Korean Journal of Botany
Vol. 25, No. 4, 153~160(1982)

Photosynthetic Characteristics of *Anabaena flos-aquae* Growing on Various Inorganic Nitrogen Sources

Maeng, Jueson
(Department of Biology, Sogang University, Seoul)

無機窒素源의 種類에 따른 藍藻類 *Anabaena flos-aquae* 光合成의 特性

孟 柱 善
(西江大學校 理工大學 生物學科)

ABSTRACT

The kinetics of ^{14}C fixation have been investigated in *Anabaena flos-aquae* growing on NH_4^+, NO_3^- and N_2-N in batch cultures. Growth rate was highest with NH_4^+, followed by NO_3^- and finally N_2. The compensation intensity(I_0) and the half-saturation irradiance(K_I) with N_2 were higher than with other N sources, but the maximum C fixation rate(P_{max}) was lower. The P_{max}/K_I ratio, which is analogous to quantum efficiency at low irradiance ranges, was also lower with N_2. All these parameters except K_I decrease with culture age, or decreasing growth rate. Since ^{14}C uptake measures net photosynthesis, the higher values of I_0 and K_I, and the low values of P_{max} and P_{max}/K_I ratio with N_2 appear to be related to the high energy demand of N_2 fixation. They may also be related to the low maximum growth rate with N_2-N.

INTRODUCTION

Many members of the blue-green algae(Cyanophyta) have a unique ability to reduce molecular N_2 to ammonia using water as reductant. They are the main agents for fixing nitrogen in both freshwater and marine environments(Wetzel, 1975; Fogg, 1978). In particular, their contribution to the total nitrogen budget is very high in flooded rice paddies with a daily average input of 0.5 kg N/ha(Stewart *et al.*, 1979) and the biomass of N_2-fixing organisms or nitrogenase activity has frequently been correlated to an increase yield of rice (Stewart *et al.*, 1979; Yamaguchi, 1979; Buresh *et al.*, 1980; Roger and Kulassoriya, 1980).

Nitrogen fixation requires reductant and ATP which, for blue-green algae, are provided by photosynthesis. They can also use NO_3^- and NH_4^+. From an energetic standpoint, the order of preference as an N source should be NH_4^+, NO_3^-, and N_2. In fact, when N_2-fixing blue-green algae were grown on these inorganic N sources, the maximum growth rate decreased in the order of the preference (Ward and Wetzel, 1980a; Rhee and Lederman, 1982) and the maximum photosynthetic rate also seemed to decrease in the same order (Ward and Wetzel, 1980b). The minimum light level to maintain growth was also higher with N_2 (Ward and Wetzel, 1980a). However, there has been no systematic investigation on how the kinetics of photosynthesis are affected by various N sources.

The present study reports on a detailed investigation of the effects of three inorganic N sources on the compensation intensity, the efficiency of light utilization, and the maximum photosynthetic rate in the N_2-fixing blue-green alga *Anabaena flos-aquae*.

MATERIALS AND METHODS

Anabaena flos-aquae obtained from D. R. S. Lean (Environment Canada, Burlington, Ontario) was grown axenically in batch culture in a modified inorganic medium of Guillard and Lorenzen (1972). Tris was replaced by phosphate buffer (10 mM) and nitrogen was supplied as NO_3^- or NH_4^+ at 1.5 mM or N_2 in air. The algae were cultured in 3-*l* Erlenmeyer flasks with a culture volume of 1.2 *l* at 23±1°C and stirred by a magnetic stirrer. Illumination was continuous at an average irradiance of 15 Wm^{-2}. pH in the medium was 7.8±0.2.

Photosynthetic rates were measured by the ^{14}C technique at 10 different irradiances ranging from 0 to 110 Wm^{-2}. The data were fitted to the following equation and the kinetic constants were determined by nonlinear regression (Dixon, 1981):

$$p=P_{max}(I-I_0)/K_I+(I-I_0) \quad (1)$$

where p is photosynthetic rate; P_{max}, the maximum photosynthetic rate; K_I, the half-saturation constant of light, or the irradance when $p=P_{max}/2$; I, irradiance; and I_0, the minimum irradiance below which no ^{14}C uptake takes place.

Nitrogen fixation was measured by acetylene reduction as described previously (Rhee and Lederman, 1982). Chlorophyll a (chl a) was extracted with 90% acetone and determined with a Turner fluorometer. The chl a standard was chl a extracted from the algae and quantified by the trichromatic equation of Strickland and Parsons (1971). Cell carbon (C) was determined by a carbon analyzer (Beckman 315B) and cell nitrogen (N) was analyzed according to Raveh and Avnimelech (1979). All measurements were made in duplicates or triplicates. Cells were counted in a Fuchs-Rosenthal Ultra Plane counting chamber in quadruplicates.

RESULTS AND DISCUSSION

Figure 1 shows the results of one of three sets of experiments. All three cultures growing on N_2, NO_3^-, and NH_4^+ followed the typical growth curve of batch cultures. The growth rate during the logarithmic phase(day 1~2) was highest in ammoium(1.88 day^{-1}) followed by nitrate (1.64 day^{-1}) and dinitrogen (1.53 day^{-1}). Similar results were obtained in nutrient-sufficient turbidostats, but the rate was lower at 1.34, 1.18, 0.95 day^{-1} for ammonium, nitrate and dinitrogen, respectively(Rhee and Lederman, 1982). The differences between the batch and the continuous culture may be due to different culture conditions; the medium for the turbidostat was buffered by a low level Tris at a pH value of 7.3±0.2 and the population density was kept below 2×10^5 cells · ml^{-1}. Variations in growth rate with different N sources were also reported for other blue-green algal species (Ward and Wetzel, 1980a).

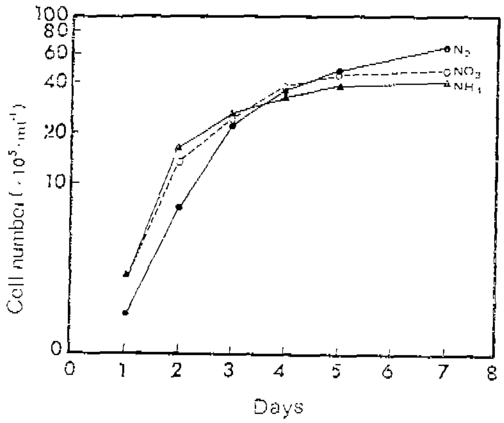

Fig. 1. The growth curve of *Anabaena flos-aquae* with NO_2^-(●), N_2(○) and NH_4^+(▲) as the N source.

Since the growth stage of each culture is different with time, it is not possible to compare the three cultures on a time scale(Fig. 1). Therefore, comparisons were made on the basis of daily average growth rate. Although the rate varies continuously with time except during the exponential phase, the average daily rate may provide a rational basis for comparison, since it reflects the physiological states of cells.

As is the case with most nutrient-sufficient batch cultures, it is not clear what initiated the decrease of growth rate to enter the stationary phase in these cultures. It was not due to N limitation, since cell N concentration did not change significantly with time; an average cell N concentration(±SE) over a 5 day period was 50.1±3.4, 45.6 ±4.9, and 51.6±5.5$\times10^{-6}$ μg-at N $cell^{-1}$ for N_2, NO_3^-, and NH_4^- cultures, respectively. Cell C/N atomic ratios ranged from 5~8, also indicating no N limitation (Redfield, 1958; Rhee, 1982). The ratio was highest for N_2-grown cells (8.1±1.9), followed by NO^-_3(7.2±0.6), and NH_4-grown cells (6.0±0.3). The ratio did not exhibit any clear change with growth rate, or culture age.

There was no significant difference in P_{max} on a per cell basis among N_2-N, NO_3-N, NH_4-N, probably except at low growth rates in the late stationary phase in which the rate was lowest for N_2-fixing cells (Fig. 2c). On a unit chl a basis, however, the

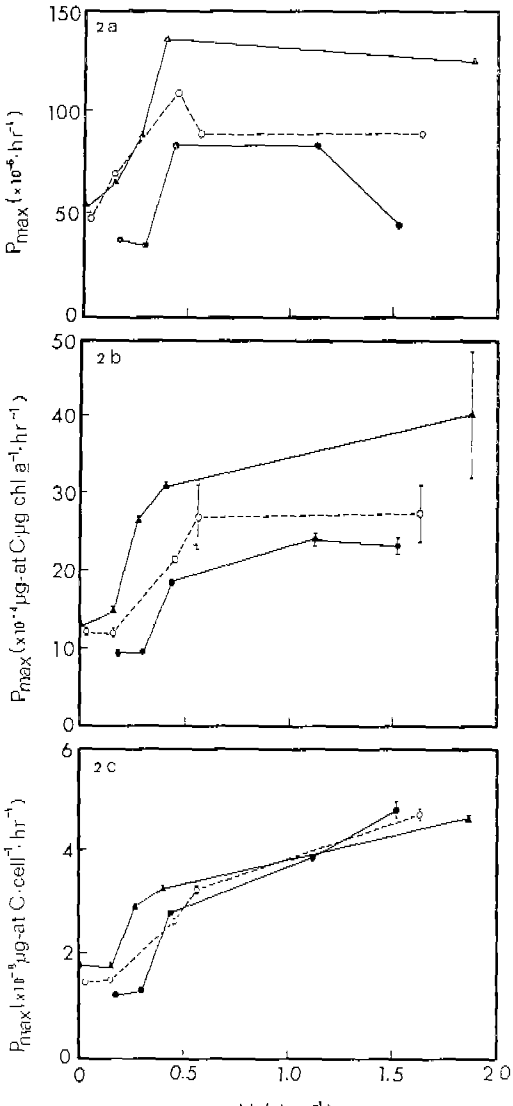

Fig. 2. The maximum pnotosynthetic rate (P_{max}) per unit cell C (2a), per chl a (2b), and per cell (2c) as a function of daily average growth rate (μ). Symbols are the same as in Fig. 1.

maximum rate was significantly less for the culture fixing N_2 than others throughout all stages of growth, although it was unclear whether there was a difference between NO_3^- and NH_4^+ (Fig. 2b). These differences in P_{max} per unit chl a reflect variations in cellular chl a; cells fixing N_2 had highest concentrations and those growing on NH_4^+ lowest. Although it is not certain why chl a content was higher in N_2-fixing cells, the higher concentration could be to maintain P_{max} per cell at a high value. On per cell C basis, P_{max} for NH_4^+ was much higher (Fig. 2a) as in *Aphanizomenon flos-aquae* (Ward and Wetzel, 1980a).

In all three cultures, P_{max} decreased with growth rate. Such decrease has also been reported with *Anacystis nidulans* and *Phormidum molle* (Daley and Brown, 1973). The cellular chl a concentration decreased in N_2 and NO_3^- cultures, but remained unchanged in NH_4^+ cultures. The declining chl a concentration is often characterized by the deterioration of cellular ultrastructure, in particular a decrease in the number of thylakoids (Daley and Brown, 1973).

The half-saturation irradiance (K_I) was significantly higher for the N_2 culture than others at least when growth rate was high, and it was higher for NH_4^+ than NO_3^- cultures (Fig. 3b). The compensation irradiance (I_0) was also higher with N_2-N than with NO_3-N or NH_4-N, but there was no significant difference between NO_3-N and NH_4-N (Fig. 3a). This difference in I_0 with N_2-N suggests that the compensation depth or the minium irradiance for photosynthesis becomes higher in summer when N/P ratios in water are low and the bloom of N_2-fixing blue-green algae occurs in many lakes. The high value of K_I also indicates that the affinity of light utilization would also decrease when N_2 is the major N source. It is known that blue-green algae can regulate their depth in response to light conditions by controlling their buoyancy primarily with gas vacuoles (Van Liere and Walsby, 1982). Therefore, the maintenance of surface blooms in many eutrophic waters may be related to these increased light requirements to maintain photosynthetic rate as suggested by Pearl and Keller(1979).

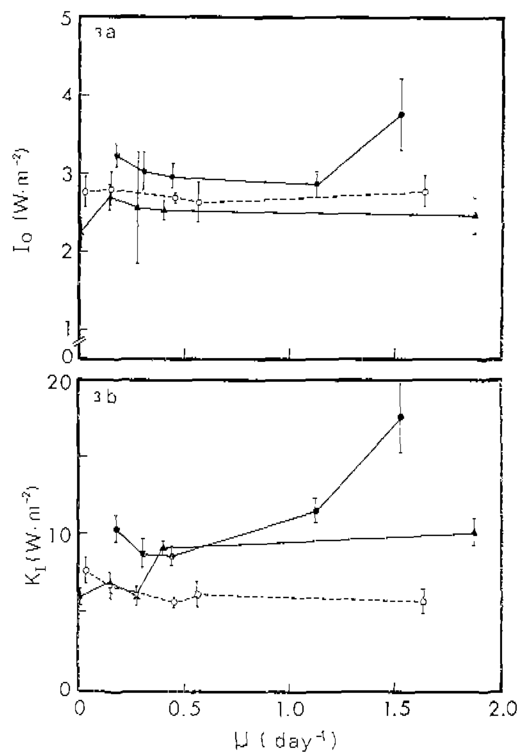

Fig. 3. The compensation irradiance, I_0(3a), and half-saturation irradiance, K_I(3b) as a function of daily average growth rate(μ). Symbols are the same as in Fig. 1.

Since ^{14}C-fixation represents net, not gross, fixation(Rhee and Gotham, 1981), the higher values of K_I and I_0 for the N_2 fixing cultures might be the results of the high energy demand for reducing nitrogen (Stanier, 1974). There is also evidence that the high values are in part due to inefficient light utilization. This is supported by the low ratio of P_{max}/K_I(Fig. 4a, b). This ratio, which is the ratio of the maximum reaction rate to the half-saturation constant in enzyme reactions at low substrate concentrations(Plowman, 1972) and thus, roughly comparable to quantum efficiency at low irradiances, was significantly lower for cells growing on N_2 than NO_3^- of NH_4^+ both on a per cell or per unit of chl a basis. It decreased in general with culture age in all cultures. The lower maximum growth rate for N_2 cultures might also be due to the same energy diversion for N_2 fixation as well as the inefficiency of light utilization. The ratio on the basis of cell number seemed to be higher with NO_3-N than NH_4-N only at a high growth rate. On a per unit chl a basis, there appeared to be little difference between them.

The light requirement for growth is different from that for photosynthesis. For this species, 15 Wm^{-2}, the light level of the present studies was saturating, since any increase above this level did not change the maximum growth rate. On the other hand, the value of(K_I+I_0) for photosynthesis ranged from 10 to 21 Wm^{-2} throughout various stages of growth(or at various growth rates), and the light saturation did not occur even above 50 Wm^{-2}. When the photosynthetic performance, the photosynthetic rate

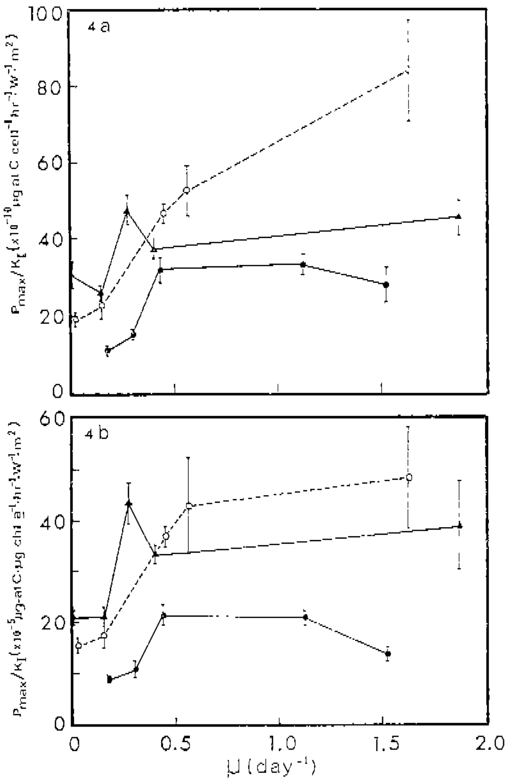

Fig. 4. The P_{max}/K_I ratio on a per cell(4a) and unit chl a (4b) basis as a function of daily average growth rate(μ). Symbols are the same as in Fig. 1.

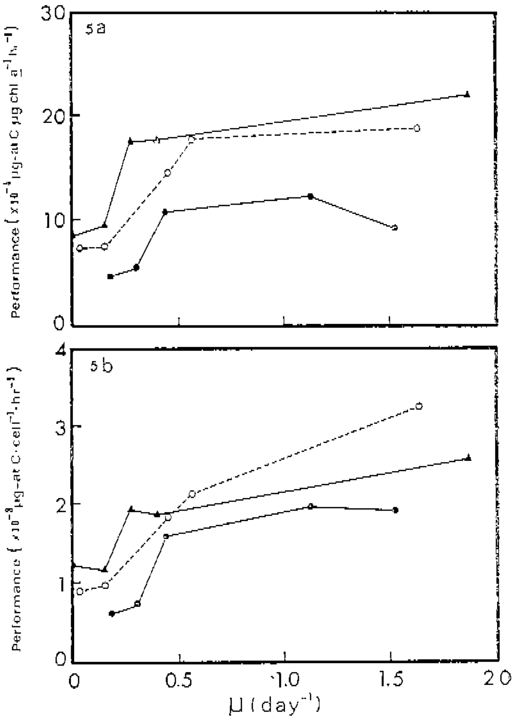

Fig. 5. Photosynthetic performance (see text) per unit chl a(5a) and per cell(5b) as a function of daily average growth rate(μ). Symbols are the same as in Fig. 1.

under growth condition(15 Wm^{-2}), was calculated by equation 1, it decreased with growth rate. Among cultures utilizing three different N sources, the performance was lowest for cells using N_2 on both per cell or unit of chl a basis (Fig. 5a, b). This is due in part to the high values of K_I and I_0 with the present data, it is difficult to draw a conclusion as to any difference between NO_3 and NH_4 cultures.

Acetylene reduction rate(per cell or cell N) decreased rapidly at the beginning of the stationary phase(Fig. 6). This sharp decrease was quite reproducible in other runs. This decrease may be due to high pO_2 to which acetylene reduction activity is very sensitive(Weare and Benemann, 1974). However, it does not necessarily mean a decrease in nitrogen fixation, because N_2 fixation is less sensitive to O_2 than acetylene reduction; the C_2H_4/N_2 ratio decreases with the increase in pO_2 to 6~8 (Ohmori and Hattori, 1979) from a theoretical conversion factor of 3. The comparison of Fig. 6

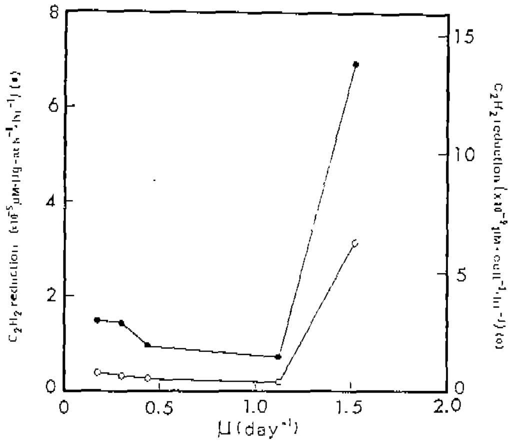

Fig. 6. Acetylene reduction rate per unit cell N(●) and per cell(○) as a function of daily average growth rate(μ).

to Fig. 2 clearly shows that there is no direct coupling between photosynthetic and acetylene reduction rates. This is not surprising since photosynthetic reserve materials can support acetylene reduction (Fogg, 1974).

摘 要

藍藻類인 *Anabaena flos-aquae*에 NH_4^+, NO_3^- 및 N_2등의 無機窒素源을 각각 공급하여 이들의 ^{14}C 固定의 kinetics를 比較 調査하였다. 生長率은 NH_4^+에서 가장 높았으며 NO_3^-, N_2 順으로 낮았다. N_2에서는 compensation intensity(I_0)와 half-saturation irradiance(K_I)가 가장 높은 반면에 炭素固定最大率(P_{max})은 낮게 分析되었고, 또한 낮은 irradiance 범위내에서는 quantum 効率과 性格이 類似한 P_{max}/K_I 値도 NH_4^+, NO_3^- 경우에 比하여 낮았다. ^{14}C 吸收率로 測定한 光合成은 純光合成率을 나타내므로 N_2가 窒素源일 때 I_0와 K_I 値가 높고 P_{max}와 P_{max}/K_I 比率이 낮은 것은 N_2를 固定하는데 많은 에너지가 所要되기 때문인 것 같고 또한 最大 生長率이 낮은 것도 이 에너지要求量과 關聯되는 것으로 보여진다.

REFERENCES

Buresh, R. J., M. E. Casselman and W. H. Patrick, Jr. 1980. Nitrogen fixation in flooded soil systems, a review. *In* Advanced in agronomy, N. C. Brady (ed.), Vol. 33. pp. 149~192. Academic Press, New York.

Daley, R. J. and S. R. Brown. 1973. Chlorophyll, nitrogen, and photosynthetic patterns during growth and senescence of two blue-green algae. *J. Phycol.* **9**: 395~401.

Dixon, W. J. 1981. Biomedical computer program P-series. University of California Press, Los Angeles.

Fogg, G. E. 1974. Nitrogen fixation. *In* Algal physiology and biochemistry, W. D. P. Stewart (ed.), pp. 560~582. University of California Press, Berkeley.

_____. 1978. Nitrogen fixation in marine environments. *Ecol. Bull.* (Stockholm) **26**: 11~19.

Guillard, R. R. L. and C. J. Lorenzen. 1972. Yellow green algae with chlorophyllide c. *J. Phycol.* **8** : 10~14.

Ohmori, M. and A. Hattori. 1979. Differential effects of oxygen on N_2 fixation and C_2H_2 reduction by *Anabaena cylindrica. Plant & Cell Physiol.* **20** : 1163~1166.

Pearl, H.W. and P.E. Keller. 1979. Nitrogen-fixing *Anabaena:* Physiological adaptations instrumental in maintaining surface blooms. *Science* **204** : 620~622.

Plowman, K. 1972. Enzyme kinetics. McGraw-Hill, New York. 171 pp.

Raveh, A. and Y. Avnimelech. 1979. Total nitrogen analysis in water, soil and plant material with persulfate oxidation. *Water Res.* **13** : 911~913.

Redfield, A. C. 1958. The biological control of chemical factors in the environment. *Amer. Sci.* **46** : 205~221.

Rhee, G-Y. 1982. Effects of environmental factors and their interactions on phytoplankton growth. *In* Advances in microbial ecology, K. C. Marshall(ed.), Vol. 6. pp. 33~71. Plenum Press, New York.

_____ and I. J. Gotham. 1981. The effect of environmental factors on phytoplankton growth: Light and the interactions of light with nitrate limitation. *Limnol. Oceanogr.* **26** : 649~659.

_____ and T. C. Lederman. 1982. Effects of nitrogen sources on P-limited growth of *Anabaena flos-aquae. J. Phycol.* (in press)

Roger, P. A. and S. A. Kulassoriya. 1980. Blue-green algae and rice. Internant. Rice Res. Inst., Los Banos, Philippines. 112 pp.

Stanier, R. Y. 1974. The relationships between nitrogen fixation and photosynthesis. *Austr. J. Expll. Biol. Med.* **51** : 3~20.

Stewart, W. D. P., P. Powell, J. K. Ladha and M. J. A. M. Sampaio. 1979. Blue-green algae (Cyanobacteria) some aspects related to their role as sources of fixed nitrogen in paddy fields. *In* Nitrogen and rice, pp. 263~286. Internat. Rice Res. Inst., Los Banos, Philippines.

Strickland, J. D. H. and T. R. Parsons. 1971. A Practical Handbook of Seawater Analysis. Fish. Res. Bd. Canada Bull. 167.

Van Liere, L. and A. E. Walsby. 1982. Interactions of cyanobacteria with light. *In* Biology of cyanobacteria, N. G. Carr and B. A. Whitton (eds.), Blackwell Pub., Oxford. 700 pp.

Ward, A. K. and R. G. Wetzel. 1980a. Interactions of light and nitrogen source among planktonic blue-green algae. *Arch. Hydrobiol.* **90** : 1~25.

_____ and _____. 1980b. Photosynthetic responses of blue-green algal populations to variable light intensities. *Arch. Hydrobiol.* **90** : 129~138.

Weare, N. M. and J. R. Benemann. 1974. Nitrogenase activity and photosynthesis in *Plectonema. J. Bacteriol.* **119** : 258~265.

Wetzel, R. G. 1975. Limnology, W. B. Saunders Co., Philadelphia. 743 pp.

Yamaguchi, M. 1979. Biological nitrogen fixation in flooded rice field. *In* Nitrogen and rice, pp. 193~206. Internat. Rice Res. Inst., Los Banos, Philippines.

(Received November 10, 1982)