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Effects of doubled atmospheric CO_2 concentration on the photosynthesis and growth of *Chlorella pyrenoidosa* cultured at varied levels of light

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ABSTRACT: *Chlorella pyrenoidosa* was cultured with 350 and 700 p.p.m.v. CO_2 at varied levels of light to see the impacts of doubled atmospheric CO_2 concentration on its growth and photosynthesis. The CO_2 enrichment did not affect the growth rate (μ), but significantly increased the cell density when light was sufficiently supplied. The CO_2 enrichment significantly depressed light-saturated photosynthesis and dark respiration in the cells grown under a high-light regime, but not those under a low-light regime. The light-saturating point for photosynthesis and photosynthetic efficiency was not affected by the CO_2 enrichment under either the high-light or low-light conditions.

KEY WORDS: ***Chlorella pyrenoidosa*, CO_2, light, photosynthesis.**

INTRODUCTION

Atmospheric CO_2 concentration is expected to be doubled during 21st century because of the industrial combustion of fossil fuels.[1] Such an increase in atmospheric CO_2 is supposed to result in a change of pH and dissolved inorganic carbon (DIC) in aquatic environments. An increase in atmospheric CO_2 from 35 to 50 Pa would increase sea-surface DIC by 3.1%, decrease pH by 0.14 units at 15°C with an alkalinity of 2.47 mol/m^3.[2] Riebesell *et al.* reported that the supply of CO_2 in seawater limited the growth of marine phytoplankton under optimal light and nutrient conditions[3] and Hein and Sand-Jensen demonstrated that doubled atmospheric CO_2 concentration stimulated marine productivity.[4] In contrast to oceanic environments, freshwater is more sensitive to atmospheric CO_2 increase because of its lower buffering capacity. The pH and DIC would be affected to a larger extent than in seawater. Therefore, ecological and physiological impacts of increased atmospheric CO_2 on freshwater algae are of general concern.

Chlorella, a common microalga in freshwater, which is used for aquaculture, has been extensively studied for its mechanism of acquisition and accumulation of CO_2 or bicarbonate. *Chlorella vulgaris* 11h was shown to take up CO_2 only, whereas *Chlorella ellipsoidea* and *Chlorella pyrenoidosa* took up HCO_3^- in addition to CO_2.[5] When the cells were cultured at 1.5–5% CO_2, photosynthetic characteristics of some green algae were significantly affected, with CO_2 affinity being lowered, the CO_2 compensation point increased and carbonic anhydrase (CA) activity decreased.[6,7] Low CO_2-grown cells of *Chlamydomonas* possessed a CO_2 concentration mechanism,[8] which involved active bicarbonate transport and increased energy (ATP) input. The energetic requirement was estimated to be 1 mol of ATP per mol inorganic carbon transported.[9] It was reported that the DIC pump formation and operation required light energy in air-grown *Scenedesmus obliquus*.[10] Although these findings are fundamental in understanding the photosynthetic physiology of these freshwater algae, they can hardly lead to any ecological implications in terms of the impacts of the atmospheric CO_2 rise, because CO_2 concentrations used in the previous studies are hundreds of times higher than the present atmospheric CO_2 concentration.

The present study aimed to investigate the effects of doubled CO_2 concentration of the atmosphere on the growth and photosynthesis of *Chlorella pyrenoidosa* when cultured at varied levels of light.

MATERIALS AND METHODS

Chlorella pyrenoidosa was obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, the Chinese Academy of Sciences,

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China. The cells were cultured in fresh Bristol's solution[11] at 50 or 200 μmol/m^2 per s (12 : 12 LD) to examine the effect of CO_2 enrichment under different light levels. All the above cultures were maintained in a CO_2 chamber (Conviron 2175; Controlled Environments Limited, Winnipeg, Manitoba, Canada) at 25°C, aerated with air of 350 (ambient) or 700 p.p.m.v. CO_2 (enriched) at a flow rate of 0.4 L/min.

Cells were harvested in the exponential growth phase, resuspended at a density of 2–5 × 10^7/mL in fresh Bristol's solution containing 2 mM $NaHCO_3$ (CO_2 concentration 44 μM) and buffered at pH 8.0 with HEPES. Photosynthetic O_2 evolution was measured by using an oxygen monitor (YSI 5300; Yellow Springs Instrument Co, Yellows Springs, OH, USA) with a Clark-type oxygen electrode. Water temperature within the assimilation chamber was controlled at 25°C by circulating water in a water jacket around the chamber with a cooling circulator (Cole Parmer Instruments Co, Chicago, IL, USA). A halogen lamp was used as a light source. Different light intensities were achieved by adjusting distances between the light source and the chamber. Parameters for *P-E* curves were analyzed according to Jassby and Platt[12] and Henley.[13]

$$P = P_{\max} \cdot \tanh(\alpha \cdot E/P_{\max}) + R_d,\ E_k = (P_{\max} + R_d)/\alpha$$

where E is irradiance; P, net photosynthetic rate at a given irradiance; $P_{\max}$, light-saturated photosynthetic rate; α, the initial slope at limiting irradiances (photosynthetic efficiency); and E_k, saturating irradiance for photosynthesis. Photosynthetically active radiation (PAR, 400–700 μm) was measured with a quantum sensor (SKP200; ELE International, Leighton Buzzard, Bedfordshire, UK).

Chlorophylls were extracted in 90% acetone and determined according to Jeffrey and Humphrey.[14] Cells were counted in 6–10 parallels of small subsamples with a hemocytometer under a microscope. Growth rate (μ) was expressed as doublings/day, determined as follows:

$$\mu = (\log_2 N_t - \log_2 N_0)/t$$

where N is the cell density (cells/mL) at the beginning (N_0) or t number of days (N_t).

Data were analyzed according to analysis of variance followed by the Duncan test. The confidence level was set at 5%.

RESULTS

The growth rate (μ) in the exponential growth phase (from the 1st day to the 5th day) of *C. pyrenoidosa* was insignificantly ($p > 0.05$) affected by CO_2 enrichment either under the low or high light ($p > 0.05$) (Fig. 1; Table 1), although the increase of PAR from 50 to 200 μmol/m^2 per s significantly ($p < 0.05$) enhanced μ-value by approximately 50%. When CO_2 concentration was elevated from 350 to 700 p.p.m.v., cell densities at the end of the culture were significantly raised under the high-light ($p < 0.05$), but showed insignificant change ($p > 0.05$) under the low-light regime.

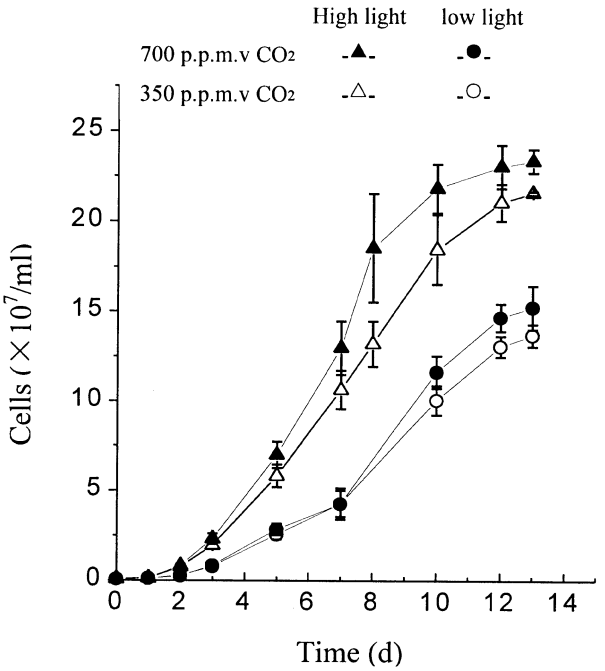

Fig. 1 Growth of *Chlorella pyrenoidosa* under high (200 μmol/m^2 per s) and low (50 μmol/m^2 per s) light in cultures aerated with air of 350 and 700 p.p.m.v. CO_2 at 25°C. Data are the mean ± SD ($n = 3$).

When *C. pyrenoidosa* was grown under varied light levels, the effects of CO_2 enrichment on its photosynthetic characteristics were dependent on light availabilities (Fig. 2; Table 2). Doubled atmospheric CO_2 concentration to 700 p.p.m.v. had a significant effect on light-saturated net photosynthesis ($P_{\max}$) and dark respiration (R_d) in the high-light cultures ($p < 0.05$; Fig. 2), but showed insignificant effects in the low-light cultures ($p > 0.05$). The enriched CO_2 reduced $P_{\max}$ and R_d by 11% and 30% in high-light cultures, respectively. The initial slope of the *P-E* curves; namely the photosynthetic efficiency (α), was not affected by the CO_2 enrichment under either low light or high light ($p > 0.05$) (Table 2). However, α was raised by approximately 62% under the low-light compared with the high-light cultures. The impact of the CO_2 enrichment on photosynthesis saturating radiation (E_k) was insignificant under either the low light or the high light ($p > 0.05$); however, the high light

Table 1 Growth rate (μ) and, biomass yield (cell density) at the end of culture of *Chlorella pyrenoidosa* grown at 350 and 700 p.p.m.v. CO_2 under varied levels of light (50 and 200 μmol/m^2 per s)

	50 μmol/m^2 per s		200 μmol/m^2 per s	
	350	700 p.p.m.v. CO_2	350	700 p.p.m.v. CO_2
μ (/day)	1.2 ± 0.2^a	1.2 ± 0.3^a	1.8 ± 0.4^b	2.0 ± 0.4^b
Cell density ($\times 10^7$/mL)	13.7 ± 0.6^a	15.2 ± 1.2^a	21.6 ± 0.1^b	23.4 ± 0.7^c

Those with different superscript are significantly different ($P<0.05$).
Data are the means ± SD ($n=3$), based on Fig. 1.
p.p.m.v., parts per million volume.

Table 2 Dark respiration (R_d), light-saturating photosynthetic rates (P_{max}), photosynthetic efficiency (α) and light saturating point (E_k) of *Chlorella pyrenoidosa* grown at 350 and 700 p.p.m.v. CO_2 under varied levels of light

	50 μmol/m^2 per s		200 μmol/m^2 per s	
	350	700 p.p.m.v. CO_2	350	700 p.p.m.v. CO_2
R_d (μmolO_2/mg chl per h)	51.7 ± 1.1^a	47.0 ± 3.7^a	22.8 ± 2.6^b	16.0 ± 3.1^c
P_{max} (μmolO_2/mg chl per h)	148.2 ± 10.8^a	136.5 ± 4.6^a	276.1 ± 9.2^b	246.7 ± 10.9^c
α (μmolO_2/mg chl per h)/(μmol/m^2 per s)	3.7 ± 0.1^a	3.5 ± 0.1^a	2.3 ± 0.1^b	2.2 ± 0.0^b
E_k (μmol/m^2 per s)	53.9 ± 2.2^a	54.3 ± 1.4^a	126.5 ± 5.7^b	118.8 ± 1.0^b

Those with different superscript are significantly different ($P<0.05$).
Data are the means ± SD ($n=3$), based on Fig. 2.
chl, chlorophyll; p.p.m.v., parts per million volume.

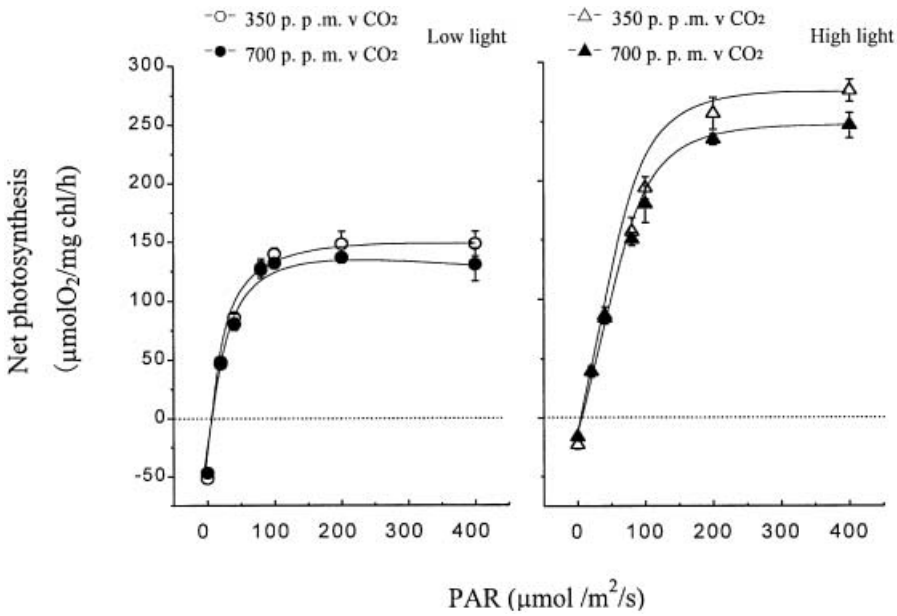

Fig. 2 Net photosynthetic O_2 evolution rates as a function of PAR in *Chlorella pyrenoidosa* grown at varied CO_2 (350, 700 p.p.m.v.) and light levels (50, 200 μmol/m^2 per s) at 25°C. Data are the mean ± SD ($n=3$).

significantly ($p<0.05$) increased the E_k value in comparison with the low light (Fig. 2; Table 2).

DISCUSSION

Inorganic carbon in water exists in the form of CO_2 (aq), HCO_3^-, CO_3^{2-}, which can reach an equilibrium as follows:

$$CO_2\ (aq) + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{2-}$$

Carbon dioxide in freshwater usually accounts for 0.6–38% of the total inorganic carbon with a pH range of 8.5–6.5.[15] When CO_2 concentration in air increases, the equilibrium will be broken, and the reactions proceed towards the right-hand side to reach a new equilibrium. Aeration with addition of CO_2 to culture raises DIC and aqueous CO_2 partial

pressure.[2] In the present study, when supply of light was sufficiently high, CO_2 enrichment to the doubled level of the present atmosphere significantly increased cell density of *C. pyrenoidosa*, but μ was not affected during the exponential growth phase. It appeared that the effects of the elevated CO_2 on the growth of this alga were dependent on light when cell density became high enough for CO_2 or DIC to be a limiting factor. Cell density was not enhanced with the CO_2 enrichment when *C. pyrenoidosa* was cultured under the conditions of low light, which could be associated with limitation of CO_2 fixation because of lowered contents of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) under low light conditions.[16] It is supposed that doubled atmospheric CO_2 concentration would affect the growth of *C. pyrenoidosa* when it grows under bright solar radiation, and such an effect would increase by a great extent when the cell density becomes high.

In the present study, CO_2 enrichment insignificantly affected the P_{max} in the low-light, but significantly decreased it in the high-light cultures. Depressed photosynthesis has also been reported in macroalgae cultured with high CO_2 and sufficient light supply.[17] *Chlorella pyrenoidosa* is capable of using bicarbonate, and possesses carbonic anhydrase (CA)[18,19] which catalyzes the interconversion of HCO_3^- and CO_2. Suppression of the synthesis of CA by high CO_2, associated with a reduced capacity in using HCO_3^-, has been documented in microalgae.[20,21] Rubisco in *Chlorella* was reported not to be affected by 5% CO_2 concentration.[19] In the present study, the activity of CA in *C. pyrenoidosa* might have been reduced, resulting in decreased P_{max} in CO_2-enriched cultures under high light. It was reported that high CO_2 concentration insignificantly affected CA activity of *Peridinium gatunense* when cultured in low light.[22] That CO_2 increase to 700 p.p.m.v. did not affect P_{max} under the low light might be attributed to the stable CA activity in *C. pyrenoidosa* of the present study. However, to what extent doubled atmospheric CO_2 concentration, a minor enrichment compared to the CO_2 concentrations used for the discovery of CA suppression, could affect the CA synthesis or its activity in *C. pyrenoidosa* under high light is still a question. E_k was not affected by the doubled CO_2 concentration, implying that such an extent of CO_2 enrichment could not influence the light requirement for photosynthetic saturation.

Photosynthetic efficiency (α) is a function of photosynthetic energy conversion efficiency.[13] Investigation with *Synechococcus* sp. indicated that photosynthetic efficiency for net O_2 evolution in low CO_2-grown cells was approximately half that of high CO_2-grown (2–4%) cells, implying that a considerable amount of light energy was used to concentrate CO_2 within the low CO_2-grown cells.[23] However, doubled atmospheric CO_2 concentration did not affect the photosynthetic efficiency of *C. pyrenoidosa* either grown under the low- or high-light regimes in the present study, indicating that 700 p.p.m.v. CO_2 in this study, 75 times lower compared with 5% CO_2, was low enough not to affect the photosynthetic efficiency.

Carbon dioxide removal by photosynthesis in density-lower cultures may be slower than CO_2 dissolution from air; therefore, the photosynthesis could not be CO_2 limited when the alga was cultured with a continuous supply of CO_2. The suppressed photosynthesis of the cells grown with the CO_2 enrichment when measured in the sealed chamber must be due to the limited CO_2 supply within the closed system. Subsequently, *C. pyrenoidosa* can perform net photosynthesis at a faster rate in the open-system culture than in the closed-system (measurement) when light is sufficiently supplied. This accounts for the discrepancy between the observed growth and measured photosynthesis.

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