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Review article

Enhanced lipid productivity coupled with carbon and nitrogen removal of the diatom *Skeletonema costatum* cultured in the high CO_2 level

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ABSTRACT

Rising CO_2 is causing noticeable environmental and social problems and thus to reduce CO_2 emission is becoming extremely urgent. Biodiesel from microalgae is considered to have immense potential in replacing fossil fuels and reducing CO_2 emission, apart from remediating eutrophic waters. How to increase biodiesel yield with low cost is the key for successful application of biofuel. In this study, we used high CO_2 concentration (5%) to culture the marine diatom *Skeletonema costatum* and aimed to enhance lipid productivity through the combination of low temperature shock. High CO_2 first inhibited the specific growth rate but then increased it after a 24 h pause of gas supply. High CO_2 increased lipid content by 26.8% and lipid productivity by 29.0%; the combination with low temperature shock resulted in a further stimulation (50.0% for lipid content and 42.5% for lipid productivity). High CO_2 also stimulated carbon and nitrogen assimilation, leading to the increase of carbon and nitrogen removal rates by 28.9% and 22.7%, respectively; while low temperature shock did not show a significant effect. High CO_2 increased maximum photochemical efficiency but reduced non-photochemical quenching, with insignificant effects of low temperature shock on either. Therefore, the stimulating effects of high CO_2 on lipid accumulation could be attributed to increased photosynthetic performance because photosynthesis can supply ATP and carbon skeleton for lipid synthesis. These findings indicate the success of the combination of high CO_2 and low temperature shock in enhancing algal lipid yield and bioremediation capacity.

1. Introduction

As a result of anthropogenic activities including the burning of fossil fuels, cement production and land use change, the atmospheric CO_2 has increased to 419 ppm in June 2021, which is 50% higher than pre-industrial levels and the highest ever recorded in human history [1]. It spent over 200 years to increase the amount of carbon dioxide in the atmosphere by 25%, and only 30 years to reach 50% higher than the pre-industrial levels [1]. The rapid increase in atmospheric CO_2 levels has led to a series of direct and indirect environmental sequences, including global warming, sea level rise, extreme weather, ocean acidification, etc. These environmental problems are causing severe ecological and social risks, referring to species loss and extinction, water supply, food security, human health, and economic growth [2]. Furthermore, these climate-related risks are predicted to increase with rising CO_2 and global warming [2]. To mitigate global climate change, most developed countries have pledged to achieve carbon neutrality by 2050. As the world's currently-largest CO_2 emitter, China has promised to reach peak emissions by 2030 and carbon neutrality by 2060. In order to reach these goals, power, transportation, and industrial sectors must be decarbonized first [3]. Therefore, carbon-zero bioenergy has been gaining attentions. On the other hand, to keep economic growth, it will be recalcitrant for decarbonization in the fields of aviation, freight transport and power-intensive industries [3]. Therefore, the balance between CO_2 emissions and sinks is essential and accordingly there is growing interest in techniques for actively removing CO_2 emissions from the atmosphere, termed negative emissions technologies (NETs). A variety of NETs have been proposed including soil carbon sequestration, forestry, wetland, blue carbon, weathering, direct air capture with carbon storage, bioenergy with carbon capture and storage (BECCS), etc. [4]. Therefore, bioenergy is the core for both carbon-neutral energy and BECCS.

Algal bioenergy is deemed to have enormous advantages compared to the first and second generation biofuels due to their high growth rates plus without competing arable land with crops [5,6]. The first thing for algal bioenergy is to select suitable species and hence all kinds of algae, including micro and macro, freshwater and seawater, have been tested for the use in bioenergy [5,7,8]. Among them, green microalgae,

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particularly *Chlorella* and *Dunaliella*, have received much more attention thanks to their high growth rates and lipid content [9,10]. Marine diatoms, contributing to approximately 40% of ocean carbon fixation, represent the most productive and environmentally flexible eukaryotic microalgae in the earth [11,12]. Furthermore, diatoms can acclimate to changes of light and temperature very well [13–15], indicating the advantages for outdoor culture. However, compared to green microalgae, fewer studies have been conducted on biofuels from diatoms.

The large-scale culture of microalgae requires massive consumptions of water, inorganic nutrients (mainly N and P) and CO_2, leading to the high production cost, severely limiting the development of algal biofuel industry. It has been showed that the combination of algae cultivation and wastewater/flue gas treatment is an effective way to reduce the cost of microalgae culture and enhance biomass yield [16,17], and the related environmental pollution can be friendly alleviated and even solved at the same time [17,18]. Although most algae possess CO_2 concentrating mechanisms (CCMs), aeration with high CO_2 usually enhances algal growth because CO_2 in media is still limited particularly for those with high cell densities [19–21]. In addition to rapid growth, high lipid content is also very desirable for biodiesel production from microalgae. Several approaches have been used to increase lipid content of algae. Among them nitrogen starvation may be the most effective and temperature regulation also shows the potential [22–24]. However, little is known on the combined effects of high CO_2 and low temperature shock on lipid synthesis and removal capacity of carbon and nitrogen in algae.

Skeletonema costatum, a ubiquitous and bloom-forming marine diatom, shows potential as biofuel [23]. Previous study demonstrated that short-term (6 h) high CO_2 treatment (5%) could stimulate the growth and lipid synthesis of *S. costatum* [25]. In the present study, we hypothesize that high CO_2 could stimulate CO_2 and nitrogen removal and the combination with temperature shock could enhance lipid synthesis and productivity of *S. costatum*. In this study, *S. costatum* was first cultured in different CO_2 levels and then transferred to low temperature to test this hypothesis.

2. Materials and methods

2.1. Algal culture and experimental design

Skeletonema costatum was isolated from coastal waters of the Yellow Sea (34.71°N, 119.49°E), China. Cells were first cultured with natural seawater enriched with F/2 medium in 1 L Erlenmeyer flasks to a high concentration of ~10^6 cells mL^{-1} (exponential growth phase) and then dispersed into twelve Erlenmeyer flasks (1 L volume) with the final concentration of 56,788 cells mL^{-1}. The ambient air (0.04%, LC) and 5% CO_2 (HC) were used for six flasks, respectively. Therefore, there are six incubations for each CO_2 treatment in this stage ($n = 6$). The 5% CO_2 was supplied by a CO_2 enricher (CE100C-2, Ruihua, China) that could maintain CO_2 levels with the variation less than 3% of the setting values. Cells of *S. costatum* could not growth in 10% CO_2 in a preliminary experiment and therefore 5% CO_2 was chosen for this study. The supply of 5% CO_2 was paused on day 5 for 24 h because continuous aeration with high CO_2 significantly inhibited growth of *S. costatum* based on the preliminary experiment. Cultures were conducted in an intelligent incubator (HP200G-3, Ruihua, China) that supply light with led bulbs (80 μmol photons m^{-2} s^{-1}) with a 12 h:12 h light dark cycle. The temperature was maintained at 20 ± 0.2 °C and the culture was aerated with a flow rate of 0.3 L min^{-1} through a 0.22 μm filter (Milipore, America). After 10 days of culture when biomass density at both CO_2 concentrations achieved or approached the peaks, half of flasks under ambient air and 5% CO_2 conditions were transferred to a 10 ± 0.2 °C intelligent incubator (HP200G-3, Ruihua, China) and the other half was kept at 20 ± 0.2 °C. Therefore, the experiment was conducted in triplicate in this stage ($n = 3$). The low temperature shock lasted for 4 h. The 10 °C shock was chosen because it is optimal to induce lipid accumulation in a short-time based on a preliminary experiment. The period of 4 h was chosen considering that this sort of periods is usually used for stress induction of *S. costatum* and longer time would lead to the decrease of algal growth [23].

2.2. Seawater carbonate system

The pH_{NBS} in media was measured at 10:30 a.m. daily with a pH meter (310P-01A, Thermo Fisher, China) that was calibrated with standard National Bureau of Standards (NBS) buffers (pH = 4.01, 7.00, and 10.01 at 25.0 °C; Thermo Fisher Scientific Inc., USA). At the same time, 15 mL media were collected for the measurement of total alkalinity (TAlk).TAlk was measured using Gran acidimetric titration method [26]. The data of pH and TAlk were applied to CO2SYS software [27] to calculate CO_2 concentration in seawater.

2.3. Biomass and growth

The cell number in each flask was counted at 10:00 a.m. every day with a plankton counter (DSJ-01, Dengxun, Xiamen, China) under the microscope (ECLIPSE Ts2-FL, Nikon, Japan). The specific growth rate (SGR) was calculated by the equation: $SGR = \ln (N_n/N_{n-1}) (t_n - t_{n-1})^{-1}$, where N_n and N_{n-1} represent the cell numbers at time t_n and t_{n-1}, respectively.

2.4. Lipid extraction and measurement

Lipid was extracted according to modified Folch method [28]. Algal cells were collected by centrifuging (8960 *g*, 10 min, 4 °C) 250 mL media and then dried in an oven (DHG-9146A, Jing Hong, China) at 60 °C to constant weight. Twelve mL chloroform-methanol (v:v = 2:1) and 3 mL NaCl solution (0.88%) were added to the algal powder of each sample and then mixed at 2000 rpm for 20 min using a Multi-tube vortex mixer (DMT-2500, China). After centrifugation (8960 *g*, 10 min, 20 °C), the upper phase was removed. Then 5 mL methanol-water (v:v = 1:1) solution was added to each tube, Mixing (2000 rpm, 20 min) were conducted again. After centrifuging (8960 *g*, 10 min, 20 °C) and removing the upper phase once more, the bottom phase was dried under a steady stream of nitrogen supplied by a Nitrogen Blowing Instrument (NDK200-1 N, Miulab, China). Lipid weight was obtained by subtracting the empty centrifuge tube with the centrifuge tube holding lipid. Lipid content (%) is the percentage of lipid weight to dry weight of algal powder. Lipid productivity (mg L^{-1} d^{-1}) = ($C_n \times L_n – C_0 \times L_0$)/t, where C_n and C_0 are cell density (mg L^{-1}) at initial and end of the culture period, respectively; L_n and L_0 are lipid content (%) at initial and end of the culture period, respectively; t is the culture period.

2.5. Elementary composition

Cells cultured under different conditions were collected and filtered onto 0.22 μm Whatman GF/F filters (25 mm in diameter) that were precombusted at 450 °C for 6 h before use. Collections were fumed with high concentration of HCl (12 mol L^{-1}) for 12 h to remove possible inorganic carbon and nitrogen and then dried in an oven (DHG-9146A, Jing Hong, China) at 60 °C for another 12 h. The contents of carbon and nitrogen in cells were measured by an Elementar Vario EL Cube (Elementar, Germany).

2.6. Removal rate of carbon and nitrogen

Removal rate of carbon and nitrogen by algae was calculated according to the following equation: R = ($C_n \times E_n – C_0 \times E_0$)/t, where R is the ramoval rate (mg L^{-1} d^{-1}) of carbon or nitrogen; C_n and C_0 are cell density (cells L^{-1}) at initial and end of the culture period, respectively; E_n and E_0 are carbon or nitrogen content (mg $cell^{-1}$) at initial and end of the culture period, respectively; t is the culture period.

2.7. Photosynthetic parameters

Photosynthetic parameters of *S. costatum*, including maximum photochemical efficiency (F_v/F_m) and nonphotochemical quenching (NPQ), were determined by a Multiple Excitation Wavelengths Chlorophyll Fluorescence Analyzer (Multi-color-PAM, Walz, Germany). According to the equations of Genty et al. [29], $F_v/F_m = (F_m—F_0) / F_m$; $NPQ = (F_m–F_m')/F_m'$, where F_m and F_0 are maximum and minimum chlorophyll fluorescence of cells after 15 min dark adaptation, respectively; F_m' is the maximum fluorescence yield under actinic light. The actinic light was set to be consistent with the culture light intensity (80 μmol photons m^{-2} s^{-1}) and the saturating pulse was 5000 μmol photons m^{-2} s^{-1} with the duration time of 0.8 s.

2.8. Photosynthetic pigments

Fifty mL media were collected and filtered with GF/F filters (Whatman, UK). Then the filtered cells were extracted with 5 mL absolute methanol at 4 °C over night. The extracts were centrifuged at 5000 *g* for 10 min at 4 °C (Universal 320R, Hettich, Germany) and then scanned with a spectrophotometer (TU-1810DASPC, China). The contents of chlorophyll *a* (Chl *a*) and carotenoid content were determined according to the equations of Wellburn [30].

2.9. Statistical analysis

The data in this study were expressed as means ± standard deviation (SD) and statistical analysis was performed using SPSS v.23. The data conformed to a normal distribution (Shapiro-Wilk, $P > 0.05$) and the variances could be considered equal (Levene's test, $P > 0.05$). Repeated measures analysis of variance (ANOVA) was used to assess the effects of CO_2 on biomass, growth rate, pH and CO_2 levels over the culture period. Two-way ANOVA was conducted to analyze the effects of CO_2 and temperature on lipid content, lipid productivity, carbon content, nitrogen content, ration of C/N, carbon removal rate, nitrogen removal rate, F_v/F_m, NPQ, Chl *a* content, and carotenoids content. Least significant difference was used for post hoc analysis. *P*-values less than 0.05 were considered statistically significant.

3. Results

3.1. Environmental parameters and growth

The changes of pH and CO_2 concentration in seawater were monitored during the culture period (Fig. 1). Repeated ANOVA analysis showed that CO_2 and culture time interacted on pH in seawater ($F_{(9,90)} = 150.388$, $P < 0.001$) and high CO_2 significantly decreased pH ($F_{(1,10)} = 6020.073$, $P < 0.001$). Under low CO_2 condition (LC, 0.04%), pH ranged from 8.11 to 8.77 during the culture period. Under high CO_2 condition (HC, 5%), it changed from 6.28 to 6.72 if the value on day 6 was excluded. The leap of pH on day 6 was due to the pause of 5% CO_2 supply on day 5 for 24 h. High CO_2 supply significantly increased CO_2 level in seawater ($F_{(1,10)} = 3406.106$, $P < 0.001$). Under high CO_2 supply, CO_2 in seawater maintained a high level (>570 μmol kg^{-1} seawater) except for that on day 6, while CO_2 level was below 20 μmol kg^{-1} seawater when supplied with ambient air.

The changes of cell density of *S. costatum* during the culture period were also detected (Fig. 2A). Cell density varied with culture time ($F_{(9,90)} = 871.912$, $P = 0.026$) and the patterns under LC and HC are different ($F_{(9,90)} = 7376.450$, $P = 0.009$). Cell density under ambient CO_2 kept increasing until day 8 and then decreased to $2.71 \pm 0.16 \times 10^6$ cells mL^{-1} by day 10. On the other hand, cell density under high CO_2 did not increase significantly until day 5, reached $2.69 \pm 0.25 \times 10^6$ cells mL^{-1} on day 9 and kept stable ($2.72 \pm 0.31 \times 10^6$ cells mL^{-1}) on day 10. In terms of specific growth rate (Fig. 2B), CO_2 and culture time also had an interactive effect on it ($F_{(1,10)} = 209.701$, $P < 0.001$). Specific growth rate under LC showed a continuous decrease trend while it increased to the peak (1.26 d^{-1}) on day 5 and then decreased with culture time under HC.

3.2. Lipid content and productivity

Lipid content in *S. costatum* cultured under different conditions ranged from 27 to 41% (Fig. 3A). Both CO_2 and temperature affected lipid content (Table 1). Post hoc LSD comparison ($P = 0.05$) showed that HC increased lipid content by 26.8% and 27.4% under control and low temperature (LT), respectively. LT increased lipid content by 18.3% under HC while the increase under LC was not statistically significant. The combination of HC and LT resulted in the largest increase of 50.0% in lipid content. CO_2 and temperature treatments also significantly affected lipid productivity (Table 1). HC increased lipid productivity by 29.0% and 17.7% under control and LT, respectively (Fig. 3B). LT stimulated lipid productivity by 21.1% and 10.5% under LC and HC,

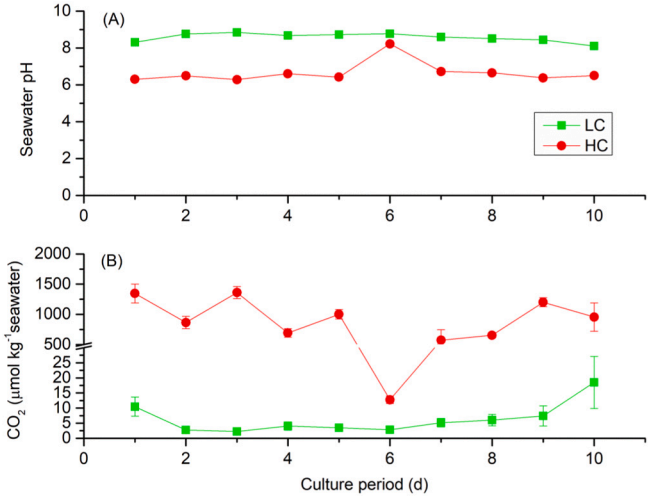

Fig. 1. The change of pH (A) and CO_2 concentration (B) in seawater during the culture period. LC and HC represent ambient air and 5% CO_2, respectively. The error bars represent the standard deviations ($n = 6$).

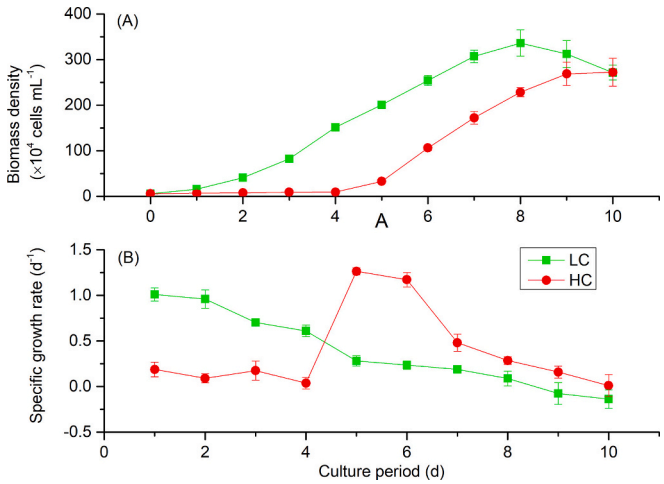

Fig. 2. Biomass density (A) and specific growth rate (B) of *S. costatum* cultured at different CO_2 levels. LC and HC represent ambient air and 5% CO_2, respectively. The error bars represent the standard deviations (n = 6).

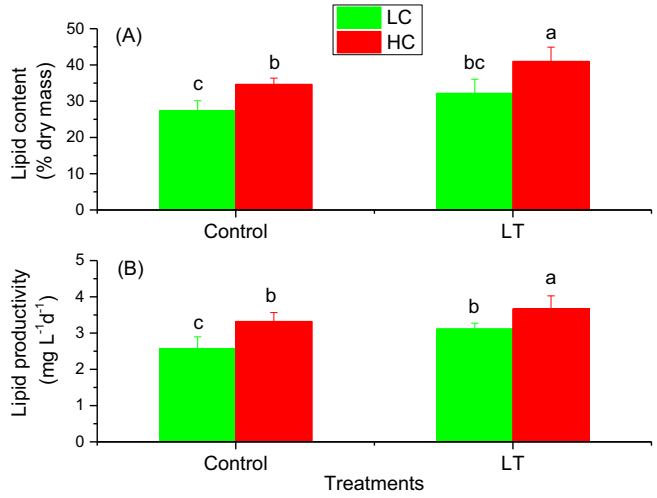

Fig. 3. Lipid content (A) and productivity (B) of *S. costatum* cultured at different CO_2 levels suffering from low temperature shock. LC and HC represent ambient air and 5% CO_2, respectively. Control and LT represents 20 °C and 10 °C, respectively. The error bars represent the standard deviations ($n = 3$). Different letters above error bars represent significant differences among treatments ($P < 0.05$).

respectively. HC combined with LT increased lipid productivity by 42.5%.

3.3. Content and removal rate of carbon and nitrogen

Carbon content of *S. costatum* cultured under different CO_2 and temperature conditions ranged from 30.5 to 39.7% (Fig. 4A). CO_2 affected carbon content while temperature did not show any significant effect (Table 1). HC increased carbon content by 27.9% and 30.2% under control and LT conditions, respectively. Nitrogen content of *S. costatum* changed from 5.0 to 6.7% (Fig. 4B). HC also increased nitrogen content (Table 2) although the increase at control temperature was not statistically significant. Temperature did not affect nitrogen content either (Table 2). In terms of the ratio of C/N (Fig. 4C), it maintained relatively stable (5.8–6.3) under different conditions; neither CO_2 nor temperature had a significant effect on it (Table 2).

Based on the increment of carbon and nitrogen in cells during the culture period, the removal rates of carbon and nitrogen from seawater by *S. costatum* were assessed (Fig. 5). CO_2 affected carbon removal rate while temperature had no effect (Table 2). High CO_2 increased carbon removal rate by 28.9% and 31.3% under control and LT, respectively (Fig. 5A). The similar pattern also occurred in nitrogen removal rate (Table 3 & Fig. 5B). High CO_2 increased nitrogen removal rate by 22.7% and 35.4% under control and LT, respectively although the increase under control was not statistically significant based on post hoc LSD analysis.

3.4. Photosynthetic performance

To explore potential reasons for the effects of CO_2 and temperature on lipid synthesis and carbon and nitrogen removal rates, the photosynthetic performance of *S. costatum* were investigated. Maximum photochemical efficiency (F_v/F_m) and non-photochemical quenching (NPQ) of photosystem II were first presented (Fig. 6). CO_2 affected F_v/F_m of cells while temperature did not have a significant effect (Table 3). High CO_2 increased it by 12.4% and 22.5% under control and LT, respectively (Fig. 6A). In contrast, high CO_2 negatively affected NPQ (Table 3) and led to 78.4% and 75.2% of decrease under control and LT, respectively (Fig. 6B). Similar to F_v/F_m, temperature did not affect NPQ either (Table 3).

In addition to F_v/F_m and NPQ, the changes of Chl *a* and carotenoid, two key photosynthetic pigments of *S. costatum*, were also detected (Fig. 7). Temperature had a main effect on content of Chl *a* and it also had an interactive effect with CO_2 (Table 4). Low temperature decreased Chl *a* content by 31.7% under LC but did not affect it at HC (Fig. 7A). HC decreased Chl *a* content by 20.8% under control temperature. Temperature also affected carotenoid (Table 4). Low temperature reduced carotenoid content by 23.9% under LC did not affect it under HC (Fig. 7B). CO_2 did not affect the content of carotenoid (Table 4).

4. Discussion

4.1. Response of algal growth to high CO_2

The current CO_2 levels in seawater are lower than half saturation constant of ribulose-1,5- bisphosphate carboxylase/oxygenase (Rubisco)

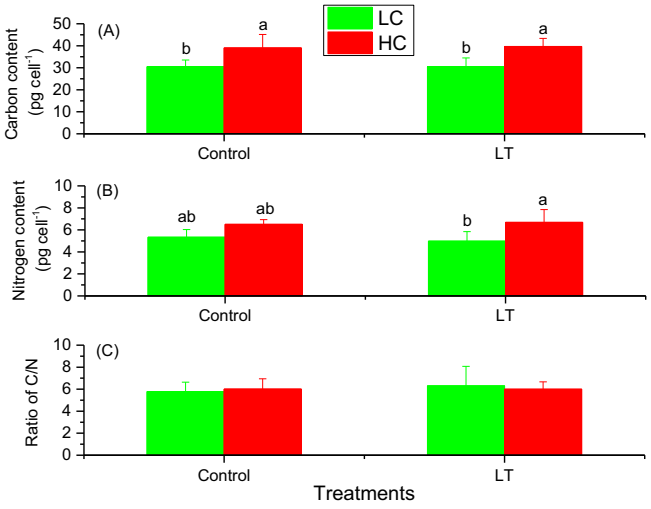

Fig. 4. The content of carbon (A), nitrogen (B) and their ratio (C) of *S. costatum* cultured at different CO_2 levels suffering from low temperature shock. LC and HC represent ambient air and 5% CO_2, respectively. Control and LT represents 20 °C and 10 °C, respectively. The error bars represent the standard deviations (n = 3). Different letters above error bars represent significant differences among treatments ($P < 0.05$).

Table 1
Two-way analysis of variance for the effects of CO_2 and temperature on lipid content, lipid productivity and carbon content of *S. costatum*. CO_2*Temp means the interactive effect of CO_2 and temperature, df means degree of freedom, F means the value of F statistic, and Sig. means *p*-value.

Source	Lipid content			Lipid productivity			Carbon content		
	df	F	Sig.	df	F	Sig.	df	F	Sig.
CO_2	1	19.003	0.002	1	38.235	<0.001	1	12.371	0.008
Temp	1	9.142	0.016	1	17.911	0.003	1	0.013	0.911
CO_2*Temp	1	0.165	0.695	1	0.854	0.383	1	0.019	0.893
Error	8			8			8		

Table 2
Two-way analysis of variance for the effects of CO_2 and temperature on nitrogen content, ratio of C/N, and carbon removal rate of *S. costatum*. CO_2*Temp means the interactive effect of CO_2 and temperature, df means degree of freedom, F means the value of F statistic, and Sig. means *p*-value.

Source	Nitrogen content			Ratio of C/N			Carbon removal rate		
	df	F	Sig.	df	F	Sig.	df	F	Sig.
CO_2	1	9.006	0.017	1	0.004	0.950	1	12.664	0.007
Temp	1	0.030	0.866	1	0.157	0.702	1	0.013	0.911
CO_2*Temp	1	0.315	0.590	1	0.174	0.688	1	0.019	0.893
Error	8			8			8		

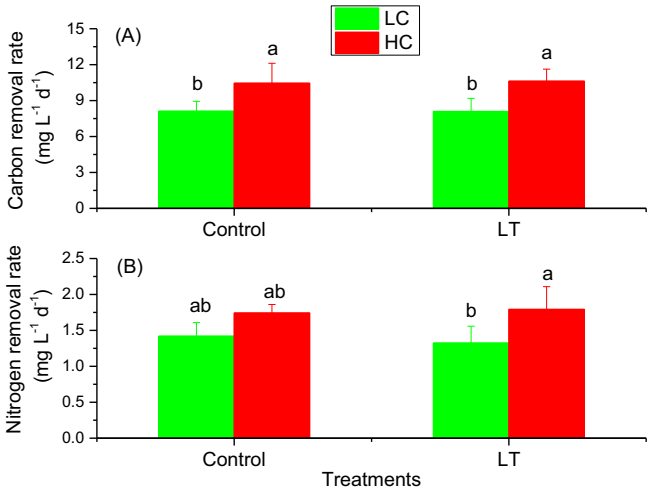

Fig. 5. Removal rate of carbon (A) and nitrogen (B) of *S. costatum* cultured at different CO_2 levels suffering from low temperature shock. LC and HC represent ambient air and 5% CO_2, respectively. Control and LT represents 20 °C and 10 °C, respectively. The error bars represent the standard deviations (n = 3). Different letters above error bars represent significant differences among treatments ($P < 0.05$).

for most marine microalgae [31,32], indicating that CO_2 is limited for algal photosynthesis. Therefore, CO_2 enrichment could usually enhance algal growth [33]. For instance, compared to ambient air, 790 ppm CO_2 enhanced growth rates of *Thalassiosira pseudonana* and *Emiliania huxleyi* by 20%–40% [34]. However, the high CO_2 level (5%) reduced specific growth rate of *S. costatum* during the first four days in this study. In fact, high CO_2 has another effect that is to decrease pH, apart from enriching CO_2 levels in waters. The decreased pH can disturb the acid-base balance both at the cell surface and within cells and could impose negative effects on algal growth [35,36]. For instance, a decrease of 0.36 units in pH reduced specific growth rate of the green macroalga *Ulva linza* cultured under P-limited conditions [36]; 0.3 units of decrease in pH also reduced specific growth rate of the cyanobacterium *Trichodesmium* [37]. In the present study, high CO_2 supply led to a decrease of about 2 units in pH and thus showed significant repressive effects on growth of *S. costatum*. Furthermore, *S. costatum* has been proven to have efficient CCMs to address CO_2 limitation and hence may benefit little from enriched CO_2 [19]. Therefore, the negative effects of decreased pH would easily surpass the positive effects of increased CO_2 on growth of *S. costatum*. The cells grew fast during the 24 h pause of high CO_2 supply on day 5, leading to the sudden peak of specific growth rate; *S. costatum* had a higher specific growth rate at HC compared to LC even after high CO_2 was resupplied, indicating robust acclimating capacity of this species to high CO_2. *S. costatum* is commonly a dominant species in coastal waters and experiences fluctuating environments, including light, pH and salinity, etc. Therefore this species has a strong capacity to acclimate to changing conditions. In fact, high CO_2 can strongly inhibit growth of some algae species, particularly for marine algae [38,39]. Engström [38] found that marine microalgae were very sensitive to low pH and all tested marine species showed decreased biomass levels when pH was below 7. Our study suggests that intermittent aeration of high CO_2 could be a solution to culture marine algae with high CO_2.

4.2. Effect of high CO_2 and low temperature on lipid synthesis

In the present study, high CO_2 enhanced the lipid content in *S. costatum*. The increased accumulation of lipid was also reported in

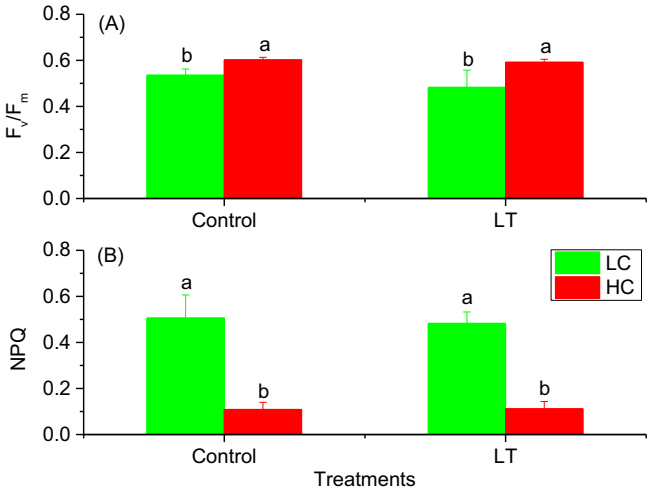

Fig. 6. F_v/F_m (A) and NPQ (B) of *S. costatum* cultured at different CO_2 levels suffering from low temperature shock. LC and HC represent ambient air and 5% CO_2, respectively. Control and LT represents 20 °C and 10 °C, respectively. The error bars represent the standard deviations (n = 3). Different letters above error bars represent significant differences among treatments ($P < 0.05$).

Table 3
Two-way analysis of variance for the effects of CO_2 and temperature on nitrogen removal rate, F_v/F_m, and NPQ of *S. costatum*. CO_2*Temp means the interactive effect of CO_2 and temperature, df means degree of freedom, F means the value of F statistic, and Sig. means *p*-value.

Source	Nitrogen removal rate			F_v/F_m			NPQ		
	df	F	Sig.	df	F	Sig.	df	F	Sig.
CO_2	1	9.231	0.016	1	26.755	0.001	1	91.273	<0.001
Temp	1	0.030	0.867	1	3.491	0.099	1	0.447	0.523
CO_2*Temp	1	0.314	0.590	1	1.535	0.250	1	0.543	0.482
Error	8			8			8		

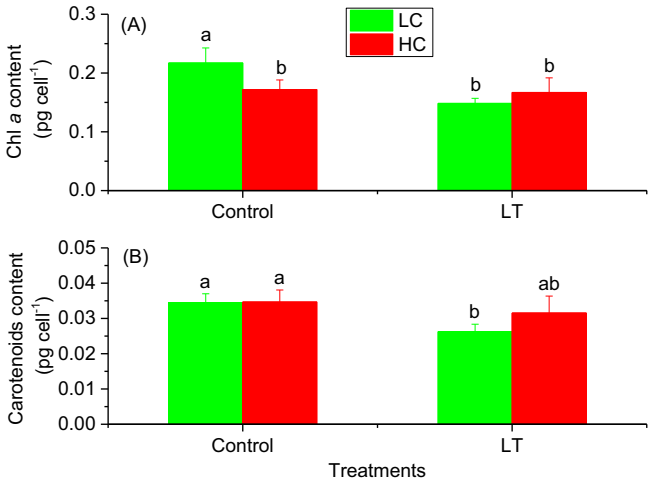

Fig. 7. Content of Chl *a* (A) and carotenoid (B) of *S. costatum* cultured at different CO_2 levels suffering from low temperature shock. LC and HC represent ambient air and 5% CO_2, respectively. Control and LT represents 20 °C and 10 °C, respectively. The error bars represent the standard deviations (n = 3). Different letters above error bars represent significant differences among treatments ($P < 0.05$).

Table 4
Two-way analysis of variance for the effects of CO_2 and temperature on Chl *a* content and carotenoid content of *S. costatum*. CO_2*Temp means the interactive effect of CO_2 and temperature, df means degree of freedom, F means the value of F statistic, and Sig. means *p*-value.

Source	Chl *a* content			Carotenoid content		
	df	F	Sig.	df	F	Sig.
CO_2	1	1.328	0.282	1	1.991	0.196
Temp	1	10.160	0.013	1	8.518	0.019
CO_2*Temp	1	7.627	0.025	1	1.695	0.229
Error	8			8		

other algae species. For instance, Gordillo et al. [40] found that compared to 0.035% CO_2, high CO_2 (1%) increased total lipids in *Dunaliella viridis* from 1.83 to 2.06 pg cell^{-1} when cultured under N-limited condition. Gao et al. [41] reported that higher CO_2 (0.2%) increased lipid content in *Ulva rigida* compared to ambient air. Lipid biosynthesis depends on photosynthesis to a large extent because both ATP generated in light reaction and carbon skeleton synthesized in dark reaction are necessary for lipid synthesis [23,42]. Therefore, increased photosynthetic rate could stimulate lipid synthesis in theory. In this study, high CO_2 increased maximum photochemistry efficiency (F_v/F_m) of *S. costatum*. Increased F_v/F_m represents increased photosynthetic activity of PSII and accelerated production of ATP. Furthermore, high CO_2 also enhanced carbon content of *S. costatum*, indicating increased carbon fixation and higher availability of carbon skeleton.

To enhance lipid content in algae, environmental stressors are usually used to induce lipid synthesis. Among them, low temperature shock is deemed to have a significant effect on lipid content, particularly on the syntheses of unsaturated fatty acids [43]. For instance, both EPA and PUFA in *Phaeodactylum tricornutum* were raised by 120% after being shifted from 25 to 10 °C for 12 h [44]. The same temperature shift for 24 h also resulted in the increased proportion of oleate in *Selenastrum capricornutum* [45]. This technique was also successful in inducing lipid synthesis in *S. costatum* in this study. The increased lipid synthesis at lower temperatures could enhance membrane fluidity and thus is deemed as a defensive mechanism of algae to deal with decreased temperatures [43]. A further increase in lipid content and productivity was achieved when combing high CO_2 and low temperature treatments in the present study, suggesting the additive effect of these two treatments. Algal lipid is ideal material for biodiesel and health-care product for human as well [46–48]. The increased lipid productivity indicates increased yield and profit when culturing *S. costatum* commercially with high CO_2 levels since the supply of high CO_2 can come from flue gas.

4.3. Increased carbon and nitrogen assimilation

High CO_2 increased F_v/F_m of *S. costatum* in this study. F_v/F_m, defined as the maximum photochemical efficiency, represents the efficiency of PSII in converting light into chemical energy. High CO_2 could accelerate the carboxylation process of Rubisco and hence the carbon fixation in dark reaction. The accelerated dark reaction could drain ATP and NADPH generated by light reaction, enhancing the operation of electron transfer in PSII and thus enhancing F_v/F_m [19]. In addition, high CO_2 could stimulate PsbA removal, contributing to vigorous PSII [11]. After acclimation to high CO_2, cells cultured at the high CO_2 level reduced their NPQ compared to those at ambient air in the end of the culture period. NPQ represents the energy used for non-photochemical process and usually increased when cells suffer environmental stress [49]. The decreased NPQ may indicate the conversion from negative to positive effects of high CO_2 on photosynthesis of *S. costatum*. Therefore, high CO_2 increased carbon assimilation of *S. costatum* in the present study. Burkhardt and Riebesell [50] also reported that carbon content of *S. costatum* collected from North Sea increased with aquatic CO_2 level. In contrast to high CO_2, low temperature showed little effects on PSII activity, indicating the high regulation capacity of *S. costatum* to low temperature. The increased carbon content resulted in increased carbon removal rate of *S. costatum* cultured under the high CO_2 condition, which could contribute to subsequent carbon sequestration, e.g., using BECCS technology. To achieve the Paris 1.5 or 2 °C target, many countries pledge to be carbon neutral by 2050 or 2060. BECCS shows the potential in carbon sequestration and accomplishing carbon neutrality [51]. This present study implies that culturing microalgae with flue gas may enhance the efficiency of BECCS, which is critical for increasing the feasibility of BECCS in mitigating climate change.

High CO_2 decreased Chl *a* of *S. costatum* at 20 °C but did not affect it at 10 °C. High CO_2 commonly result in the decreased Chl *a*, termed "pigment economy" [36], because there is no need to synthesize more Chl *a* to capture photons under high CO_2 conditions given the enhanced photochemical efficiency of PSII. However, this phenomenon of "pigment economy" did not occur at low temperature in the present study, probably due to higher requirement for Chl *a* to maintain photosynthetic activity at lower temperature.

In addition to carbon assimilation, high CO_2 also enhanced nitrogen assimilation in *S. costatum* in the present study. Higher CO_2 levels have been found to increase nitrate reductase activity in macroalgae, such as *Ulva rigida*, *Hizikia fusiforme*, and *Sargassum muticum* [52–54], which could contribute to increased nitrogen assimilation. In addition, two of the three reductive steps in NO_3^- assimilation occur in the chloroplast and require the participation of ferredoxin [55]. Enhancement of non-cyclic electron transport can increase the turnover rate of ferredoxin, supporting NO_3^- assimilation. In the present study, high CO_2 increased F_v/F_m and thus non-cyclic electron transport, which may contribute to increased NO_3^- assimilation. The increased NO_3^- assimilation indicates the increased bioremediation capacity of *S. costatum* when cultured with high CO_2 levels.

5. Conclusion

Our study investigated the combined effects of high CO_2 and low temperature shock on lipid productivity and carbon and nitrogen removal rates of the marine diatom *S. costatum* for the first time. The high CO_2 level enhanced lipid synthesis and thus lipid productivity of cells. The content of carbon and nitrogen and removal rates of CO_2 and nitrogen were also stimulated by high CO_2. The combination with low

temperature shock increased lipid productivity and removal rates of carbon and nitrogen further. Microalgae are considered to have remarkable advantages in terms of their use in biofuel, health-care product and bioremediation. The treatment of high CO_2 and low temperature shock can enhance the features of *S. costatum* in these fields. Future work can be done in the aspect of selecting more marine algae with high tolerance to low pH because mariculture can tremendously reduce the use of freshwater and be conducted conveniently in coastal areas.

Abbreviations

BECCS bioenergy with carbon capture and storageCCMsCO_2 concentrating mechanismsF_v/F_mmaximum photochemical efficiencyHChigh CO_2 condition (5%)LClow CO_2 condition (0.04%)LTlow temperatureNETsnegative emissions technologiesNPQnonphotochemical quenching

CRediT author contribution statement

Shuyu Xie: Investigation, Methodology, Writing- Original draft preparation, Writing- Reviewing and Editing. Fan Lin: Investigation, Methodology, Writing- Reviewing and Editing. Xin Zhao: Methodology, Writing- Reviewing and Editing. Guang Gao: Conceptualization, Methodology, Formal analysis, Visualization, Writing- Original draft preparation, Writing- Reviewing and Editing, Supervision, Funding acquisition.

Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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