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Research note

Effects of doubled atmospheric CO_2 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 CO_2 to determine the impact of doubled atmospheric CO_2 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 CO_2 cultures. Both the low- and high-CO_2-grown cells showed reduced light-dependent O_2 evolution rate and photochemical efficiency (F_v/F_m) owing to photoinhibition when exposed to high photon flux density. However, high-CO_2-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*, CO_2, growth, photoinhibition, photosynthesis.

Human activities and industrial combustion of fossil fuels have increased the global CO_2 concentration in the atmosphere. It has been anticipated that the atmospheric CO_2 concentration will be doubled to 700 ppmv during this century (King *et al*. 1992), which may trigger global warming. The doubled atmospheric CO_2 concentration would raise the CO_2 in surface seawater by 100%, HCO_3^- by 6%, and reduce pH by 0.279 (Stumm and Morgan 1996). It is important to assess the ecological impact of increasing atmospheric CO_2 on photosynthesis and growth of aquatic plants (Bowes 1993). Riebesell *et al*. (1993) showed that enriched CO_2 concentrations could promote the growth of marine phytoplankton, and Hein and Sand-Jensen (1997) demonstrated that atmospheric CO_2 increase could raise oceanic primary production by phytoplankton. Elevated CO_2 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 okamurae* Yendo and *Gloiopeltis 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 CO_2 rise. The chemistry of freshwater is more sensitive to atmospheric CO_2 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 CO_2 from air associated with the increasing atmospheric CO_2. Thus, ecological and physiological impacts of atmospheric CO_2 rise on freshwater algae are of general concern.

Chlamydomonas reinhardtii Dangeard, a well-known freshwater green alga, exhibited lower CO_2 affinity and high CO_2 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 CO_2 (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 CO_2 increase on the organism, because the CO_2 concentrations in those studies were a hundredfold of the present atmospheric CO_2 level. The present study aimed to investigate the effects of doubled atmospheric CO_2 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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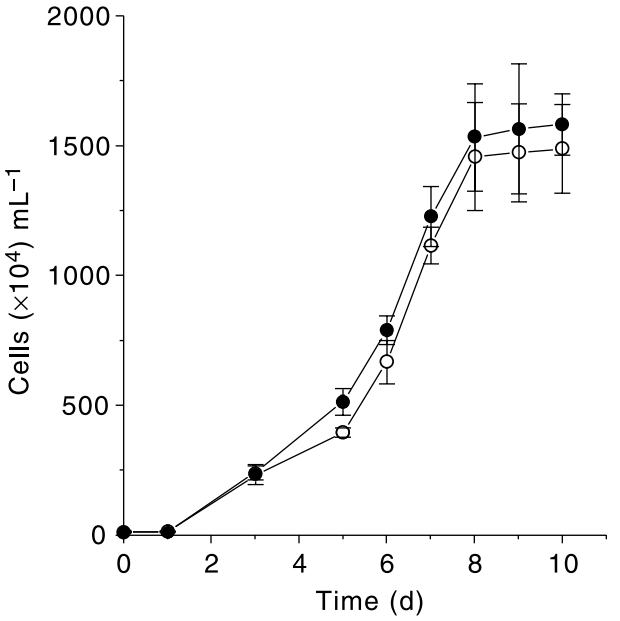

Fig. 1. Growth of *Chlamydomonas reinhardtii* at 350 (○) and 700 (●) ppmv CO_2. Data are the means of triplicate cultures ± SD.

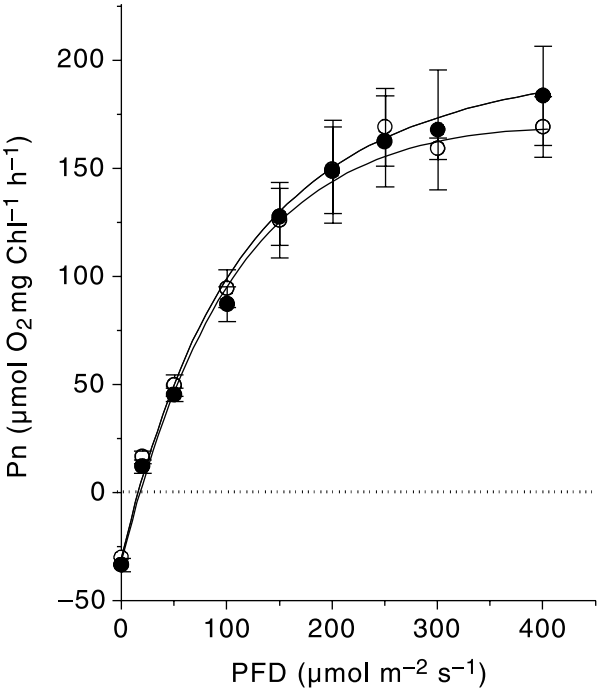

Fig. 2. Net photosynthesis (Pn) as a function of photon flux density (PFD) of *Chlamydomonas reinhardtii* grown under 350 (○) and 700 (●) ppmv CO_2. Measured at the log phase at 25°C. Data are the means for triplicate cultures ± SD.

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 CO_2) or CO_2-enriched air (700 ppmv CO_2) in a plant CO_2 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:

$$\mu = \ln X_2 - \ln X_1 / t_2 - t_1$$

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 O_2 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 $NaHCO_3$ 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 O_2 concentration in the chamber was reduced to 20% by sparging N_2 for about 20 s before the measurements. The rates of photosynthesis were expressed as μmol O_2 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 by 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 CO_2. 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 CO_2 (*t*-test, $P > 0.1$). The final cell density at stationary phase was 1.487×10^7 mL^{-1} at 350 ppmv CO_2 and 1.582×10^7 mL^{-1} at 700 ppmv CO_2, 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 CO_2. Net photosynthetic rates were very similar in the two types of culture, with the maximal

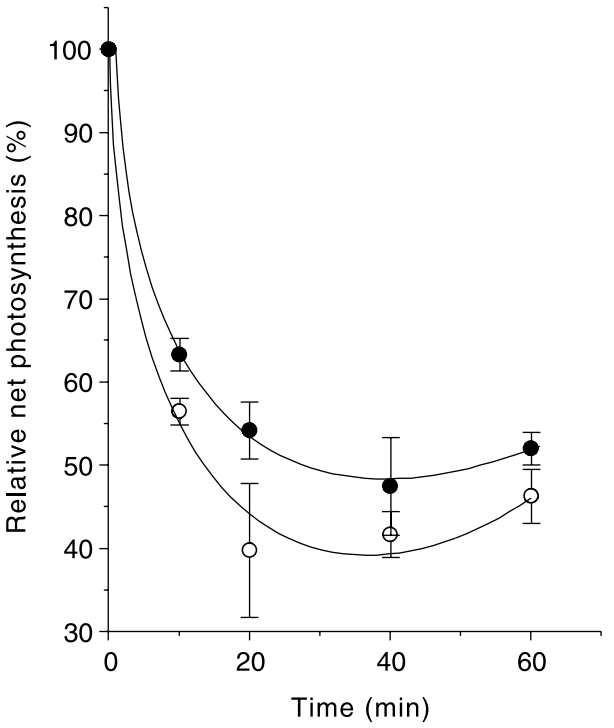

Fig. 3. Effects of exposure to high PFD (1000 μmol photons m^{-2} s^{-1}) on the photosynthesis of *Chlamydomonas reinhardtii* grown under 350 (○) and 700 (●) ppmv CO_2. The net photosynthetic rate at zero time was 170 μmol O_2 mg^{-1} Chl h^{-1}. Data are the means for triplicate cultures ± SD.

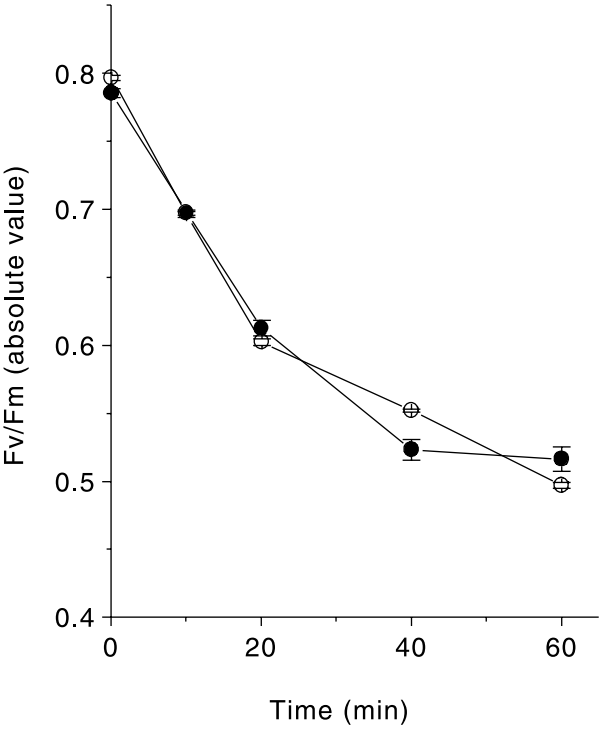

Fig. 4. The photochemical efficiency (F_v/F_m) of *Chlamydomonas reinhardtii* grown under 350 (○) and 700 (●) ppmv CO_2 concentrations as a function of exposure time to high PFD (1000 μmol m^{-2} s^{-1}). Data are the means for triplicate cultures ± SD.

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_k) 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 m^{-2} s^{-1}), decreased markedly with time. The low-CO_2-grown cells showed 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 photoinhibitory recovery.

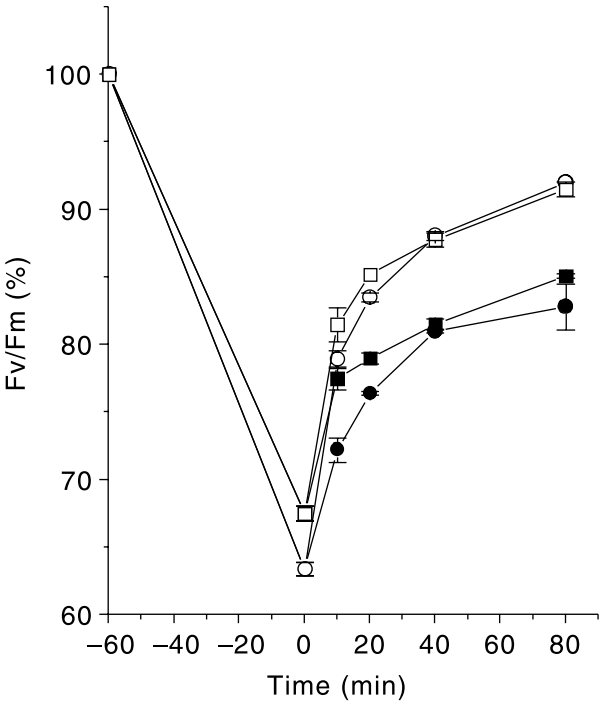

Fig. 5. Recovery of the photochemical efficiency (F_v/F_m) in dim light (○,□) and in darkness (●,■) after 60 min high PFD exposure in *Chlamydomonas reinhardtii* grown under 350 (●,○) and 700 (■,□) ppmv CO_2 concentrations. Data are the means for triplicate cultures ± SD.

Algae use ribulose bisphosphate carboxylase/oxygenase (Rubisco) to fix CO_2, but CO_2 is not the only carbon source for aquatic photosynthesis. Inorganic carbon in water exists in the form of CO_2 (aq), HCO_3^- and CO_3^{2-}, which can reach an equilibrium as follows:

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

The CO_2 in fresh water usually accounts for 38–0.6% of the total inorganic carbon within a pH range of 6.5–8.5. When CO_2 concentration in air increases, the equilibrium is broken, and the reactions proceed toward the right-hand until it reaches a new equilibrium. Doubled atmospheric CO_2 concentration resulted in doubled concentration of CO_2 (aq), but only increased HCO_3^- by 0.6%, and reduced pH by 0.298 in fresh water (Stumm and Morgan 1996). *Chlamydomonas reinhardtii* was proven to be capable of using HCO_3^- for photosynthesis and possess CO_2 concentrating mechanism when CO_2 in the bulk was not enriched; when grown in 5% CO_2, the HCO_3^- pumping mechanism and CA-mediated diffusion of CO_2 were negatively affected (Badger *et al*. 1980; Imamura *et al*. 1983; Moroney *et al*. 1985). That doubled atmospheric CO_2 (700 ppmv) in the present study did not influence the growth and photosynthesis implies that such a minor increase of CO_2 (compared with 5%) could not affect HCO_3^- utilization and/or carbonic anhydrase activity when natural conditions meet those in our experiments.

Chlamydomonas reinhardtii grown in 350 ppmv CO_2 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 CO_2 on the photochemical property of the alga. Spalding *et al*. (1984) showed that CO_2 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 CO_2 uptake rates (Krause 1988), or a decrease in F_v/F_m (Falk and Samuelsson 1992). In the present study, both the light-dependent O_2 evolution rate and F_v/F_m were decreased when *C. reinhardtii* cells were exposed to high light. Doubled atmospheric CO_2 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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