Centrifuge it at 8000 rpm for 10 min and collected supernatant. 0.5 mL supernatant was mixed with 0.5 mL water and subsequently mixed with 5 mL Coomassie G-250 solution. 5 mins later, recorded the absorptance at $OD_{595}$. The soluble sugar content of the samples was calculated as follows[3]:

$$Soluble\ protein\ content(mg/g) = (C \times V_1)/(10^6 \times V_2 \times W) \qquad (8)$$

C: Value calculated from standard curves;

$V_1$: The volume of the fixed volume of the lettuce filtrate;

$V_2$: The volume of sample supernatant added when recording the absorptance.

W: the weight of sample.

**Phytotoxicity of the carbon dots (CDs)**

The content of MDA in lettuce of each treatment was determined by the MDA (malondialdehyde, MDA, ContentAssay Kit, Colorimetric Method) content kit produced by

Shanghai Sangon Biological Engineering Company. The SOD activity in lettuce seedlings was determined by the NBT reduction method[4].

**Figures**

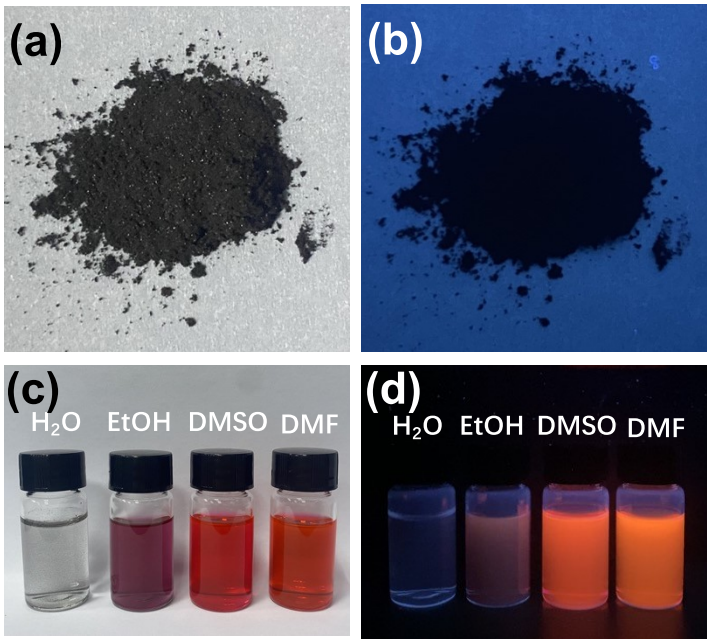


**Fig. S1.** (a-b) Powder of CDs under environmental light and 365 nm light respectively; (c-d) CDs dispersed in water, EtOH, DMSO and DMF under environmental light and 365 nm light respectively.

**Fig. S2.** PL spectra of the CDs dispersed in (a) DMSO and (b) DMF.

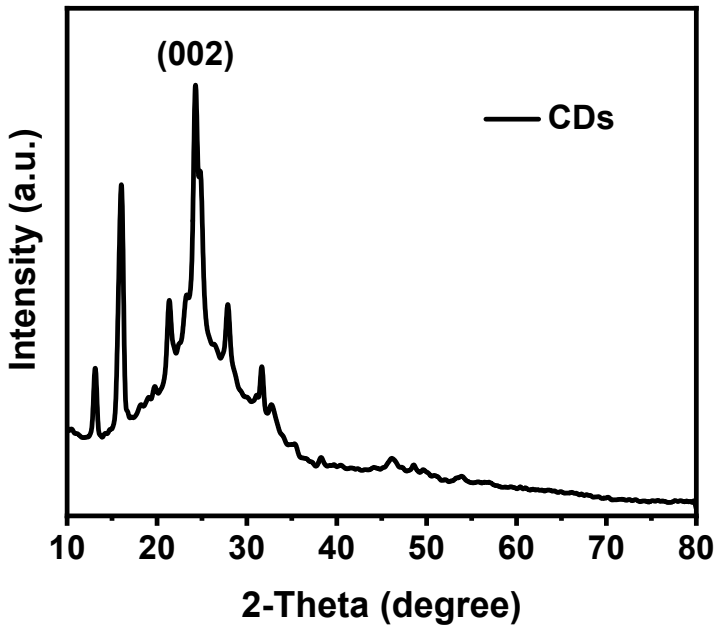


**Fig. S3.** XRD spectra of the CDs.

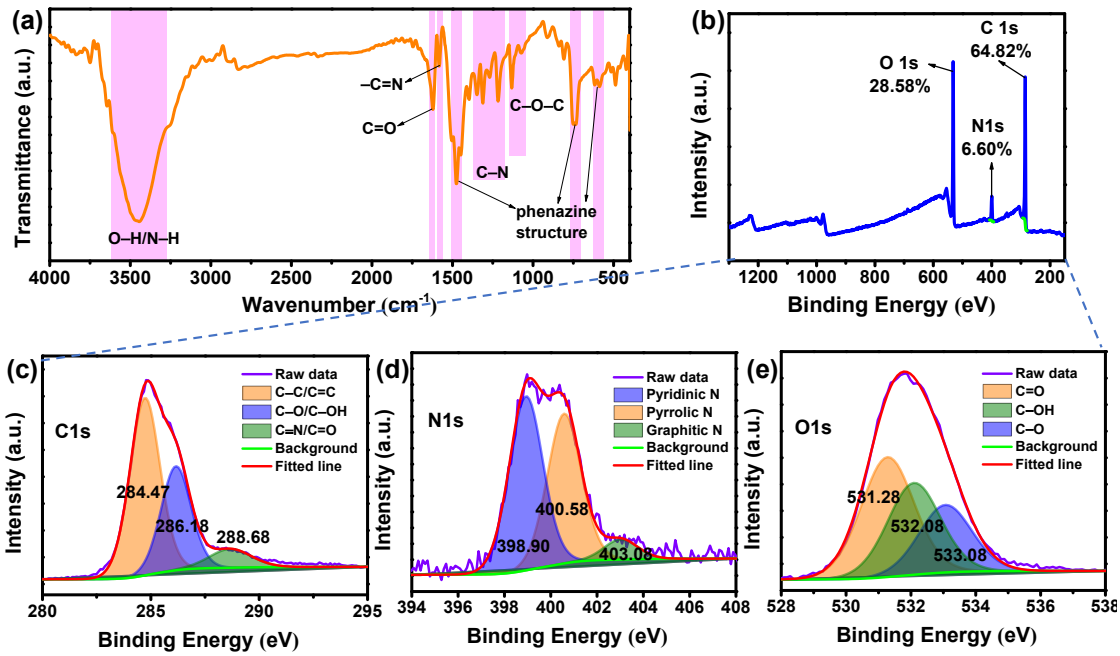


**Fig. S4.** (a) FTIR spectra and (b-e) XPS results of the CDs.

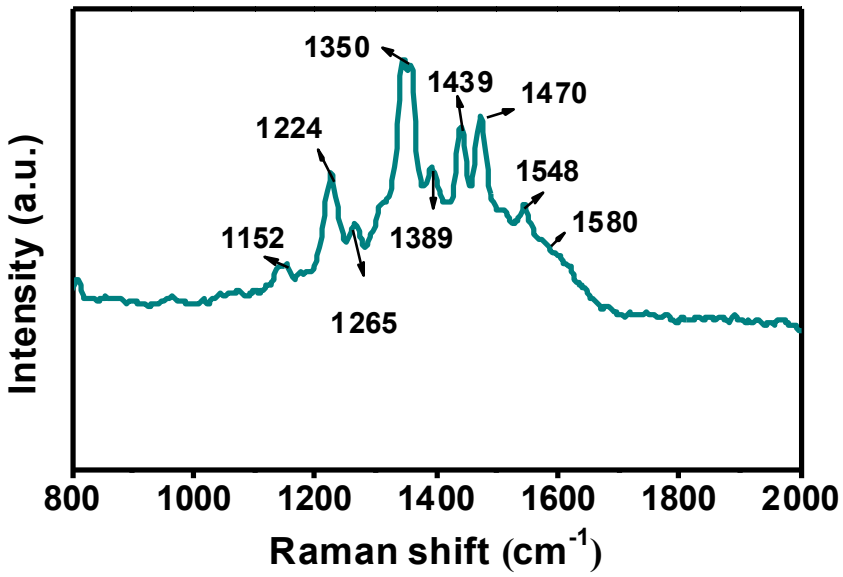


**Fig. S5.** Raman spectrum of CDs.

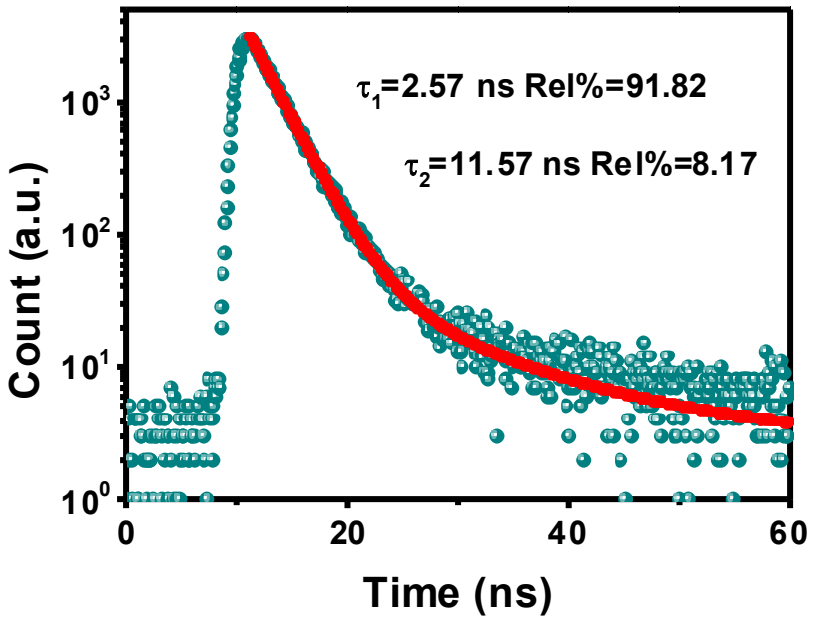


**Fig. S6.** The excited-state fluorescent decay curve of CDs monitored at 598 nm emission.

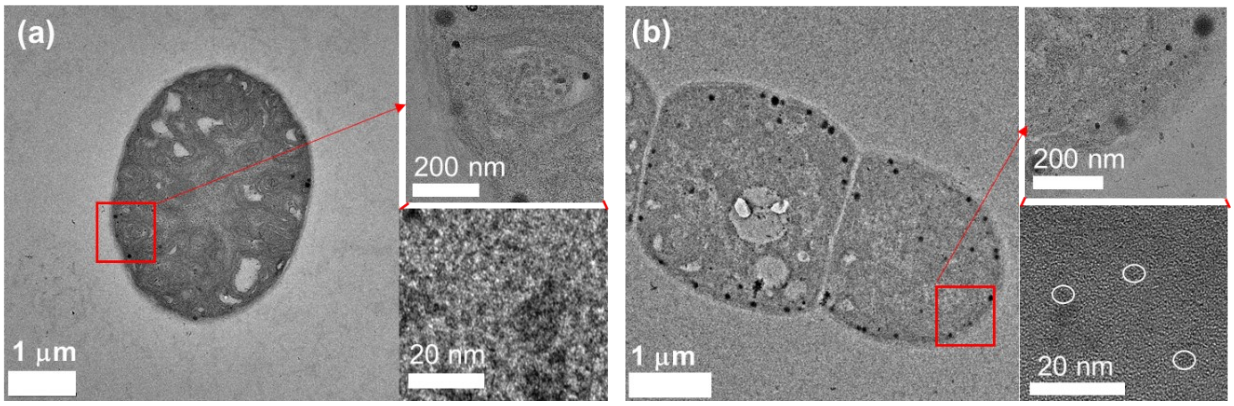


**Fig. S7.** TEM images of (a) pure microalgae and (b) CDs hybrid with microalgae.

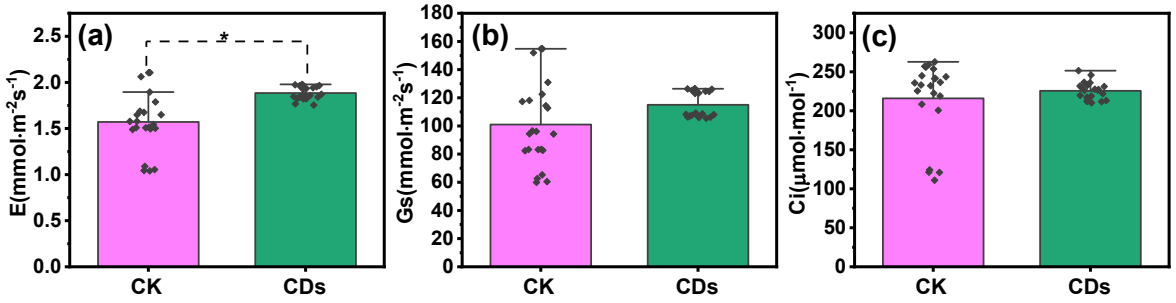


**Fig. S8.** Photosynthetic indexes including (a) E, (b) Gs, and (c) Ci of lettuce (CK is group with pure microalgae filtrate treatment, while CDs in group with CDs/microalgae filtrate treatment; "*" means significant difference ($P < 0.05$, n > 5).

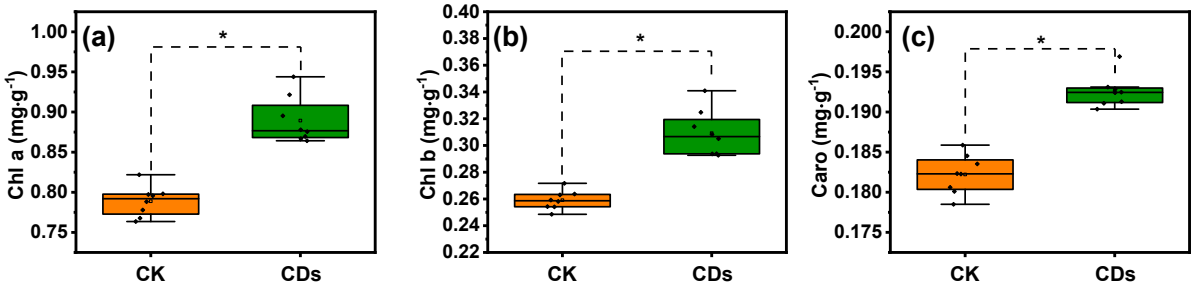


**Fig. S9.** (a) The Chl a, (b) Chl b and (c) carotenoids content of lettuce (CK is group with pure microalgae filtrate treatment, while CDs in group with CDs/microalgae filtrate treatment; "*" means significant difference ($P < 0.05$, n > 5).

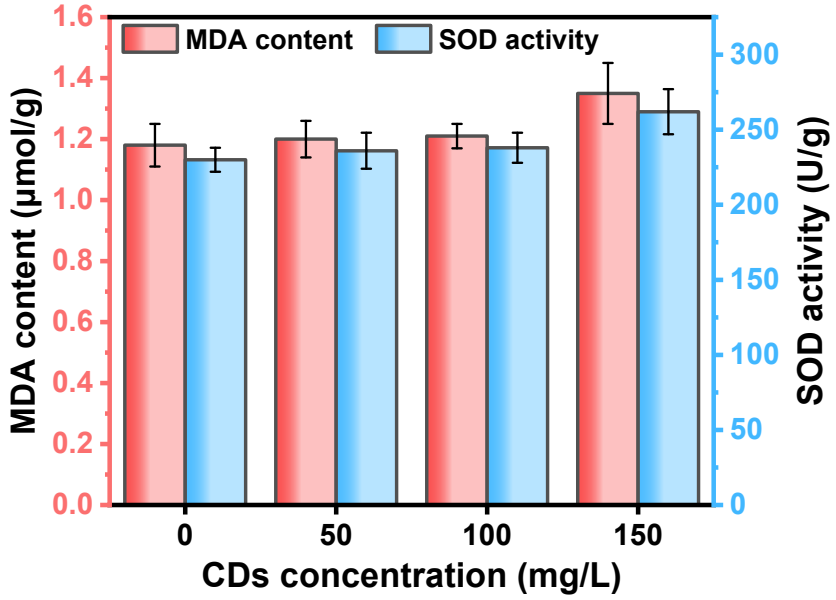


**Fig. S10**. MDA content and SOD activity of different treated lettuce.

## Reference

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