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Supplementary Information

Monoethanolamine and microalgae collaboration enhances carbon sequestration and accumulation of high-value eicosapentaenoic acid

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Materials and Methods

1.1 Microalgae species and culture conditions

The microalgae species used in this study was *Nannochloropsis gaditana* KC–3, which was isolated and purified from the Bohai Sea and maintained in the authors' laboratory. KC–3 was cultured in filtered seawater supplemented with BG11 medium (Liu et al. 2020) in 250 mL flasks containing 150 mL of medium at 25 °C under 40 μmol/m^2/s. The flasks were shaken on a shaker table at 200 rpm for 6 min every 4 h.

1.2 Effects of MEA on TIC content in culture medium

The experiment was conducted to investigate the effects of MEA on CO_2 uptake and solubility in the culture medium. The culture medium was filtered seawater supplemented with BG11 without autoclaving. The experiments were performed in 250 mL flasks containing 150 mL of medium with free gas exchange through a filter paper-lined sealing film. The CO_2 used in this study was atmospheric and no additional CO_2 was used. MEA was added at concentrations of 0, 10, 20, 30, and 40 mg/L. The experimental assay was then initiated. All experiments were performed at 25°C under a light intensity of 40 μmol/m^2/s. The flasks were shaken on a shaker table at 200 rpm for 6 min every 4 h. TIC levels in the culture media were measured periodically until three consecutive measurements showed similar values, indicating the achievement of steady-state conditions.

1.3 MEA microalgae collaboration system

Compared to the above experimental conditions, the major difference is that the medium was autoclaved and the inoculation concentration of KC–3 was set to

$1*10^7$cells/mL. The culture medium was filtered seawater supplemented with BG11 and autoclaved. The experiments were performed in 250 mL flasks containing 150 mL of medium with free gas exchange through a filter paper-lined sealing film. The CO_2 used in this study was atmospheric and no additional CO_2 was used. MEA was added at various concentrations, then microalgae were inoculated and the experimental test was initiated. All experiments were performed at 25°C under a light intensity of 40 μmol/m^2/s. The flasks were shaken on a shaker table at 200 rpm for 6 min every 4 h.

1.4 Total inorganic carbon detection

The total inorganic carbon (TIC) of the solution was measured by centrifuging 10 mL of the algal culture at 5,500 rpm for 5 min at 4°C. The resulting supernatant was collected and TIC was quantified using a TC/TOC/TIC detector (Multi N/C 2100S, Analytik Jena) (Hou et al., 2023).

1.5 Determination of cell density

To determine the cell density under each treatment, 0.5 ml of the algal culture was collected for cell counting using a call counter (IA1000, Countstar, Shanghai, China) (Zhang et al., 2023).

1.6 Determination of cell dry weight

For the determination of the dry cell weight (DCW) of algae, 8 mL of cultured cells were collected, washed with distilled water, and filtered by passing through a 0.45 μm filter membrane. Before and after filtration, the membrane was dried at 105°C to constant weight (W_0 and W_1, g). The algae weight was calculated using the following equation: DCW (g/L) = ($W_1 – W_0$) / 0.008.

1.7 Microalgal composition analysis

To investigate the impact of MMC on algal biochemical composition, protein, lipid, and total carbohydrate were quantified. Microalgal cells were harvested by centrifugation at 8000 rpm for 10 min at 4 °C. The microalgal cell pellet obtained was washed twice with phosphate buffer solution. Protein content was determined using a bicinchoninic acid protein assay kit (Solarbio Science & Technology, Beijing, China). Total carbohydrate contents were determined by anthrone sulphuric acid method (Luo et al., 2023). The total lipids contents were determined using the method of Bligh and Dyer with a slight modification (Sun et al., 2015).

1.8 Determination of EPA

For fatty acid (FA) analysis, transmethylation reactions of the extracted lipid fractions were performed using 0.5 M KOH-methanol solution. FA methyl esters were then extracted with *n*-hexane and analyzed by modified gas chromatography (Hao et al., 2022). Samples were examined by a gas chromatography (GC-2010 Pro, Shimadzu, Kyoto, Japan) equipped with a Supelco RT-2560 column (100 m × 0.25 mm × 0.20 μm). The injector and detector temperatures were set at 230 and 260 °C, respectively. The oven temperature was set to hold at 100 °C for 5 min, then rise in temperature at a rate of 3 °C min−1 up to 240 °C, and finally held at 240 °C for 30 min. The carrier gas was high-purity nitrogen，with a flow rate of 30 ml/min, and the shunt ratio was 20:1. The eicosapentaenoic acid methyl ester (Sigma-Aldrich, St. Louis, MO, USA) were used to identify and quantify the EPA in the algal cells.

A series of standard points within the concentration range of 100~700 mg/L with a gradient of 100 mg/L were selected for the purpose of experimental calibration. The standard curve for EPA gas-phase detection was constructed by measuring the response signals at each concentration point and fitting the data linearly. The standard curve's mathematical expression is y=0.0013x+16.661, where y is the detected response signal and x is the substance concentration (Fig. S1).

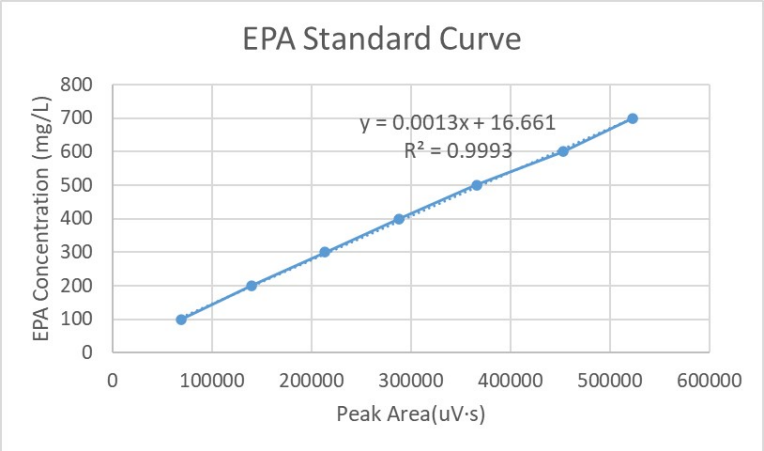

Figure S1. The standard curve for EPA gas-phase detection

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