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# Influence of $CO_2$, light and watering on growth of *Nostoc flagelliforme* mats

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**Abstract**

The terrestrial blue-green alga (cyanobacterium), *Nostoc flagelliforme*, was cultured in air at various levels of $CO_2$, light and watering to see their effects on its growth. The alga showed the highest relative growth rate at the conditions of high $CO_2$ (1500 ppm), high light regime (219–414 $\mu$mol $m^{-2}s^{-1}$) and twice daily watering, but the lowest rate at the conditions of low light (58–114 $\mu$mol $m^{-2}s^{-1}$) and daily twice watering. Increased watering had little effect on growth rate at 350 ppm $CO_2$, but increased by about 70% at 1500ppm $CO_2$ under high light conditions. It was concluded that enriched $CO_2$ could enhance the growth of *N. flagelliforme* when sufficient light and water was supplied.

*Nostoc flagelliforme* (Berk. & Curtis) Bornet & Flahault is an edible terrestrial blue-green alga (cyanobacterium) that is distributed on arid or semiarid steppes of northern and west-northern parts of China. The Chinese studies on the ecology, physiology, biochemistry and resources of this species have been recently reviewed (Gao, 1998).

In China, increased market demands and the exploitation of land associated with the economic growth are diminishing the resources of *N. flagelliforme* (Gao, 1998). However, *N. flagelliforme* has not yet been successfully farmed. Therefore, growth of the alga has been of general concern with regard to its large scale cultivation. Its growth is favored by diel fluctuations in temperature rather than a constant temperature (Chu et al., 1989). The mean increase in biomass over a period of 14 days was about 20% and the maximum was 30% under moist conditions at 10–25 °C and about 20 $\mu$mol $m^{-2}$ $s^{-1}$. Hu et al. (1987), by culturing *N. flagelliforme* on several kinds of soil, found that filaments of *N. flagelliforme* elongated faster with periodical fertilization compared to those observed in the field. Nevertheless, little has been documented on the effects of watering, light and $CO_2$ on its growth. However, the only inorganic carbon source for the photosynthesis of air-exposed *N. flagelliforme* is $CO_2$, so growth might be limited by the present atmospheric $CO_2$ concentration.

The present study aims to investigate the influence of $CO_2$ concentration, light intensity and watering on growth of *N. flagelliforme* in order to understand their individual and combined effects when developing a farming technology for the organism.

## Materials and methods

*Nostoc flagelliforme* was collected at Siziwangqi, Inner Mongolia, in 1995 and stored dry before use in experiments in 1998. Twenty mats (each about 0.5 g d. wt) were gently rinsed 3 times with tap water, then soaked in BG-11 medium in an incubator at 25 °C and 40 $\mu$mol $m^{-2}s^{-1}$for 9–10 h, a period shown previously to be long enough for the physiological recovery of the alga (Scherer et al., 1984; Gao et al., 1998). Each mat was then stretched evenly on a transpar-

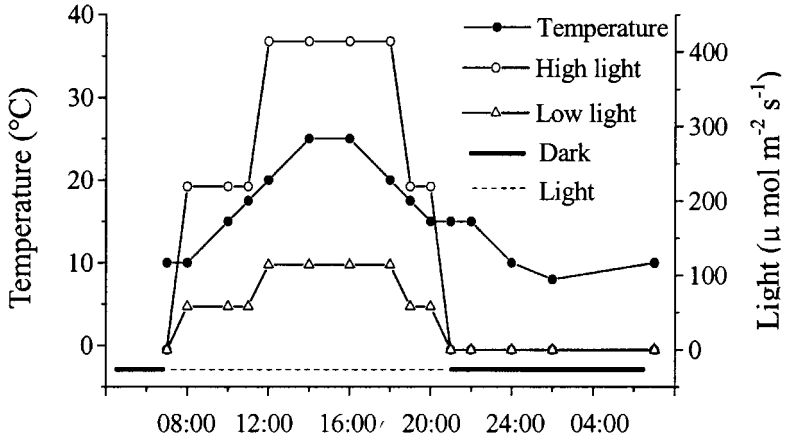


*Figure 1.* Diurnal changes of temperature and light in the $CO_2$-chamber.

ent plastic sheet, surface water droplets were removed with tissue paper before placed in a $CO_2$-chamber (E7, Coviron). The $CO_2$-chamber can simulate daily fluctuations of light and temperature. The patterns of diel light and temperature changes in this study were set as in Figure 1. Day and night cycles were set as 14: 10 h. The highest temperature at noon was 25 °C; and the lowest temperature at night was 8 °C. The highest light intensities during the daytime were 414 $\mu$mol $m^{-2}s^{-1}$ and 114 $\mu$mol $m^{-2}s^{-1}$ in the high and low light zones, respectively. The chamber was divided into two parts, high light zone and low light zone by adjusting the height of the shelf from the light source. Ten mats were placed in the high light zone and the other ten in the low light zone. The experiments were carried out at $CO_2$ concentrations of 350 and 1500 ppm. $CO_2$ concentration within the $CO_2$-chamber was controlled with fluctuations of less than 3%.

Watering was carried out daily once or twice by soaking the algal mats in BG-11 medium for 30 min at 0700 or/and 1730 h at 25 °C and 40 $\mu$mol $m^{-2}s^{-1}$ in another incubator. Relative water content (RWC) of the mat was calculated as follows:

$$RWC = (Wt\text{-}Wd)/(Ww\text{-}Wd) \times 100\%,$$

where Wd is dry weight (80 °C, 10 h); Wt, instantaneous weight of sample measured at a particular time interval; Ww, initial wet weight.

Changes in mass (dry weight) of a mat were used to assess its growth. To determine the dry weight, the mats were taken out from the chamber, placed by a fan to make them air-dried evenly at room temperature of 25 °C and relative humidity of about 50%, weighed to the nearest 0.1 mg ($W_a$). Then, a small amount of each mat (approx. 10%) was cut off and the remaining mat was weighed again ($W_b$). The cut-off parts were dried in an oven (80 °C) for 10 h, cooled in a desiccator and its dry weight ($W_c$) measured. The ratio of dry to air-dry weight was determined as $W_c/(W_a\text{-}W_b)$. Then the dry weight (D) of a mat was calculated from air-dried weight as follows:

$$D = W_c/(W_a\text{-}W_b)*W_a,$$

Relative growth rate ($\mu$,% $day^{-1}$) was calculated as:

$$\mu = (100\mathrm{In}(D_t/D_0))/t$$

where $D_0$ is initial dry weight, $D_t$ is dry weight after t number of days.

## Results and discussion

The patterns of water loss after watering are shown in Figure 2. The mats in the morning lost about 75% water under high light and 67% under low light in 6 h, the corresponding half-times for desiccation being about 3.7 and 4.3 h, respectively (Table 1). The mats that were re-watered in afternoon lost about 71% water under high light and 53% under low light by the end of the light period, the corresponding half-times for desiccation being 1.9 and 2.5 h, respectively (Table 1). The maximal rates of water loss per hour from the mats were about 17% and 12% in the morning and about 25% and 20% in the afternoon in the high and low light regimes, respectively. Elevation of $CO_2$ to 1500 ppm did not affect the water-loss process of the alga compared with that under the ambient $CO_2$ level ($p < 0.1$, t-test). Morning and afternoon

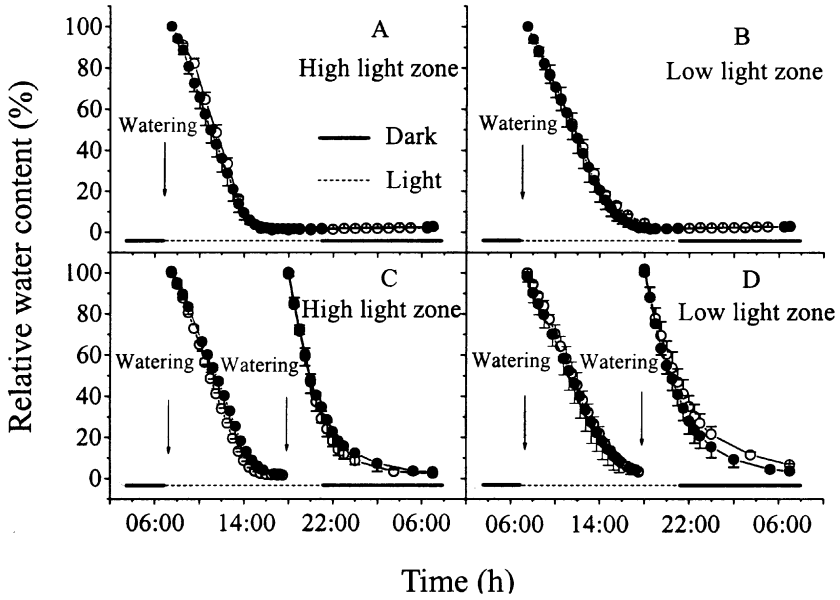


*Figure 2.* Relative water content of *Nostoc flagelliforme* cultured under ambient (350 ppm, ○), and elevated $CO_2$ concentration (1500 ppm, ●), in the $CO_2$-chamber. The first watering was at 0700 (A, B, C and D), and the second watering at 1730 every day (C, D).

*Table 1.* Relative water contents (RWC,%) of *N. flagelliforme* mats in the middle (noon) and at the end (evening) of the light period and their half-desiccation time (h) at different levels of $CO_2$, light and daily watering. The relative water content was 100% at the beginning of the light period. The data were based on Figure 2

| Different light zone | First watering | | | Second watering | |
|---|---|---|---|---|---|
| | RWC (%) | | Half-desiccation time (h) | RWC (%) | Half-desiccation time (h) |
| | noon | evening | | evening | |
| High | 39.3 ± 4.2 | 1.4 ± 0.1 | 3.7 ± 0.2 | 29.1 ± 3.5 | 1.9 ± 0.3 |
| * High | 36.5 ± 3.7 | 1.6 ± 0.3 | 3.5 ± 0.1 | 34.6 ± 4.0 | 1.9 ± 0.1 |
| Low | 48.0 ± 2.4 | 1.8 ± 0.1 | 4.3 ± 0.3 | 46.9 ± 6.4 | 2.5 ± 0.4 |
| * Low | 47.6 ± 1.9 | 1.7 ± 0.1 | 4.1 ± 0.6 | 40.8 ± 5.7 | 2.4 ± 0.1 |

* Data in these lines are for the mats under 1500 ppm $CO_2$.

watering resulted in different rates of water loss, only because of the different patterns of temperature. In the morning, temperature was low in the initial phase and increased slowly from 8 to 25 °C toward noon. In the afternoon, temperature was high in the initial phase and decreased afterwards. This gave rise to faster half desiccation in the early afternoon. High light at the beginning of afternoon could also contribute to the faster desiccation.

Growth was monitored as changes in mass of the mats (Figure 3). The biomass of each mat increased by about 5 ∼ 31% in 15 days of culture (Table 2). Chu (1989) reported that the mean filament elongation of *N. flagelliforme* was from 12 to 20% in 14 days at 10–25 °C and about 20 $\mu$mol $m^{-2}$ $s^{-1}$. The elongation of the filaments expressed as a percentage change may be equivalent to the increase in biomass in terms, provided the filament mass to length ratio is constant. The mean relative growth rates of *N. flagelliforme* in the high light zone were respectively 1.18 and 0.96 at ambient $CO_2$ concentration of 350 ppm, and 1.13 and 1.80 at 1500 ppm $CO_2$ (Table 2) for once and twice daily watering, respectively. Increasing watering from once to twice per day resulted in 68% enhancement of the growth at the high $CO_2$ concentration ($p$ <0.01, t-test). The mean growth rates regardless of watering time were 1.13∼1.80 and 0.33∼0.35 for the high and low light zones, respectively. Increased light enhanced

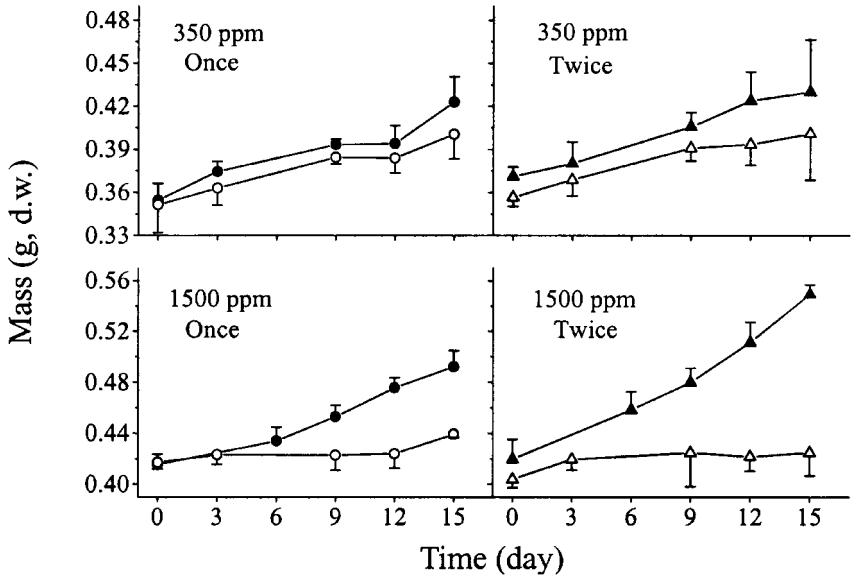


*Figure 3.* Growth of *Nostoc flagelliforme* under ambient $CO_2$ concentration (about 350 ppm) and elevated $CO_2$ concentration (1500 ppm). Each datum represents the mean of 5 mats ± SD. Solid and open symbols represent those cultured under high light and low light zones, respectively.

*Table 2.* Growth of *N. flagelliforme* at different levels of $CO_2$, light and watering. Values of $\mu$ and biomass increase are the mean ± SD of 5 mats

| Concentration of $CO_2$ (ppm) | Light zone Light zone | Daily watering | $\mu$ | Biomass increase in 15 days (%) |
|---|---|---|---|---|
| 350 | high | once | $1.18 \pm 0.39$ | $19.5 \pm 6.92$ |
| 350 | low | once | $0.88 \pm 0.54$ | $14.4 \pm 9.08$ |
| 350 | high | twice | $0.96 \pm 0.50$ | $15.8 \pm 8.09$ |
| 350 | low | twice | $0.76 \pm 0.67$ | $12.5 \pm 10.7$ |
| 1500 | high | once | $1.13 \pm 0.27$ | $18.6 \pm 4.82$ |
| 1500 | low | once | $0.35 \pm 0.10$ | $5.33 \pm 1.59$ |
| 1500 | high | twice | $1.80 \pm 0.31$ | $31.2 \pm 6.27$ |
| 1500 | low | twice | $0.33 \pm 0.34$ | $5.20 \pm 5.21$ |

growth by about 150 ~ 400%, and the difference was significant ($p$ <0.01, t-test). That is, *N. flagelliforme* grew faster under high than low light. The light compensation point for photosynthesis was 35–107 $\mu$mol $m^{-2}s^{-1}$, and the photosynthesis-saturated PFD was 205–437 $\mu$mol $m^{-2}s^{-1}$, depending on the water content (Qiu & Gao, unpublished data). The light intensities at noon in the present study were 414 and 114 $\mu$mol $m^{-2}s^{-1}$ for the high and low light regimes. Photosynthesis could be almost saturated in the high light zone during the middle of the day, but unsaturated in the low light zone for the whole light period. Twice daily watering in the low light zone resulted in decreased growth, because the enhanced respiratory loss exceeded photosynthetic gain.

In the low light regime, the effects of elevated $CO_2$ concentration on the growth of *N. flagelliforme* were non-significant ($p$ >0.1, t-test), regardless of the level of watering. Under conditions with high light and twice daily watering, elevation of the $CO_2$ concentration significantly ($p$ <0.05, t-test) increased the growth of *N. flagelliforme*, with an increase of about 31%, about twice the value for that at 350 ppm $CO_2$ (Table 2). Ther was little effect ($p$ >0.1, t-test) in the absence of increased daily watering. *N. flagelliforme* grew by 13% to 20% at 350 ppm $CO_2$ and by 31% at 1500 ppm $CO_2$. Presumably the elevated $CO_2$ con-

centration under high light increased photosynthesis to an extent that water became a limiting factor. More water might be required by the alga if higher levels of light and $CO_2$ were supplied. This also implies that enriched $CO_2$ would enhance the growth of *N. flagelliforme* when light and water were sufficiently supplied.

The results suggest that growth of *N. flagelliforme* could be $CO_2$-limited in nature, especially after rain or in the morning when the algal mats are moist with dew and the light is sufficiently high for its photosynthesis. Any natural or artificial increase in $CO_2$ rise will probably benefit photosynthetic production and growth of the alga.

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