

Supplementary Materials

Carbon mass balance in *Arthrospira platensis* culture with medium recycle and high CO₂ supply

Applied Sciences

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1. pK₂ determination with KOH titration

The stoichiometric equilibrium constant, K_2 , between bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) was measured with KOH titration. After the preparation of the modified SOT medium by addition of 0.27 mol L^{-1} NaHCO_3 , 5-M KOH was added in small quantities to draw the relationship between KOH addition and pH (Fig. S1). The pK₂ was estimated from the intercept between the half of the equivalence point, $1/2 V_{\text{eq}}$, and the titration curve, which was approximately 9.74.

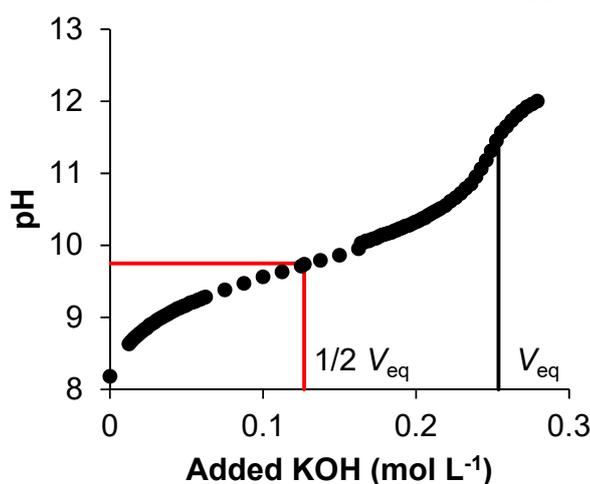


Figure S1. Modified SOT medium titration with KOH

When the two dissociating ions (HCO_3^- and CO_3^{2-}) exist at the same concentration, $K_2 = [\text{H}^+]$, and thus $\text{p}K_2 = \text{pH}$. On top of the titration, the K_2 value was confirmed by addition of 0.135 mol L^{-1} of sodium bicarbonate (NaHCO_3) and 0.135 mol L^{-1} of sodium carbonate (Na_2CO_3). The resulting pH was 9.80. Therefore, pK₂ 9.8 was assumed in this study.

2. CO₂ partial pressure in the absorption columns

The initial partial pressure of CO₂, $p_{\text{CO}_2}^0$ (Pa), in the headspace was,

$$p_{\text{CO}_2}^0 = y_{\text{CO}_2} \cdot p \quad (\text{S1})$$

where y_{CO_2} is CO₂ molar fraction (0.55 for Run1; 1 for Run 2 and 3), and p is the total headspace

pressure, which was assumed to be 1.013×10^5 Pa throughout the experiment. The gas bag was attached to 0.2- μm air-filter (Aervent-50, Millipore, USA), which was observed to prevent headspace gas intrusion into the gas bag, while permitting gas permeation from the gas bag into the headspace when headspace CO_2 was absorbed. The headspace CO_2 partial pressure at time t , $p_{\text{CO}_2}(t)$, gradually decreased with CO_2 absorption following the equation below:

$$p_{\text{CO}_2}(t) = \frac{nRT}{V_g} = \frac{(n_0 - \text{CO}_2^{\text{abs}} + x\text{CO}_2^{\text{abs}})RT}{V_g} = p_{\text{CO}_2}^0 - \frac{(1-x)\text{CO}_2^{\text{abs}}RT}{V_g} \quad (\text{S2})$$

where n is the amount of CO_2 molecules in the headspace at time t (mol), V_g is the volume of headspace (L), n_0 is the initial amount of CO_2 molecules (mol), CO_2^{abs} is the cumulative amount of CO_2 molecules transferred from gas to liquid since the beginning of a 24-hour CO_2 absorption period till time t (mol), and x is the CO_2 molar fraction in the gas bags. Since *A. platensis* is known to lack the capability of nitrogen fixation [37], the amount of nitrogen gas in the headspace was assumed to have remained constant.

3. CO_2 absorption capacity

The amount of CO_2 absorption over a 24-hour absorption period was estimated (Figure S1) based on the following calculations. Firstly, according to Henry's law, when equilibrium is reached, the relationship between gaseous CO_2 and dissolved CO_2 is as follows:

$$[\text{CO}_2]_{\text{sat}} = H^{cp} \times p_{\text{CO}_2} \quad (\text{S3})$$

where $[\text{CO}_2]_{\text{sat}}$ is the saturation concentration of CO_2 (mol L^{-1}), H^{cp} is Henry's law solubility constant ($3.30 \times 10^{-7} \text{ mol L}^{-1} \text{ Pa}^{-1}$) [38] and p_{CO_2} is the partial pressure of CO_2 (Pa).

According to Henry's law, the solubility of CO_2 into absorbent relies on the balance between the partial pressure of CO_2 in the gas phase and $[\text{CO}_2]$ in the liquid phase (Eq. S3). The partial pressure of CO_2 , in this experiment, decreased with the absorption of CO_2 , since lower concentrations of CO_2 were contained in the gas bags attached to the headspace (Table 1; see Supplementary Material Section 2 for detailed calculation). On the other hand, the absorbent's DIC increased with the absorption of CO_2 , reducing pH and rising $[\text{CO}_2]$. In this study, it was shown that pH could be described by the DIC concentration (Fig. 4-a). Therefore, the amount of CO_2 absorption can describe both CO_2 partial pressure and absorbent's $[\text{CO}_2]$ in this experiment. The amount of CO_2 absorption to reach the equilibrium (Eq. S3) is the maximum absorption capacity of the absorbent at a certain initial DIC concentration during an absorption period.

Secondly, the CO_2 partial pressure in the headspace of the absorption column was expressed by simplifying Eq. S2 as:

$$p_{\text{CO}_2}(t) = g(\text{CO}_2^{\text{abs}}) \quad (\text{S4}).$$

Thirdly, the liquid CO_2 concentration, $[\text{CO}_2]$, during a 24-hour CO_2 absorption period, was calculated based on the following sequences of equations. At the beginning of a 24-hour absorption period, the initial DIC concentration, DIC_0 was:

$$\text{DIC}_0 = c_0 + \Delta c_0 \quad (\text{S5})$$

where c_0 is the initial DIC concentration on Day 0 (ca. 0.27 mol L^{-1}) and Δc_0 is the difference in DIC from Day 0 (mol L^{-1}). During a 24-h absorption period, DIC concentration changed over time:

$$DIC(t) = c_0 + \Delta c_0 + \frac{CO_2^{abs}}{V_L} = DIC_0 + \frac{CO_2^{abs}}{V_L} \quad (S6)$$

where $DIC(t)$ is DIC concentration at time t (h; $0 \leq t \leq 24$), CO_2^{abs} is the cumulative amount of CO_2 absorbed into liquid till time t (mol), V_L is the volume of absorbent (0.4 L). In Eq. 7, ΔDIC was used as the difference in DIC concentration from Day 0. The annotation was modified to include CO_2^{abs} in order to take into account the CO_2 change during a 24-hour absorption period:

$$\Delta DIC = \Delta c_0 + \frac{CO_2^{abs}}{V_L} = DIC_0 - c_0 + \frac{CO_2^{abs}}{V_L} \quad (S7).$$

The ΔDIC in Eq. 7 was replaced with Eq. S7:

$$\begin{aligned} & [H^+]^4 + (K_1 + c_0 + c_b)[H^+]^3 \\ & + \left(K_1 K_2 + K_1 c_b - K_w - K_1 \left(DIC_0 - c_0 + \frac{CO_2^{abs}}{V_L} \right) \right) [H^+]^2 \\ & + K_1 \left(K_2 c_b - K_w - K_2 c_0 - 2K_2 \left(DIC_0 - c_0 + \frac{CO_2^{abs}}{V_L} \right) \right) [H^+] - K_1 K_2 K_w \\ & = 0 \end{aligned} \quad (S8)$$

and it was simplified to:

$$[H^+] = h(CO_2^{abs}) \quad (S9)$$

which estimates the proton concentration when CO_2^{abs} is absorbed. From the proton concentration, the liquid CO_2 concentration at time t can be estimated with the following, based on Eq. 1:

$$[CO_2] = \frac{DIC_0 + \frac{CO_2^{abs}}{V_L}}{1 + \frac{K_1}{h(CO_2^{abs})} + \frac{K_1 K_2}{h(CO_2^{abs})^2}} = \frac{DIC_0 + \frac{CO_2^{abs}}{V_L}}{1 + \frac{K_1}{h(CO_2^{abs})} + \frac{K_1 K_2}{h(CO_2^{abs})^2}} \quad (S10).$$

This equation was simplified to:

$$[CO_2] = f(CO_2^{abs}) \quad (S11).$$

Finally, using Eq. S4 and S11, Eq. S3 can be expressed as:

$$[CO_2]_{sat} = f(CO_2^{abs}) = H^{cp} \times g(CO_2^{abs}) \quad (S12).$$

By solving Eq. S12 for CO_2^{abs} , the total amount of CO_2 to reach the equilibrium between the liquid phase and the headspace (or the absorption capacity) can be estimated. Readers should note that, if fresh gas is continuously supplied, Eq. S4 is not necessary and can be substituted to a single value of CO_2 gas partial pressure. The resulting calculated curves as well as the experimental values are depicted in Figure S2.

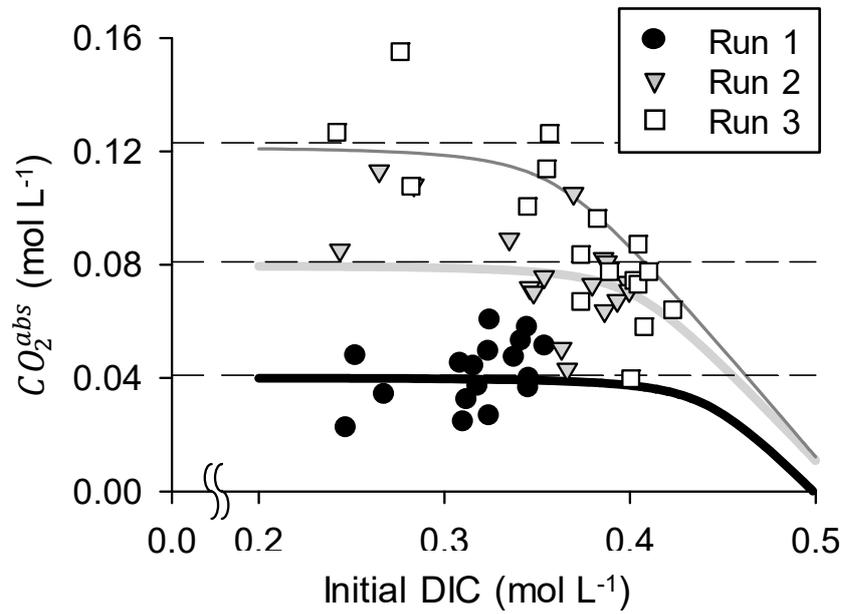


Figure S2. Calculated and experimental amount of the absorbed CO_2 over 24h (CO_2^{abs}) at different initial dissolved inorganic carbon (DIC). Plots: experimental values; solid lines: calculated values; and dashed lines: theoretical 100% absorption of supplied CO_2

4. Supplementary figures

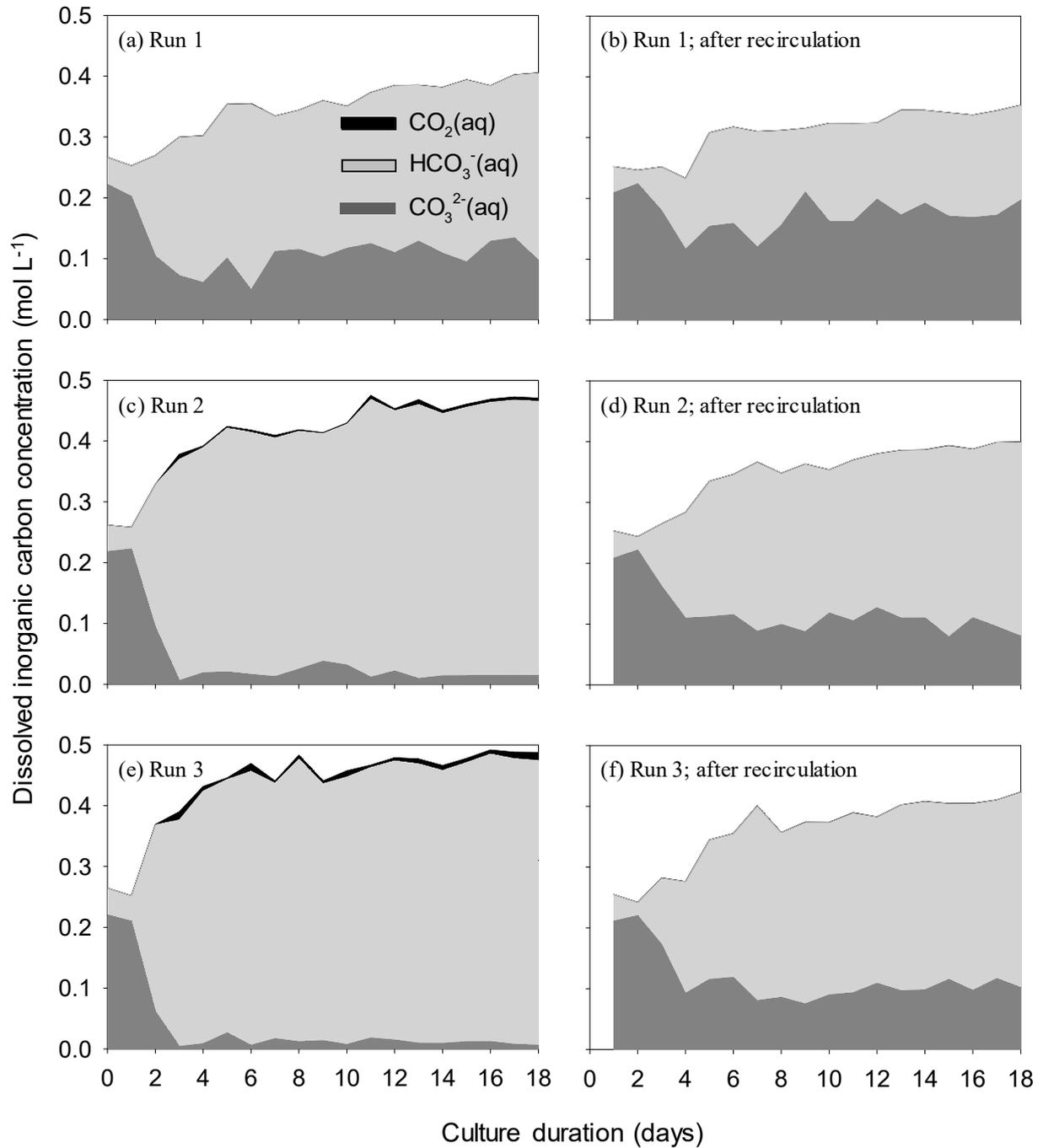


Figure S3. Dissolved inorganic carbon concentrations in CO₂ absorption columns; (a, c, e) after CO₂ supply, and (b, d, f) after medium recycle from the photobioreactors. Low pH after CO₂ supply induced decrease of CO₃²⁻ fraction and increase of HCO₃⁻ and CO₂ fractions

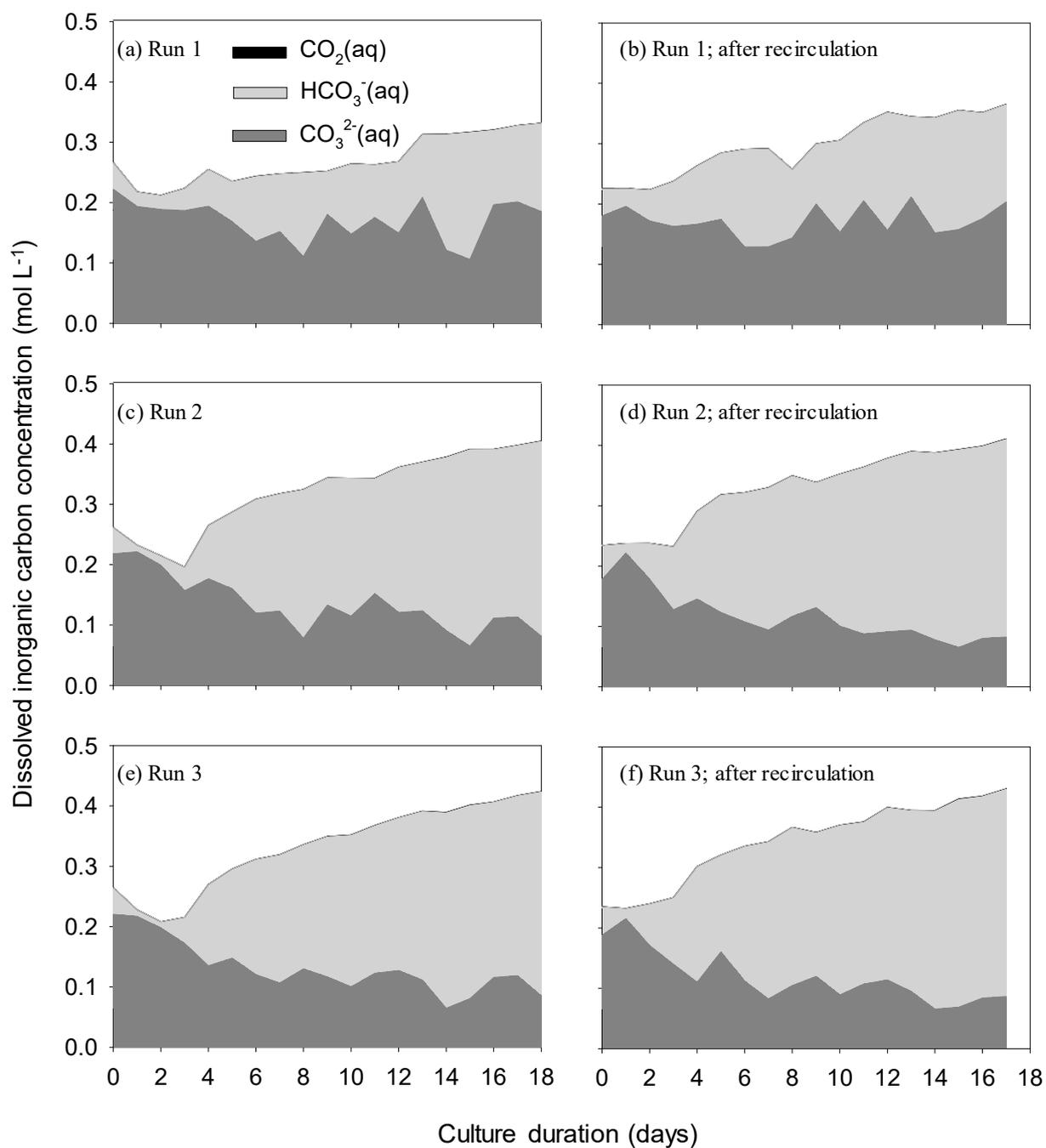
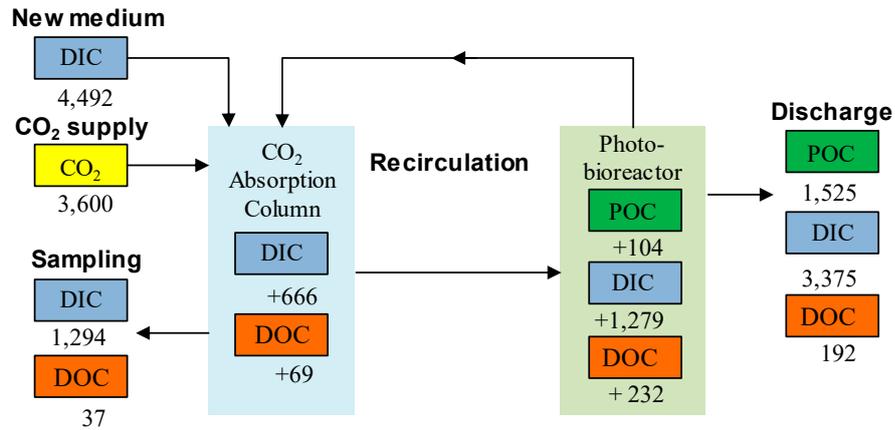
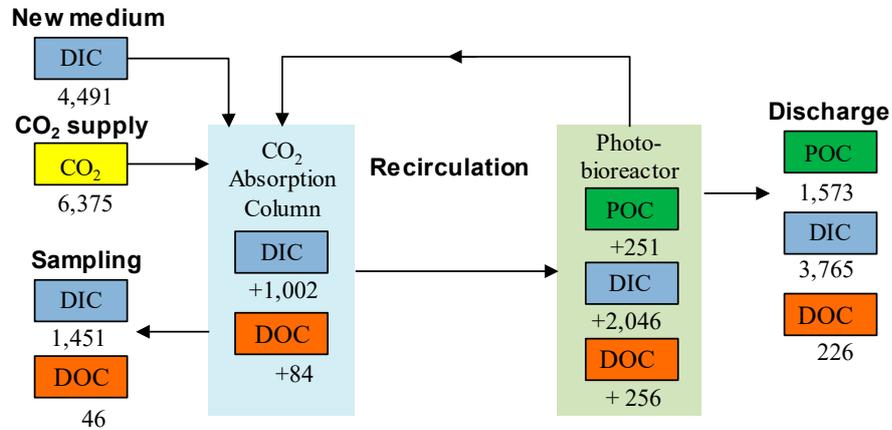


Figure S4. Dissolved inorganic carbon concentration in photobioreactors; (a, c, e) after 1-day incubation, and (b, d, f) after medium input from CO₂ absorption columns. Slight reduction in total DIC after incubation was observed.

(a) Run 1: 0.20 gC L-PBR⁻¹ d⁻¹



(b) Run 2: 0.39 gC L-PBR⁻¹ d⁻¹



(c) Run 3: 0.59 gC L-PBR⁻¹ d⁻¹

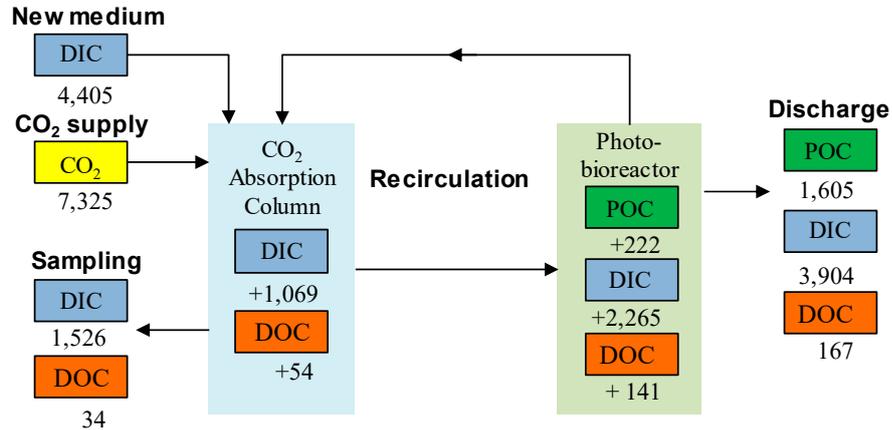


Figure S5. Carbon mass flux during 18-day operation. CO₂ supply rate of (a) Run 1, (b) Run 2, and (c) Run 3. Each box represents total cumulative mass (in milligram) of carbon fraction introduced or discharged over 18 days in the forms of CO₂, dissolved inorganic carbon (DIC), dissolved organic carbon (DOC) or algal biomass (particulate organic carbon; POC). The mass inside the CO₂ absorption column and photobioreactor represent differences from the initial state

References

1. Fujisawa, T.; Narikawa, R.; Okamoto, S.; Ehira, S.; Yoshimura, H.; Suzuki, I.; Masuda, T.; Mochimaru, M.; Takaichi, S.; Awai, K.; et al. Genomic structure of an economically important cyanobacterium, *Arthrospira (Spirulina) platensis* NIES-39. *DNA Res.* **2010**, *17*, 85–103.
2. Sander, R. Compilation of Henry's law constants (version 4.0) for water as solvent. *Atmos. Chem. Phys.* **2015**, *15*, 4399–4981.