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EFFECTS OF CO_2 ENRICHMENT ON THE BLOOM-FORMING CYANOBACTERIUM *MICROCYSTIS AERUGINOSA* (CYANOPHYCEAE): PHYSIOLOGICAL RESPONSES AND RELATIONSHIPS WITH THE AVAILABILITY OF DISSOLVED INORGANIC CARBON[1]

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***Microcystis aeruginosa* Kütz. 7820 was cultured at 350 and 700 $\mu L \cdot L^{-1}$ CO_2 to assess the impacts of doubled atmospheric CO_2 concentration on this bloom-forming cyanobacterium. Doubling of CO_2 concentration in the airflow enhanced its growth by 52%–77%, with pH values decreased and dissolved inorganic carbon (DIC) increased in the medium. Photosynthetic efficiencies and dark respiratory rates expressed per unit chl *a* tended to increase with the doubling of CO_2. However, saturating irradiances for photosynthesis and light-saturated photosynthetic rates normalized to cell number tended to decrease with the increase of DIC in the medium. Doubling of CO_2 concentration in the airflow had less effect on DIC-saturated photosynthetic rates and apparent photosynthetic affinities for DIC. In the exponential phase, CO_2 and HCO_3^- levels in the medium were higher than those required to saturate photosynthesis. Cultures with surface aeration were DIC limited in the stationary phase. The rate of CO_2 dissolution into the liquid increased proportionally when CO_2 in air was raised from 350 to 700 $\mu L \cdot L^{-1}$, thus increasing the availability of DIC in the medium and enhancing the rate of photosynthesis. Doubled CO_2 could enhance CO_2 dissolution, lower pH values, and influence the ionization fractions of various DIC species even when the photosynthesis was not DIC limited. Consequently, HCO_3^- concentrations in cultures were significantly higher than in controls, and the photosynthetic energy cost for the operation of CO_2 concentrating mechanism might decrease.**

Key index words: **blue-green alga; carbon dioxide; cyanobacterium; dissolved inorganic carbon; elevated CO_2; growth; *Microcystis aeruginosa*; photosynthesis**

Atmospheric CO_2 has risen in the last hundred years as a result of human activities such as fossil fuel burning, cement manufacture, land use, and deforestation (Bowes 1993, Stumm and Morgan 1996). Research on the impacts of CO_2 enrichment has mainly focused on terrestrial plants, and studies of aquatic species lag at least 10 years behind (Bowes 1993). Based on laboratory experiments, Riebesell et al. (1993) predicted that the atmospheric CO_2 rise would enhance the growth of marine phytoplankton. Hein and Sand-Jensen (1997) investigated marine phytoplankton communities in the field and also suggested that the elevation of atmospheric CO_2 would enhance the marine primary production. However, the impact of increased atmospheric CO_2 on productivity in fresh waters is poorly understood.

Most lakes are supersaturated with CO_2 and thus are net sources of CO_2 to the atmosphere (Cole et al. 1994, Maberly 1996). This supersaturation is a result of inputs of organic carbon (dissolved and particulate) and inorganic carbon (from plant root and soil respiration) from previously deposited lake sediments and new inputs from the catchment (Cole et al. 1994). Carbon speciation and pH in lakes vary on a diel, episodic, and seasonal basis. The concentration and speciation of dissolved inorganic carbon (DIC) is strongly linked to the pH value through equilibrium reactions among CO_2, H_2CO_3, HCO_3^-, and CO_3^{2-}. Massive diffusion resistance in waters imposes far greater limitations on CO_2 supply than in aerial systems. Phytoplankton photosynthesis removes CO_2 from the aquatic environment and results in a rise of pH. It also creates local areas that are far from equilibrium with the atmosphere and permits large diurnal fluctuations in pH, DIC, and O_2. Diel variations of up to 1.8 pH units have been recorded in a productive English lake, and the daily changes in concentration of inorganic carbon vary between 4 and 63 $\mu mol \cdot L^{-1}$ (Maberly 1996). The seasonally large biomass of phytoplankton can also cause the pH to rise and the concentration of inorganic carbon to decrease, particularly in summer when phytoplankton activity is high and the availability of inorganic carbon for phytoplankton is reduced because of stratification (Maberly 1996). The situation is further complicated in that two DIC forms (CO_2 and HCO_3^-) are used, with

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different affinities for photosynthesis (Kaplan and Reinhold 1999). All these make CO_2-enrichment experiments with freshwater species technically difficult.

The enzyme RUBISCO of cyanobacteria showed low affinities for CO_2, with K_c values of 200–300 $\mu mol \cdot L^{-1}$ (Colman 1989). However, they possess effective CO_2-concentrating mechanisms (CCMs) that raise the intracellular CO_2 level around RUBISCO, which could be 1000-fold higher than the external medium (Kaplan and Reinhold 1999). The expression of CCMs in cyanobacteria is influenced by the external DIC concentration. It has been well established that ambient air CO_2 could induce the operation of CCM, whereas cells grown on high levels of CO_2 have a repressed ability to transport DIC (Kaplan et al. 1980, Miller et al. 1984, Mayo et al. 1986). Synthesis of the HCO_3^- transport system seems to be repressed by growth at high DIC concentrations, whereas a capacity for active CO_2 transport remains (Abe et al. 1987, Badger and Gallagher 1987, Miller and Canvin 1987). Although the response of cyanobacteria to large changes in the concentration of CO_2 (1%–5% CO_2) has been examined, little study has focused on their responses to increased atmospheric CO_2.

The occurrence of cyanobacterial blooms has increased apparently over the last few decades (Oliver and Ganf 2000). These blooms degrade the recreational value of water surfaces, impair water supply, cause deoxygenation of the water by decomposition, and lead to fish kills. *Microcystis aeruginosa* has been widely recognized as one of the most common bloom-forming cyanobacteria all over the world. The present study investigates its ecophysiological responses to atmospheric CO_2 rise to assess effects of CO_2 enrichment on freshwater blooms.

MATERIALS AND METHODS

Growth conditions. *Microcystis aeruginosa* Kütz. (strain 7820, Freshwater Algae Culture Collection, China) was cultured at 40 μmol photons$\cdot m^{-2} \cdot s^{-1}$ in a plant growth chamber (E7, Conviron, Winnipeg, Canada). The plant growth chamber can stimulate daily fluctuations of light and temperature. Day and night cycles were set as 14:10-h light:dark. The highest temperature was 28° C at noon, and the lowest temperature was 22° C at night. *Microcystis aeruginosa* was grown in 500-mL Erlenmeyer flasks with 400 mL BG_{11} medium (Stanier et al. 1971) and aerated with sterile filtered ambient (350 $\mu L \cdot L^{-1}$) or elevated CO_2 (700 $\mu L \cdot L^{-1}$) air at 200 $mL \cdot min^{-1}$. Aeration was used either in or above the culture medium.

Preparation of DIC-free reaction medium. Bis-Tris Propane or CAPS 30 $mmol \cdot L^{-1}$ were added to BG_{11} medium, adjusted to pH 1.0 with 1 N HCl, aerated with pure N_2 gas for 30 min, and then adjusted back to pH 8.0 (or 10.0) with fresh supernatant supersaturated NaOH solution.

General analyses. Cell growth was determined by hemocytometer (3–6 counts for each sample). Chl *a* content was extracted with 80% acetone (Inskeep and Bloom 1985). The pH value was estimated with a pH meter (420A, Orion, Allometrics, Baton Rouge, LA, USA). Samples (5 mL each) were processed with an ultrasonic cleaner (8892, Cole-Parmer Inc., Vernon Hills, IL, USA) at ambient temperature for 2–3 min without disrupting the cells then precipitated by centrifugation (680*g*, 10 min). Total carbon, DIC, and dissolved organic carbon in the cell-free supernatant medium were measured with a total organic carbon analyzer (TOC-5000A, Shimadzu, Kyoto, Japan). DIC speciation and concentration were determined according to Stumm and Morgan (1996).

The dissolution of CO_2 into BG_{11} medium buffered with 30 $mmol \cdot L^{-1}$ Bis-Tris Propane (pH 8.0) or 30 $mmol \cdot L^{-1}$ CAPS (pH 10.0) was measured at 25° C. The flow rate of aeration was set at 200 $mL \cdot min^{-1}$. DIC and pH values were determined at intervals. The relationship between CO_2 dissolution and DIC for a known time interval is as follows:

$$Y = A + B \cdot X \tag{1}$$

where A represents the initial DIC concentration ($mg \cdot L^{-1}$); B, the rate of CO_2 dissolution ($mg \cdot L^{-1} \cdot h^{-1}$); X, the time (h); Y, the DIC concentration at time X ($mg \cdot L^{-1}$).

Photosynthetic measurements. Photosynthetic activities were assayed with a Clark Oxygen Electrode (Rank Brothers, England) with cells in the exponential growth phase. Samples were harvested by centrifugation and resuspended in fresh BG_{11} medium. The oxygen electrode was calibrated with air-equilibrium distilled water for full scale and by adding a little $Na_2S_2O_4$ powder for the zero point. Temperature was controlled with a Polystat refrigerated bath (Cole-Parmer Instrument Co.) at 25° C. Illumination was provided by a 500-W halogen lamp. Irradiance was measured with a quantum sensor (LI-185B, Li-Cor Inc., Lincoln, NE, USA) and controlled by changing the closure of the diaphragm and the distance of lamp from the assimilation chamber and was always elevated from zero to the maximum. Photosynthetic responses to DIC concentration were measured at 25° C and 700 μmol photons$\cdot m^{-2} \cdot s^{-1}$. DIC-free reaction solution (30 $mmol \cdot L^{-1}$ Bis-Tris Propane, pH 8.0) was prepared as described above. Pelleted cells were washed with reaction medium three to four times. Known amounts of $NaHCO_3$ were added to the cell suspension after depletion of DIC in the medium, as shown by the cessation of O_2 evolution, to determine the relationship of photosynthesis to CO_2 or DIC concentration. The CO_2 supply rate from spontaneous dehydration of HCO_3^- was estimated according to Miller and Colman (1980).

Photosynthetic activities (P) [μmol $O_2 \cdot$(mg chl *a*)$^{-1} \cdot min^{-1}$ or μmol $O_2 \cdot cell^{-1} \cdot min^{-1}$] were determined as follows:

$$P = 1000 \cdot A \cdot D \cdot F/(32 \cdot B \cdot C \cdot E) \tag{2}$$

where A represents the saturated O_2 concentration ($mg \cdot L^{-1}$) at a given temperature and Cl^- concentration; B, the transverse distance that the recorder pen moved from full-scale to zero point during calibration (cm); C, chl *a* content or cell density of samples ($mg \cdot L^{-1}$ or $cell \cdot L^{-1}$); D, the transverse distance that the recorder pen moved during measurement (cm); E, the distance that the chart paper moved forward during measurement (cm); and F, the chart speed ($cm \cdot min^{-1}$).

Parameters for the photosynthetic response to irradiance (P-I curves) were analyzed according to Henley (1993):

$$P = P_m \cdot \tanh(\alpha \cdot I/P_m) + R_d,$$
$$-I_k = P_m/\alpha, I_c = -R_d/\alpha \tag{3}$$

where I represents irradiance; P, photosynthetic activity at certain irradiance; P_m, light-saturated photosynthesis; I_k, saturating irradiance for photosynthesis; and I_c, light compensation point. The ascending slope at limiting irradiances, α, was calculated to assess the photosynthetic efficiency.

Parameters for the photosynthetic response to DIC were obtained by fitting net photosynthetic rates at various levels of DIC with the Michaelis-Menten formula:

$$v = V_{max} \cdot [S]/(K_{0.5}(DIC) + [S]) \tag{4}$$

where $K_{0.5}$(DIC) is the DIC concentration required to give half maximal photosynthetic rate and V_{max} is the inorganic carbon-saturated photosynthesis.

RESULTS

Growth and changes in the medium. Doubling the CO_2 concentration in the airflow enhanced the growth of *M. aeruginosa* 7820 (Fig. 1A). When being aerated at the surface or at the bottom of cultures, cell densities were, respectively, 77% and 52% higher than controls in the stationary phase ($P < 0.05$, *t*-test). Specific growth rates in the exponential phase were about 0.50–0.60 d^{-1} and were not affected significantly by raising the CO_2 concentration ($P > 0.05$, *t*-test). The enhancement was seen during the late exponential phase. The pH values of the medium were influenced significantly ($P < 0.05$, ANOVA) by the CO_2 concentration in the airflow and were lowered by an average of 0.21 pH units by doubling of CO_2 (Fig. 1B). The surface aeration reduced pH more than did subsurface aeration. The pH values of cultures increased by about 2.5 pH units during the whole growth period. Final pH values were 10.0 and 9.6, respectively, for the surface and subsurface aerations. The DIC concentrations in the medium increased with time and were elevated to a higher level with CO_2 enrichment during the growth period (Fig. 1C). The dissolved organic carbon contents in the medium also increased with the growth of the cells from the late exponential phase (Fig. 1D).

Photosynthetic characteristics. The photosynthetic responses of *M. aeruginosa* 7820 to irradiance are shown in Figure 2. No photoinhibition was observed at irradiances up to 930 μmol photons·m^{-2}·s^{-1}. Saturating irradiance for photosynthesis was low with I_k values of 105–143 μmol photons·m^{-2}·s^{-1}. Light compensation points were 18–24 μmol photons·m^{-2}·s^{-1}. Light-saturated photosynthetic rates were about 9.84–10.40 μmol O_2·(mg chl *a*)$^{-1}$·min^{-1} or 6.38–10.89 μmol O_2·(10^{10} cells)$^{-1}$·min^{-1}, and dark respiratory rates were about −1.34 to −1.86 μmol O_2·(mg chl *a*)$^{-1}$·min^{-1} or −1.19 to −1.63 μmol O_2·(10^{10} cells)$^{-1}$·min^{-1}. The photosynthetic efficiencies (α) were about 0.069–0.092 (μmol O_2·(mg chl *a*)$^{-1}$·min^{-1})·(μmol photons·m^{-2}·s^{-1})$^{-1}$ or 0.062–0.076 (μmol O_2·(10^{10} cells)$^{-1}$·min^{-1}) ·(μmol photons·m^{-2}·s^{-1})$^{-1}$. Doubling the CO_2 concentration had less effect on light-saturated photosynthetic rates

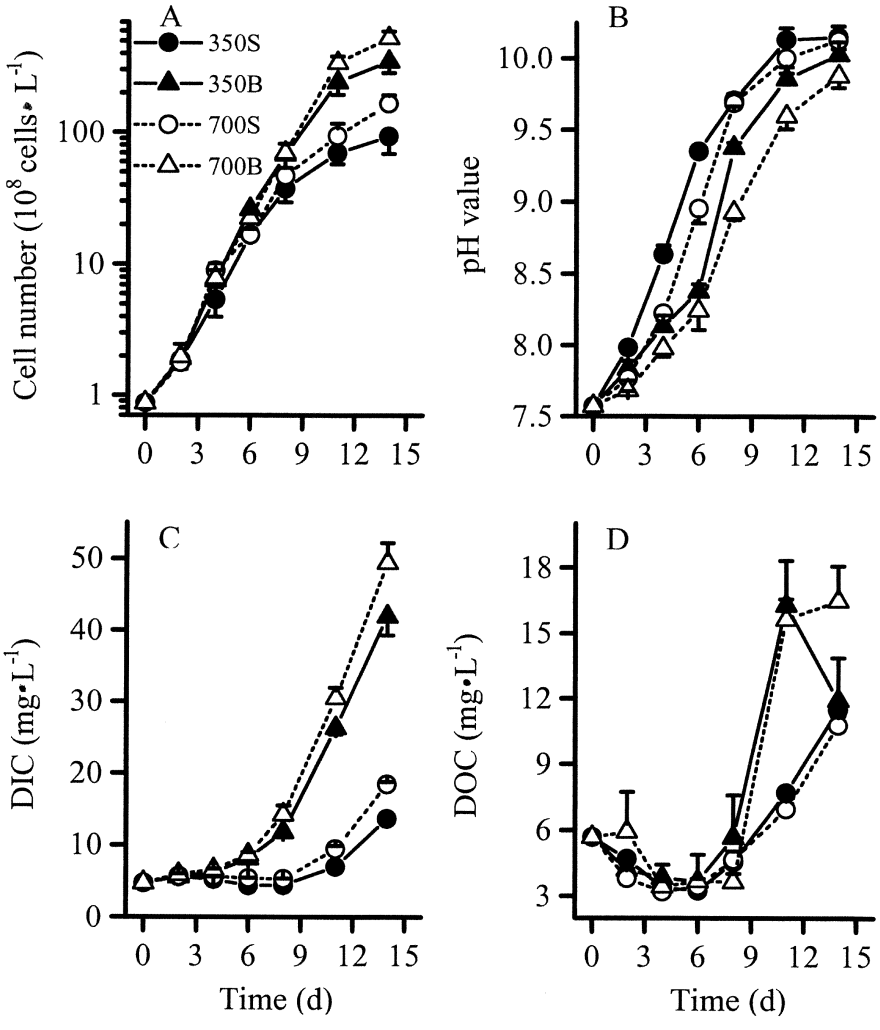

FIG. 1. Growth of *Microcystis aeruginosa* 7820 and changes of pH value, dissolved inorganic carbon (DIC), and dissolved organic carbon (DOC) in cultures. 350S and 700S, aerating 350 and 700 μL·L^{-1} CO_2 to the surface of cultures, respectively; 350B and 700B, aerating 350 and 700 μL·L^{-1} CO_2 to the bottom of flasks, respectively. Data are means ± SD ($n = 3$).

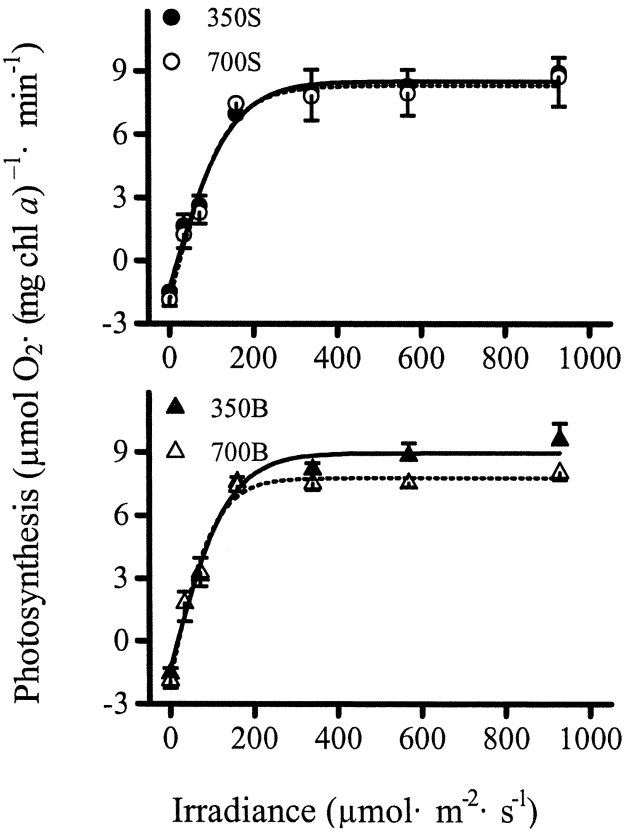

FIG. 2. Photosynthesis of *Microcystis aeruginosa* 7820 as a function of irradiance at 25° C. Data are means ± SD ($n = 5$). Other details and symbols as in Fig. 1.

on the basis of chl *a*, dark respiratory rates and photosynthetic efficiencies normalized to cell number, and light compensation points ($P > 0.05$, Tukey multiple comparison) (Tables 1 and 2). Photosynthetic efficiencies and dark respiratory rates expressed per unit chl *a* tended to increase with the doubling of CO_2. However, light-saturated photosynthetic rates normalized to cell number and saturating irradiance for photosynthesis tended to decrease with the increase of DIC concentration in the medium.

The observed rates of photosynthetic O_2 evolution in *M. aeruginosa* 7820 at pH 8.0 were compared with the maximal rate of CO_2 supply from uncatalyzed dehydration of HCO_3^- (Fig. 3). The rates of photosynthesis exceeded that of the HCO_3^- to CO_2 conversion. This indicates that this organism used HCO_3^- in addition to or instead of CO_2 for its photosynthesis. The apparent photosynthetic affinities for DIC and CO_2 were high. The cells required only 27.7–55.4 μmol·L^{-1} DIC or 0.61–1.21 μmol·L^{-1} CO_2 to give half maximal photosynthetic rate at pH 8.0 (Fig. 4, Table 3). The DIC-saturated photosynthetic rates were 7.88–9.60 μmol O_2·(mg chl *a*)$^{-1}$·min^{-1} or 10.82–14.42 μmol O_2·(10^{10} cells)$^{-1}$·min^{-1}. Doubling of the CO_2 concentration in the airflow had less effect on the DIC-saturated photosynthetic rates and photosynthetic apparent affinities for DIC and CO_2 ($P > 0.05$, Tukey multiple comparison) (Fig. 4, Table 3).

Pigment composition and content. The *in vivo* absorption spectra of *M. aeruginosa* 7820 cultured at ambient and elevated CO_2 showed the same pattern. The CO_2 enrichment had little effect on the contents of chl *a* (439 and 679 nm), carotenoids (490 nm), and phycocyanin (620 nm). Absorption spectra of lipophilic pigments also confirmed that it did not affect the relative contents of chl *a* and carotenoids. However, the chl *a* content per cell tended to decrease at elevated CO_2.

Dissolution of CO_2 in BG_{11} medium. The speed of CO_2 dissolution doubled when CO_2 concentration in the airflow increased from 350 to 700 μL·L^{-1} (Fig. 5). It was 1.8 times at pH 8.0 and 1.7–2.1 times that of control at pH 10.0 (Table 4). Subsurface aeration resulted in faster dissolution of CO_2 compared with surface aeration, with the former 12.1 and 4.9–6.2 times the latter at pH 8.0 and 10.0, respectively. The speed of CO_2 dissolution increased when pH value increased from 8.0 to 10.0. It reached to 4.9–5.6 and 2.3–2.5 times of those at pH 8.0 with the surface and subsurface aerations, respectively.

Diurnal changes of pH value and DIC concentration. Daily variation of pH value showed similar patterns independent of CO_2 concentrations in the airflow in that it rose to the highest values during the light period but decreased to the lowest during the dark period. Daily variation of DIC concentration showed the opposite patterns. That assimilation of CO_2 was faster than its dissolution resulted in decreased DIC concentration during the light period. The dissolution and

TABLE 1. Parameters for the photosynthetic irradiance responses (P-I) curves of *Microcystis aeruginosa* 7820 cultured under ambient and elevated CO_2.

Treatments	P_m μmol O_2·(mg chl *a*)$^{-1}$ · min^{-1}	α (μmol O_2 · (mg chl *a*)$^{-1}$· min^{-1}) · (μmol photons · m^{-2}·s^{-1})$^{-1}$	R_d μmol O_2·(mg chl *a*)$^{-1}$·min^{-1}	I_k μmol photons·m^{-2}·s^{-1}	I_c μmol photons·m^{-2}·s^{-1}
350S	9.84 ± 0.70[a]	0.069 ± 0.004[a]	−1.34 ±0.21[a]	143 ± 9[a]	19±4[a]
700S	10.18 ± 1.00[a]	0.077 ± 0.004[a,b]	−1.86 ± 0.19[b,c]	133 ± 11[a,b]	24 ± 3[a]
350B	10.40 ± 0.56[a]	0.079 ± 0.012[a,b]	−1.43 ± 0.22[a,c]	134 ± 25[a]	18 ± 3[a]
700B	9.56 ± 0.43[a]	0.092 ± 0.013[b]	−1.79 ± 0.36[b,c]	105 ± 15[b]	20 ± 4[a]

Photosynthetic oxygen evolution was measured in fresh BG_{11} medium at 25° C and normalized to chl *a*. Values are means ± SD ($n = 5$).

[a,b] Those with different superscript letters are significantly different ($P < 0.05$, Tukey multiple comparison.)

TABLE 2. Parameters for the photosynthetic irradiance responses (P-I) curves of *Microcystis aeruginosa* 7820 cultured under ambient and elevated CO_2.

Treatments	P_m μmol O_2·(10^{10} cells)$^{-1}$·min^{-1}	α (μmol O_2·(10^{10} cells)$^{-1}$·min^{-1})·(μmol photons·m^{-2}·s^{-1})$^{-1}$	R_d μmol O_2·(10^{10} cells)$^{-1}$·min^{-1}	I_k μmol photons·m^{-2}·s^{-1}	I_c μmol photons·m^{-2}·s^{-1}
350S	10.89 ± 2.00[a]	0.076 ± 0.012[a]	−1.45 ± 0.22[a]	143 ± 9[a]	19 ± 4[a]
700S	8.79 ± 0.55[a]	0.067 ± 0.006[a]	−1.63 ± 0.33[a]	133 ± 11[a,b]	24 ± 3[a]
350B	8.73 ± 0.98[a,b]	0.067 ± 0.019[a]	−1.19 ± 0.25[a]	134 ± 25[a]	18 ± 3[a]
700B	6.38 ± 1.25[b]	0.062 ± 0.018[a]	−1.21 ± 0.42[a]	105 ± 15[b]	20 ± 4[a]

Photosynthetic oxygen evolution was measured in fresh BG_{11} medium at 25° C and normalized to cell number. Values are means ± SD (n = 5).

[a,b,c] Those with different superscript letters are significantly different ($P < 0.05$, Tukey multiple comparison.)

respiratory release of CO_2 resulted in the rise of DIC during the dark period. In a light:dark cycle, DIC concentrations and cell densities in the CO_2-enriched cultures were higher, but the pH values were lower than the controls.

In the exponential phase, CO_2 and HCO_3^- concentrations in the medium (Table 5) were higher than those required to saturate the photosynthesis (Fig. 4). Thus, inorganic carbon was not a limiting factor for photosynthesis during the period of culture. The variations of DIC in cultures with the subsurface aeration in a light:dark cycle were 0.331 and 0.273 mg·L^{-1} at ambient and elevated CO_2, respectively. CO_2 dissolution was nearly fast enough to support photosynthetic CO_2 fixation. Therefore, the decrease in DIC concentration was less during the light period. Its rise was also less in the dark period, implying that the dissolution of CO_2 in the medium nearly reached equilibrium concentration. When aerated at the surface of the cultures, the CO_2 dissolution was slower than that being aerated to the bottom of flask (Table 4). The removal of DIC by CO_2 fixation was much faster than the CO_2 dissolution, which resulted in rapid decrease of DIC in the medium during the light period. During the dark period, DIC concentrations increased because of respiratory CO_2 release and CO_2 dissolution. The variations of DIC in a light:dark cycle were 0.880 and 0.555 mg·L^{-1} at ambient and elevated CO_2, respectively.

In the stationary phase, DIC in cultures was mainly in the form of HCO_3^- and CO_3^{2-} and the availability of free CO_2 was very low (Table 5). The HCO_3^- concentrations in cultures aerated at the surface decreased to 40–50 μmol·L^{-1} after 4–6 h in the light, and then the removal of DIC by CO_2 fixation slowed down. This was different from subsurface aeration where it remained constant in the light. The removal of DIC could reach 7–13 times the rate of CO_2 dissolution at pH 10.0. Although the dissolution of CO_2 increased with the increase of pH value, its rise could not account for the slowdown of DIC removal in the

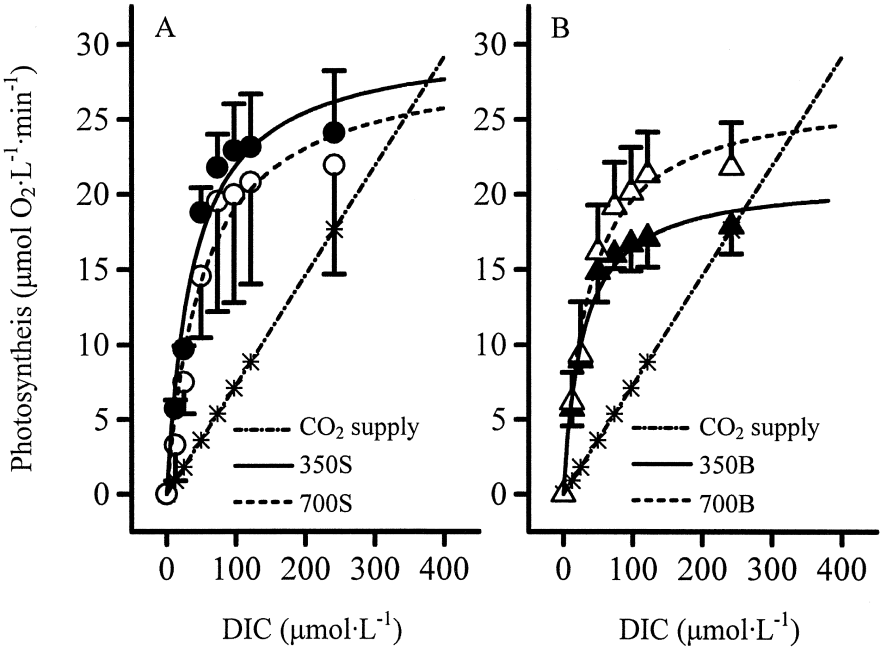

FIG. 3. Comparison of the observed photosynthetic rates of *Microcystis aeruginosa* 7820 at pH 8.0 with the theoretical photosynthetic rates that maximally could be supported by the uncatalyzed conversion of HCO_3^- to CO_2. Data are means ± SD (n = 3). Other details and symbols as in Fig. 1.

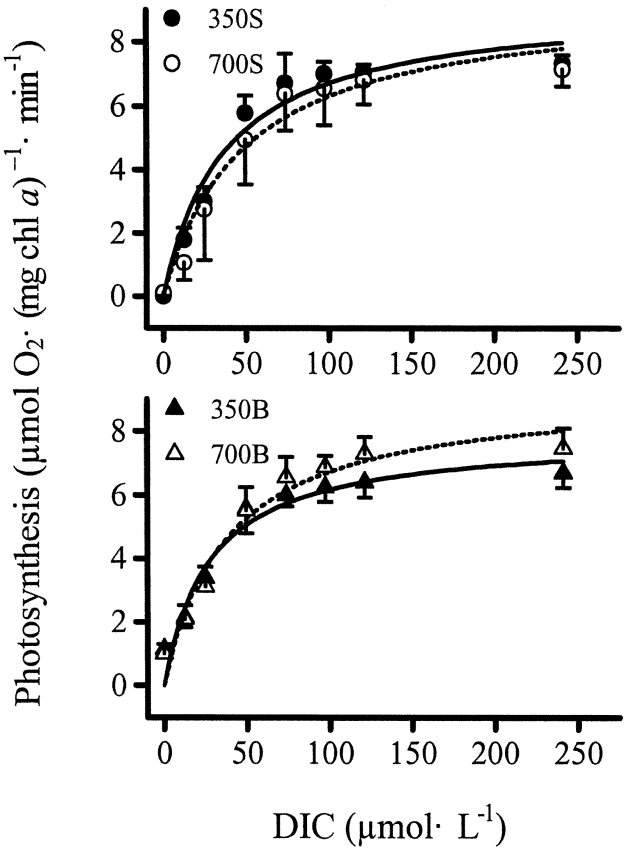

FIG. 4. Photosynthetic CO_2 assimilation as a function of DIC concentration for *Microcystis aeruginosa* 7820. The measurements were conducted at 700 μmol photons·m^{-2}·s^{-1} and 25° C. Data are means ± SD (n = 3). Other details and symbols as in Fig. 1.

light. It appeared that cultures being aerated at the surface were carbon limited in the stationary phase. Constant DIC removal suggested that cultures with subsurface aeration were not carbon limited. However, doubling of the CO_2 concentration in the airflow enhanced CO_2 dissolution, lowered the pH of cultures, and influenced ionization fractions of various DIC species. Thus, HCO_3^- concentrations in cultures with the elevated CO_2 were 2.3–2.5 times higher than those of the controls (Table 5).

DISCUSSION

Most inland waters are net sources of CO_2 to the atmosphere when averaged over time, that is, CO_2 concentrations are above air equilibrium (Cole et al. 1994, Maberly 1996). The DIC concentrations of natural fresh waters could be as high as 1.5–2.2 mmol·L^{-1} (Hutchinson 1967, Colman 1989). Cyanobacteria grown in these waters could be expected to have a repressed ability to transport DIC and consequently maintain a low CO_2 affinity. However, the growth of phytoplankton populations in fresh water is accompanied by a decrease in ambient DIC, which may fall to micromolar concentrations, and these low concentrations may be maintained for long periods of time because of the low rate of CO_2 diffusion from air. Meanwhile, the decrease in DIC concentration is accompanied by an increase of pH values, which may rise over pH 10.0, and also by an increase in dissolved O_2 to levels of 100%–200% of the air-equilibrium value. Thus, carbon limitation might develop in intense cyanobacterial blooms even in lakes with high concentrations of DIC. The growth of *M. aeruginosa* 7820 at varied levels of CO_2 indicated that inorganic carbon might limit its growth during the formation of water blooms, especially when the phytoplankton populations were dense.

The atmospheric CO_2 partial pressure is predicted to double in this century. It will not have a parallel effect on the CO_2 concentration in fresh waters because most of them are net sources of CO_2 to the atmosphere (Cole et al. 1994, Maberly 1996). With an increase in CO_2, plants will differ in growth and competitive interactions. The photosynthetic mechanism of a species is the major determinant of how it will respond to changes in CO_2. *Microcystic aeruginosa* has the ability to take up HCO_3^- (Talling 1976), which was also confirmed in this study. Thus, it seems that ambient DIC would not limit its growth. However, this study has made it clear that cultures aerated at the surface were carbon limited in the stationary phase. Thus, doubling of CO_2 concentration in the airflow enhanced its photosynthesis via the increase in the availability of DIC and thereafter enhanced its growth significantly. In addition, the operation of the CCM is dependent on the supply of photosynthetic energy (Ogawa et al. 1985, Kaplan et al. 1987, Sültemeyer et al. 1993, Kaplan and Reinhold 1999). Cyclic and linear electron flows have been suggested to support the

TABLE 3. Parameters for the photosynthetic responses of *Microcystis aeruginosa* 7820 cultured under ambient and elevated CO_2 to dissolved inorganic carbon.

Treatments	V_{max} μmol O_2·(mg chl a)$^{-1}$·min^{-1})	V_{max} (μmol O_2·(10^{10} cells)$^{-1}$·min^{-1})	K_m(DIC) (μmol·L^{-1})	K_m(CO_2) (μmol·L^{-1})
350S	9.28 ± 0.51[a]	14.19 ± 5.02[a]	38.6 ± 9.8[a]	0.85 ± 0.21[a]
700S	9.60 ± 0.72[a]	14.42 ± 5.71[a]	55.4 ± 28.5[a]	1.21 ± 0.62[a]
350B	7.88 ± 0.56[a]	11.77 ± 2.11[a]	27.7 ± 1.9[a]	0.61 ± 0.04[a]
700B	9.29 ± 0.95[a]	10.82 ± 2.32[a]	38.2 ± 9.8[a]	0.84 ± 0.21[a]

Photosynthetic oxygen evolution was measured at 25° C and pH 8.0 in BG_{11} medium buffered with 30 mM Bis-Tris Propane. Values are means ± SD (n = 3).

[a]: Those with different superscript letters are significantly different ($P < 0.05$, Tukey multiple comparison.)

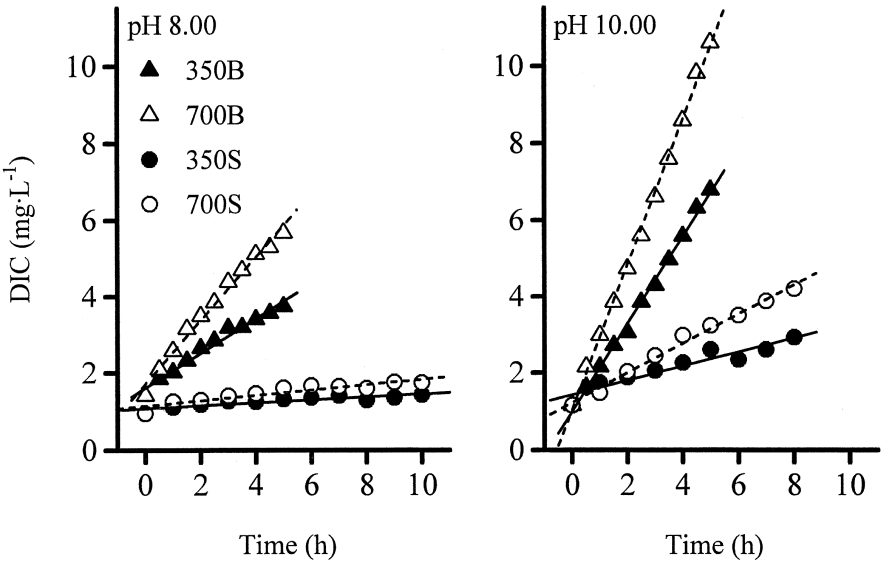

FIG. 5. The dissolution of CO_2 in BG_{11} medium buffered, respectively, with 30mM Bis-Tris Propane (pH 8.0) and 30 mM CAPS (pH 10.0) at 25° C. Other details and symbols as in Fig. 1.

CO_2 and HCO_3^- transports, respectively (Li and Canvin 1998). When *M. aeruginosa* 7820 was not limited by the availability of DIC, doubling of the CO_2 concentration in the airflow could enhance the dissolution of CO_2, lower the pH values of cultures, and influence ionization fractions of various DIC species. Thus, bicarbonate concentrations in cultures were significantly higher than controls (Table 5). Assuming identical internal bicarbonate concentrations and identical transmembrane electrical potential at the plasmalemma, an increased external bicarbonate concentration would decrease the free energy gradient that has to be overcome in pumping bicarbonate into the cell and also the free energy gradient driving any leak of bicarbonate out of the cell. For the first of these to have an impact on energy costs, the stoichiometry of ATP consumed to HCO_3^- transported would have to decrease when the energy gradient for HCO_3^- is smaller. The energy cost of net bicarbonate influx would also decrease if there were less leakage when the external bicarbonate concentration was higher. These were consistent with the increased photosynthetic efficiencies normalized to chl *a* when DIC concentration was increased in the medium (Table 1). Similar results about energy cost and DIC concentration for growth have already been published for cyanobacteria (Beardall 1991) and eukaryotic algae (Kübler and Raven 1995).

This study shows that doubling of CO_2 concentration enhanced the growth of *M. aeruginosa* 7820 by 52%–77%. Cells grew faster at elevated CO_2, and the cell size was less than in control cultures. Thus, the enhancements would be less than those mentioned above if dry weight was used to estimate its growth. Although dry weight was not determined in this study, this point is supported partly by the growth rate on the basis of chl *a* content (increased by 24%–55%). Kimball (1983) analyzed 430 prior observations and concluded that crop species had 33% greater yields at doubling of atmospheric CO_2. It has also been suggested that the photosynthetic rate of C_3 species was usually 10%–50% greater than that of their ambient CO_2-grown counterparts (Cure and Acock 1986, Jiang et al. 1997). These works are mainly about C_3 higher plants (without a CCM) and may be of little relevance to *Microcystis*.

Microcystis aeruginosa 7820 has been cultured at low irradiance (40 μmol photons·m^{-2}·s^{-1}) in this study because optimal growth of phytoplankton is often achieved at irradiances of 40–60 μmol photons·m^{-2}·s^{-1} (Whit-

TABLE 4. Coefficients for the linear regression formula (Y = A + B·X) of data presented in Figure 5.

PH	Treatments	A (mg·L^{-1})	B (mg·L^{-1}·h^{-1})	R^2	Sign. Lev.
8.0	350S	1.086 ± 0.041	0.037 ± 0.007	0.764	$4.40E^{-4}$
	700S	1.151 ± 0.056	0.068 ± 0.010	0.853	$5.00E^{-5}$
	350B	1.610 ± 0.070	0.453 ± 0.024	0.976	$1.32E^{-8}$
	700B	1.718 ± 0.094	0.830 ± 0.032	0.987	$8.22E^{-10}$
10.0	350S	1.436 ± 0.110	0.185 ± 0.023	0.901	$9.00E^{-5}$
	700S	1.234 ± 0.071	0.384 ± 0.015	0.990	$3.49E^{-8}$
	350B	1.003 ± 0.071	1.143 ± 0.024	0.996	$3.89E^{-12}$
	700B	1.061 ± 0.080	1.890 ± 0.027	0.998	$1.26E^{-13}$

TABLE 5. The concentration (μmol·L^{-1}) and speciation of DIC in the medium in the exponential and stationary phase.

	Cultures in the exponential phase				Cultures in the stationary phase			
	8:30		18:30		8:30		18:30	
Treatments	HCO_3^-	CO_2	HCO_3^-	CO_2	HCO_3^-	CO_2	HCO_3^-	CO_2
350S	480	5.97	443	3.19	195	0.023	33	0.001
700S	512	9.47	477	4.68	281	0.048	40	0.001
350B	496	8.04	493	4.90	851	0.104	222	0.006
700B	523	11.97	510	6.74	2828	2.012	774	0.042

ton and Peat 1969, Silvey et al. 1972). Under natural conditions, photosynthesis often takes place at reduced irradiance (relative to noon at the surface) and is confined to the euphotic zone. Saturating irradiance for photosynthesis in cyanobacteria seems to be lower than for many other algae, and *M. aeruginosa* may be able to maintain autotrophic growth at relatively lower irradiance than their potential competitors (Fogg 1965). The activity of CCMs in eukaryotic algae is affected by the ambient photon flux density, and acclimation to decreasing light level resulted in a loss of some part of the ability to take up inorganic carbon (Kübler and Raven 1995). Several observations on higher land plants indicate that CO_2 enrichment enhances photosynthesis and growth even at limiting irradiance, and sometimes the relative enhancement is greater under such conditions (Sionit et al. 1982, Cure and Acock 1986, Allen 1990, Gifford 1992). Most measurements of the effects of elevated CO_2 on photosynthesis have used relatively high irradiance, where CO_2 supply or RUBISCO capacity is the major limitation. Meanwhile, the levels of nitrogen and phosphorus used in this study are higher than those in natural fresh waters. The annual average concentrations of total nitrogen and total phosphorus in Chaohu Lake, Dianchi Lake, Donghu Lake, and Taihu Lake (all known to have freshwater blooms and eutrophication) in China could reach 7.5 $mg \cdot L^{-1}$ and 0.417 $mg \cdot L^{-1}$, respectively (Li et al. 1995, Gou and Yan. 1999, Jin et al. 1999, Zhang et al. 1999). However, plant-level studies that have examined the interaction between elevated CO_2 and mineral nutrition indicate almost without exception that though growth is depressed in a nutrient-poor environment, it is still stimulated by CO_2 enrichment, often to the same degree as in nutrient-sufficient conditions (Wong 1979, Conroy et al. 1986, Cure and Acock 1986, Norby et al. 1986, Allen 1990, Hocking and Meyer 1991, Wong and Osmond 1991, Gifford 1992). Data cited here are mainly on the acclimated responses of higher plants to increased CO_2, and it is not clear, at least in some cases, if the data refer to "balanced" or "unbalanced" growth.

Manipulations of carbon and pH value have resulted in shifts in species dominance to cyanobacteria under conditions of inorganic carbon limitation in field (Shapiro 1990). This ability to compete at low CO_2 concentrations has been construed as the means by which cyanobacteria attain dominance. However, whether CO_2 limitation is essential for the formation of cyanobacterial blooms seems to be contradictory (Shapiro 1997). Nevertheless, carbon limitation may still have a major impact on buoyancy regulation. It is clear that two major aspects are relevant to the bloom formation of cyanobacteria, namely their abundance and buoyancy regulation. Controlled migrations to specific depths permit some cyanobacteria in oligotrophic waters to grow at the expense of nutrients retained at depth. Three mechanisms have been involved in the buoyancy regulation in *Microcystis* cells: 1) regulation of gas vesicle production; 2) changes in cell ballast, particularly carbohydrate; and 3) destruction of gas vesicles by turgor pressure collapse (Reynolds and Walsby 1975, Kromkamp and Mur 1984, Thomas and Walsby 1985, Konopka et al. 1987, Kromkamp et al. 1988, Klemer 1991, Oliver 1994). Although CO_2 limitation of photosynthesis can promote buoyancy in the short term by preventing the collapse of turgor-sensitive gas vesicles and/or by limiting polysaccharide accumulation, sustained carbon limitation restricts buoyancy regulation by limiting gas vesicle and polysaccharide synthesis (Oliver 1994, Klemer et al. 1996). Undoubtedly more information is required on the buoyancy regulation in *Microcystis* cells by inorganic carbon, especially the elevation of atmospheric CO_2.

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