Research note

Effects of doubled atmospheric CO₂ concentration on the growth and photosynthesis of *Chlamydomonas reinhardtii* (Volvocales, Chlorophyceae)

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SUMMARY

The freshwater microalga, *Chlamydomonas reinhardtii* Dangeard, was cultured under 350 and 700 ppmv CO₂ to determine the impact of doubled atmospheric CO₂ concentration on its growth and photosynthesis. No significant difference was observed in the specific growth rate, photosynthetic efficiency, maximal net photosynthetic rate and light-saturating point between the low and high CO₂ cultures. Both the low- and high-CO₂-grown cells showed reduced light-dependent O₂ evolution rate and photochemical efficiency (F_v/F_m) owing to photoinhibition when exposed to high photon flux density. However, high-CO₂-grown cells were less photoinhibited, and showed better recovery in dim light or darkness during the initial period of the recovery process.

Key words: *Chlamydomonas reinhardtii*, CO₂, growth, photoinhibition, photosynthesis.

Human activities and industrial combustion of fossil fuels have increased the global CO₂ concentration in the atmosphere. It has been anticipated that the atmospheric CO₂ concentration will be doubled to 700 ppmv during this century (King et al. 1992), which may trigger global warming. The doubled atmospheric CO₂ concentration would raise the CO₂ in surface seawater by 100%, HCO₃⁻ by 6%, and reduce pH by 0.279 (Stumm and Morgan 1996). It is important to assess the ecological impact of increasing atmospheric CO₂ on photosynthesis and growth of aquatic plants (Bowes 1993). Riebesell et al. (1993) showed that enriched CO₂ concentrations could promote the growth of marine phytoplankton, and Hein and Sand-Jensen (1997) demonstrated that atmospheric CO₂ increase could raise oceanic primary production by phytoplankton. Elevated CO₂ concentrations enhanced the growth of the marine red algae, *Porphyra yezoensis* Ueda (Gao et al. 1991) and *Gracilaria* spp. (Gao et al. 1993), raised the photosynthetic activity of the intertidal marine macroalgae, *Enteromorpha linza* (Linnaeus) J. Agardh, *Ishige okamurai* Yendo and *Gloiopteltis furcata* (Postels et Ruprecht) J. Agardh while exposed and desiccated in air (Gao et al. 1999). However, little has been documented on freshwater algae in relation to atmospheric CO₂ rise. The chemistry of freshwater is more sensitive to atmospheric CO₂ rise, because its buffering capacity is lower than seawater (Stumm and Morgan 1996). Consequently, the pH of freshwater would be reduced and its inorganic carbon composition would be altered by an extent greater than seawater owing to dissolution of CO₂ from air associated with the increasing atmospheric CO₂. Thus, ecological and physiological impacts of atmospheric CO₂ rise on freshwater algae are of general concern.

*Chlamydomonas reinhardtii* Dangeard, a well-known freshwater green alga, exhibited lower CO₂ affinity and high CO₂ compensation point (Moroney et al. 1985), and lost the activity of carbonic-anhydrase (Kimpel et al. 1983; Patel and Merrett 1986) when grown in high CO₂ (5%). These findings are fundamental to the understanding of the photosynthetic physiology of *C. reinhardtii*, but hardly lead to predictions of the ecological impact of atmospheric CO₂ increase on the organism, because the CO₂ concentrations in those studies were a hundredfold of the present atmospheric CO₂ level. The present study aimed to investigate the effects of doubled atmospheric CO₂ concentration (700 ppmv) on the growth and photosynthesis of *C. reinhardtii*.

*Chlamydomonas reinhardtii* (FACHB 479) was obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, the Chinese Academy of Sciences, Wuhan, China. The strain was isolated in the 1970s (Song et al. 1999) and has been maintained.

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since then in the Freshwater Algae Culture Collection. The alga was grown at 25°C in 500 mL flasks with 300 mL Bristol’s medium (Fujita 1972) aerated at a rate of 0.3–0.4 L min⁻¹ with ambient air (350 ppmv CO₂) or CO₂-enriched air (700 ppmv CO₂) in a plant CO₂ chamber (Conviron 125 L; Controlled Environments Limited, Winnipeg, Canada). Illumination of 60 µmol photons m⁻² s⁻¹ (12:12 LD) was provided with white fluorescent lamps. The specific growth rates at log phase were calculated by the following formula:

\[ \mu = \frac{\ln X_2 - \ln X_1}{t_2 - t_1} \]

where \( X_2 \) and \( X_1 \) are the number of cells at \( t_2 \) and \( t_1 \) days, respectively.

Photosynthetic oxygen evolution was measured with a Clark-type O₂ electrode (YSI 5300; Yellow Springs Instrument Co., Inc., Yellow Springs, USA). Cultures at the log phase of growth were harvested by centrifugation, and re-suspended in fresh medium to which NaHCO₃ was added to 1.0 mmol L⁻¹, a concentration resulting in non-limited photosynthesis (Spalding et al. 1983; Moroney and Tolbert 1985; Krupa et al. 1990). The cells in 5 mL fresh medium were transferred to the electrode chamber which was equipped with a water jacket for temperature control (25°C). Various light intensities were obtained by adjusting the distance from the electrode chamber. The O₂ concentration in the chamber was reduced to 20% by sparging N₂ for about 20 s before the measurements. The rates of photosynthesis were expressed as µmol O₂ mg⁻¹ Chl h⁻¹. Chlorophyl contents were determined according to Jeffrey and Humphrey (1975).

For the determination of photoinhibition, cells harvested at log phase and re-suspended in fresh medium were exposed to high photon flux density (PFD) of 1000 µmol photons m⁻² s⁻¹ at 25°C. A sample was drawn out at various time intervals and tested for photosynthetic activity at 300 µmol photons m⁻² s⁻¹ at 25°C. The photosynthetic activity before the high light exposure was used as control to estimate the degree of photoinhibition. Recovery treatments were followed by placing the high-PFD-exposed samples under dim light (50 µmol m⁻² s⁻¹) or in complete darkness at 25°C, stirred for periods of up to 80 min. At various time intervals, photochemical efficiency (\( F_v / F_m \)) was measured using a Plant Efficiency Analyzer (PEA MK2; Hansatech Instruments Ltd, King’s Lynn, UK). Cells were dark-adjusted for 10 min before the measurements. The maximal (\( F_m \)), variable (\( F_v \)) and non-variable fluorescence yield (\( F_o \)) were determined to estimate the photochemical properties of the alga.

Figure 1 shows the growth of \( C. \) reinhardtii at 350 and 700 ppmv CO₂. The specific growth rate (µ) of the alga was about 1.8 day⁻¹; no significant difference was found between the cultures aerated with 350 and 700 ppmv CO₂ (t-test, \( P > 0.1 \)). The final cell density at stationary phase was 1.487 \times 10⁷ mL⁻¹ at 350 ppmv CO₂ and 1.582 \times 10⁷ mL⁻¹ at 700 ppmv CO₂, respectively; the difference was not significant (t-test, \( P > 0.1 \)).

Figure 2 shows the light-dependent photosynthetic oxygen evolution of \( C. \) reinhardtii grown under 350 and 700 ppmv CO₂. Net photosynthetic rates were very similar in the two types of culture, with the maximal
rates (P_max) being 159 and 168 µmol O_2 mg^-1 Chl h^-1 for cells grown at 350 and 700 ppmv CO_2, respectively. There was no significant difference in P_max (t-test, P > 0.05). The light saturation points (I_l) were 147 and 156 µmol photons m^-2 s^-1 in low and high-CO_2-grown cells, respectively, without significant differences (t-test, P > 0.05). Similarly, no significant difference was observed in the photosynthetic efficiency (α) (t-test, P > 0.05). Therefore, doubled CO_2 concentration under the growth conditions tested did not affect these photosynthetic characteristics of _C. reinhardtii_.

The net photosynthetic activity of _C. reinhardtii_ grown under low and high CO_2 concentrations, after exposure to high PFD (1000 µmol photons m^-2 s^-1), decreased markedly with time. The low-CO_2-grown cells showed that the net photosynthesis reduced to a larger extent compared with the high-CO_2-grown cells. Significant differences between the two cultures were observed at the point of 10 min and longer exposure times (t-test, P < 0.05) (Fig. 3). Net photosynthesis was inhibited to the lowest levels after 20 min in low-CO_2-grown cells and 40 min in high-CO_2-grown cells. Half-time (the time required for the net photosynthesis to be reduced by 50% of its initial value) of photosynthetic inhibition was about 17 min in the low-CO_2 and about 30 min in the high-CO_2-grown cells. Net photosynthesis was inhibited in 40 min by about 60% in the low-CO_2 and by about 50% in the high-CO_2-grown cells. The PSII photochemical efficiency (F_v/F_m) was also reduced following the exposure to high PFD (Fig. 4). The F_v/F_m values were reduced by ca 34–38% in 60 min. After the algal cells were transferred from high light to dim light or complete darkness, photosynthetic recovery was observed immediately (Fig. 5). During the first 20 min of the recovery, the photochemical efficiency (F_v/F_m) of the low or high-CO_2-grown cells was restored to 83.5% or 85.2% in dim light, and to 76.4% or 79.0% in darkness, respectively. There were significant differences between low- and high-CO_2-grown cells within the first 20 min (t-test, P < 0.05) in dim light or darkness. The F_v/F_m was restored to ca 92% or 84% in 80 min in dim light or darkness, respectively. The recovery was faster in dim light than in darkness. Significant differences were observed between dim light and dark treatments (t-test, P < 0.05) in both low and high-CO_2-grown cells. In short, high-CO_2-grown cells showed better recovery compared with the low-CO_2-grown cells at the initial recovery phase.

No significant influences were observed on the specific growth rate and photosynthetic characteristics of _C. reinhardtii_ when grown at doubled atmospheric CO_2 concentration. However, cells grown at the elevated CO_2 level showed less photoinhibition and better photo-inhibitory recovery.
Algae use ribulose bisphosphate carboxylase/oxygenase (Rubisco) to fix CO2, but CO2 is not the only carbon source for aquatic photosynthesis. Inorganic carbon in water exists in the form of CO2 (aq), HCO3– and CO32–, which can reach an equilibrium as follows:

\[ \text{CO}_2 (\text{aq}) + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \rightleftharpoons 2\text{H}^+ + \text{CO}_3^{2-} \]

The CO2 in fresh water usually accounts for 38–0.6% of the total inorganic carbon within a pH range of 6.5–8.5. When CO2 concentration in air increases, the equilibrium is broken, and the reactions proceed toward the right-hand until it reaches a new equilibrium. Doubled atmospheric CO2 concentration resulted in doubled concentration of CO2 (aq), but only increased HCO3– by 0.6%, and reduced pH by 0.298 in fresh water (Stumm and Morgan 1996).

Chlamydomonas reinhardtii grown in 350 ppmv CO2 was more easily photoinhibited when exposed to high PFD than that grown in 700 ppmv. This can be attributed to the effects of the doubled CO2 on the photochemical property of the alga. Spalding et al. (1984) showed that CO2 concentration influenced not only the carbon metabolism, but also the photochemical properties of C. reinhardtii. It is generally agreed that the primary site of the photoinhibitory response is located on PSII (Vonshak et al. 1996), which is reflected by a reduction in oxygen evolution or CO2 uptake rates (Krause 1988), or a decrease in Fv/Fm (Falk and Samuelsson 1992). In the present study, both the light-dependent O2 evolution rate and Fv/Fm were decreased when C. reinhardtii cells were exposed to high light. Doubled atmospheric CO2 concentration must have had effects on the PSII photochemical property of C. reinhardtii in view of the photoinhibition and its recovery.

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REFERENCES


