Journal of Environmental Sciences Vol.17, No.6, pp.937—941, 2005

ISSN 1001—0742
CN11—2629/X

Article ID: 1001-0742(2005)06-0937-05 **CLC number**: X131.2 X522 **Document code**: A

Influence of phosphorus on *Microcystis* growth and the changes of other environmental factors

JIN Xiang-can[1,*], CHU Zhao-sheng[1], YI Wen-li[2], HU Xiao-zhen[1]

(1. Research Center for Lake Ecology and Environments, Chinese Research Academy of Environmental Sciences, Beijing 100012, China. E-mail: jinxc@craes.org.cn; 2. Department of Resources and Environments, Northwest Sci-Tech University of Agriculture and Forestry, Shanxi 712100, China)

Abstract: The growth processes of *Microcystis aeruginosa* (FACHB-41) in simulated Taihu Lake water with different phosphorus concentrations were investigated using laboratory microcosms. The algal biomass increased with the increase of phosphorus concentration when it was lower than 0.445 mg/L, while the dissolved oxygen (DO) and pH increased, dissolved inorganic nitrogen (DIN) and light intensity underwater(I) decreased. Responding to the changes of the "environmental factors", the cellular carbohydrate and its ratio to cellular protein decreased generally as phosphorus increased. However, when phosphorus concentration was higher than 1.645 mg/L, the biomass, the "environmental factors", the cellular carbohydrate and its ratio to cellular protein did not change likewise.

Since the environmental factors and the physiological and biochemical responses are important factors, the change of environmental factors and cell physiology and biochemistry induced by phosphorus may become the key factors that steer the growth and dominance of *Microcystis* under certain conditions. To sum up, phosphorus not only stimulate the growth of *Microcystis* directly by supplying nutrient element, but also has complex interactions with other "environmental factors" and play important roles in the growth processes of *Microcystis*.

Keywords: *Microcystis aeruginosa*; microcosm; phosphorus

Introduction

In recent decades, harmful blue-green algal blooms in lakes and reservoirs have been increasing in frequency, duration, geographic extent and severity worldwide due to excessive nutrient input as nitrogen and phosphorus. Concerns about blue-green algal blooms are growing because the blooms often cause significant problems, such as bad odors, water deoxygenation and the clogging of filter in water supply systems, furthermore, some algae may produce toxins that are dangerous to aquatic organisms, domestic animals and human beings.

Algal blooms often form by abnormal growth of blue-green algae. Phosphorus is generally considered as the shortest nutrient for algal growth in temperate and subtropical lakes (Kalff, 2001). The statistic results in many studies showed a linear relationship between biomass (chlorophyll-*a*) and total phosphorus (TP) in lakes of these zones where phosphorus was the primarily deficient nutrient (Kalff, 2001). Furthermore, numerous studies indicated that phosphorus concentration increase or the TN:TP ratio decline was the key influencing factor on the phytoplankton community structure (Smith, 1983; Bulgakov, 1999; Oliver, 2000).

However, the influencing mechanism of phosphorus on algal growth is complicated. During the growth processes, environmental factors such as pH value, inorganic carbon (IC) and light intensity underwater(I) are variable, and the change of these factors may influence the growth and the dominance of *Microcystis*. For example, high pH/low IC (Shapiro, 1984; 1990; 1997), low light (Dokulil, 2000) and buoyancy regulation (Reynolds, 1987) were reported to favor the dominance of the blue-green algae.

Taihu Lake is a large shallow lake (2338 km^2) in which *Microcystis* blooms occur frequently (Cai, 1994). However, the studies about the phosphorus influences on the growth process of *Microcystis* in Taihu Lake are few. Since the factors as light, wind, temperature and zooplankton, which are inconstant in natural systems, can be controlled in laboratory microcosms (Okada 1980), they are convenient for the investigation of algal growth processes. This study was to apply laboratory microcosms to study the complex impacts of phosphorus on *Microcystis* growth in the simulated Taihu Lake water, especially to study the changes of environmental factors and the cell physiological and biochemical responses during the processes.

1 Materials and methods

1.1 Materials

Since the chemical compositions of natural lake water may change with seasonal successions and keeping time, simulated lake water was used in this study. Simulated Taihu Lake water was prepared as following steps: (1) Solution containing main ions (presented in Table 1), which were the same as that in Gonghu (31°24′36.2″N; 120°14′12.8″E) in the northeast of Taihu Lake, was equilibrated with the sediments sampled from Gonghu at a solution/sediment ratio of 100:1 (V/V); (2) after equilibration for a week at 4℃, the solution (pH 8.2—8.4) was filtered (medium-speed filter paper, Fuyang Company, China); (3) $NaNO_3$ and K_2HPO_4 were added to simulate lake water of different nutrient levels.

Microcystis aeruginosa (FACHB-41) was supplied by Freshwater Algae Culture Collection, Institute Hydrobiology, Chinese Academy of Sciences. It was not bacteria free, but the bacterial biomass was low (< 2%) when it was grown in the simulated lake water.

Table 1 Concentration of main ions determined in Gonghu

Cations	Concentration, mmol/L	Anions	Concentration, mmol/L
Mg^{2+}	0.398	Cl^-	1.000
Ca^{2+}	0.926	NO_3^-	0.0727
Na^+	1.400	SO_4^{2-}	0.675
K^+	0.208	HCO_3^-	1.825

1.2 Culture experiments

Foundation item: The National Basic Research Program (973) of China (No. 2002CB412301); * Corresponding author

Microcosm system used in this study is schematically shown in Fig.1. A glass column with 50 cm high and 20 cm diameter was placed in water bath of constant temperature with white fluorescent lamps illuminating above. Sterilized air (filtered through 0.2 μm filter) was blown to the water surface to supply CO_2 for the culture system.

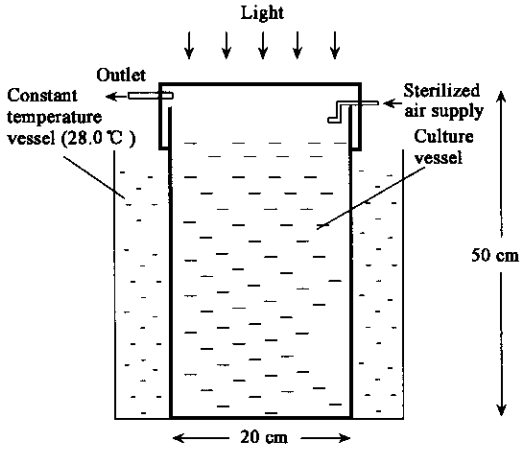

Fig.1 Schematics of microcosm system

Microcystis was grown in 11 L simulated lake water at different phosphorus concentration (0.045, 0.145, 0.445 and 1.645 mg/L) under a 12 h light:12 h dark cycle at 28℃ in the microcosms. The incident light intensity was 10000 lx. *Microcystis* was cultivated in MA medium until exponential phase before inoculated into the microcosms, and then grown in the simulated lake water for 2 d to decrease cellular phosphorus content (or cell P quota, Q_p). The inoculated population density is 5×10^4 cells/ml. The glass column with simulated lake water was autoclaved at 121℃ for 30 min and then blown with sterilized air for one day before inoculation. The algal biomass, pH, DO, total phosphorus (TP), dissolved total phosphorus (DTP) and dissolved inorganic nitrogen (DIN) were analyzed every one or two days, and the Q_p, cellular carbohydrate (C_c) and cellular protein (C_p) were determined every two or three days. Flasks were used in the experiments too. The culture conditions of flasks were the same as microcosms except for illumination and the volume. Flasks (500 ml) were illuminated around at 2000 lx and the volume of simulated lake water was 200 ml.

The biomass of *Microcystis* was determined by spectrometric method using 650 nm wavelength, and the value was converted to dry weight (dw) through Equation (1):

$$dw(g/L) = 0.352A, \quad (1)$$

dw is the dry weight of *Microcystis* and A is absorbance. pH and DO in the simulated lake water were determined potentiometrically. Phosphorous and nitrogen were analyzed by colorimetric method (USEPA, 1971). The simulated lake water was digested with acid potassium persulfate for TP determination. The algal suspension was filtered (Whatman/C) and the filtrate was digested to determine the DTP and DIN. The alga on the filter was washed three times by minus-phosphorous MA culture medium and then digested to determine Q_p. Carbohydrate was determined with the anthrone reagent employing D-glucose as standard, and protein was determined using Folin-ciocalteu method with bovine serum albumin as standard, both according to Herbert *et al*. (Hebert, 1971). Since the changing of light intensity underwater in microcosm experiments can not be investigated during the algal growth, independent experiments were carried out to investigate light changing with algal biomass.

2 Results

2.1 Biomass

As shown in Fig. 2, the biomass of *Microcystis* cultivated in simulated lake water in microcosm experiments was lower than those in flask experiments at all phosphorus concentration level, and the difference was more pronounced at higher phosphorous concentration. The growth curves also indicated that the exponential phase in microcosm experiments was shorter than those in flask experiments. Furthermore, there was a long "linear phase" before stationary phase in microcosm experiments with the slopes (k) increased with the phosphorus concentration (Table 2).

Table 2 Linear regression of growth curves of *Microcystis* in microcosm experiments at "linear phase" (7—15 d)

Phosphorus concentration, mg/L	0.045	0.145	0.445	1.645
k, g/(L·d)	8.18×10^{-4}	1.04×10^{-3}	1.16×10^{-3}	1.61×10^{-3}
R^2 ($n = 9$)	0.917	0.937	0.967	0.907

Note: k is the slope and R^2 is correlation coefficient

The biomass in microcosm experiments increased with the phosphorus concentration in the range of 0.045—0.445 mg/L. However, when the concentration attained 1.645 mg/L, the biomass did not increase any more, and there was a growth lag on the first several days although the slope of growth curves at "linear phase" was the highest one (Table 2). Unlike microcosm experiments, the algal biomass increased with phosphorus concentration in flask experiments with concentrations from 0.045 to 1.645 mg/L.

2.2 DIN, DTP and Q_p in microcosm experiments

It can be seen from the DIN curves in Fig. 3 that although the N:P ratio changed from 222 to 6.07 when the phosphorus concentration increased from 0.045 to 1.645 mg/L, nitrogen was enough for algal growth during the process in four microcosm experiments. Unlike nitrogen, phosphorus might be depleted in these experiments when the phosphorus concentration was low. The changing of Q_p and DTP (Fig.3 and Fig. 4) reflected the assimilation processes during *Microcystis* growth. *Microcystis* cells absorbed phosphorus quickly and reserved high-level phosphorus on the day 3 after inoculation, and Q_p fell down with DTP consuming (since the algal biomass was too low to determine the Q_p accurately on the former 2 d, the phosphorus reserving process is not illustrated in Fig. 4). The results also showed that the changing of Q_p lagged to that of DTP for a few days. In the microcosm experiments of 0.045 and 0.142 mg/L phosphorus, DTP was consumed quickly and Q_p reached the lowest level a few days later. It suggested that phosphorus limitation occurred in these two experiments. In the microcosm experiment of 0.445 mg/L phosphorus, DTP was not depleted until stationary phase and Q_p remained high, which indicated that phosphorus was enough though DTP was used up. In the microcosm experiment of 1.645 mg/L phosphorus, both DTP and Q_p were high until stationary

phase, which implied that phosphorus was enough for *Microcystis* growth.

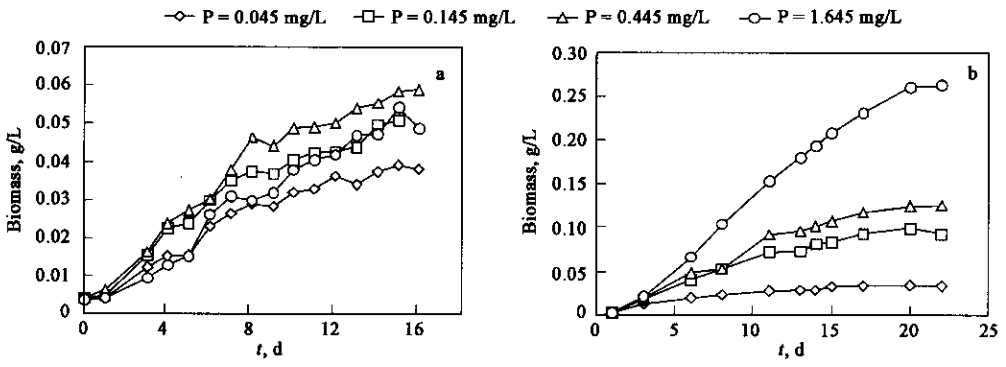

Fig.2 *Microcystis* growth in microcosm experiments(a) and flask experiments(b) with different phosphorus concentrations

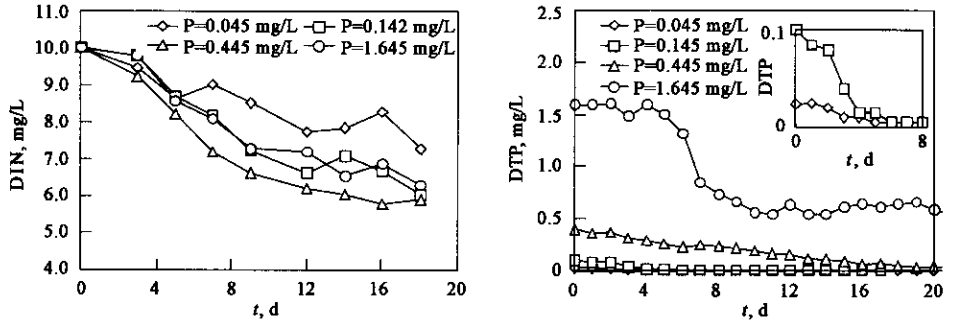

Fig.3 Dissolved inorganic nitrogen(DIN) and dissolved total phosphorus(DTP) in the simulated lake water during the growth processes in microcosm experiments

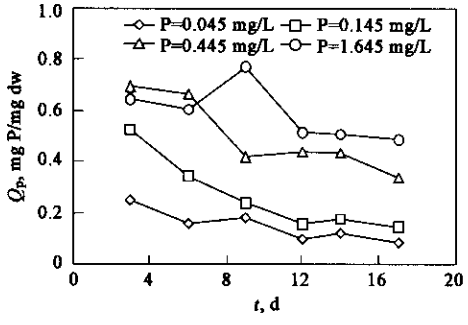

Fig.4 Cellular phosphorus content (Q_p) of *Microcystis* during the growth processes in microcosm experiments

2.3 pH and DO in microcosm experiments

As shown in Fig.5, pH and DO had "diurnal cycles" in the microcosm experiments with four different phosphorous concentrations. They began to increase after the lamp was lit and decrease when the lamp was turned off, and they fell down to its lowest value, which were close to the last of 24 h, i.e. the beginning of lighting. The changing of pH/DO (Fig.5) during the algal growth processes was slightly prior to the changing of biomass. They increased quickly when *Microcystis* started to grow and reached the maximum at the exponential phase, then began to decrease at the stationary phase.

The changes of pH/DO were strongly correlated with phosphorus concentration in microcosm experiments, and the increase of pH/DO was accelerated until phosphorus concentration reached 0.445 mg/L.

2.4 Light intensity at different depths in microcosms

The changes of light intensity at the different depths in the microcosm(the column center) versus algal density(Fig. 6) suggested that *Microcystis* was the most important substance absorbing light in microcosms, and the increase of algal biomass might result in a significant self-shading for *Microcystis* growth. It also implied that the increase of phosphorus concentration might result in a significant self-shading for *Microcystis* growth indirectly.

2.5 C_c(dry weight percentage) and the C_c/C_p ratio

Like the change of pH/DO, the change of C_c and C_c/C_p with time also showed "diurnal cycles"(Fig.7). C_c and C_c/C_p increased when the "day" came and decreased as soon as the "night" came, and they increased more remarkable in the first 2 h of lighting. During *Microcystis* growth processes, C_c increased at exponential and "linear phase" while began to decrease at the stationary phase, while C_c/C_p increased continuously during the whole growth processes(Fig.7).

The values of C_c and C_c/C_p showed a general increase trend with the increase of phosphorus concentration from 0.045 to 0.445 mg/L, and the increase trend stopped at 1.645 mg/L.

3 Discussions

The direct influence of phosphorus on algal growth can be estimated by Monod equation or Droop's equation by measuring DTP and Q_p. However, during *Microcystis* growth, the environmental factors such as pH, IC, DO, DIN and I kept on changing in microcosms, such changes would influence *Microcystis* growth greatly. Therefore, the algal growth differences in microcosm experiments result not only from the changes of DTP and Q_p, but also from the changes of environmental factors. Response to the changes of environmental factors, physiological and biochemical changes may play important roles on the growth of *Microcystis*.

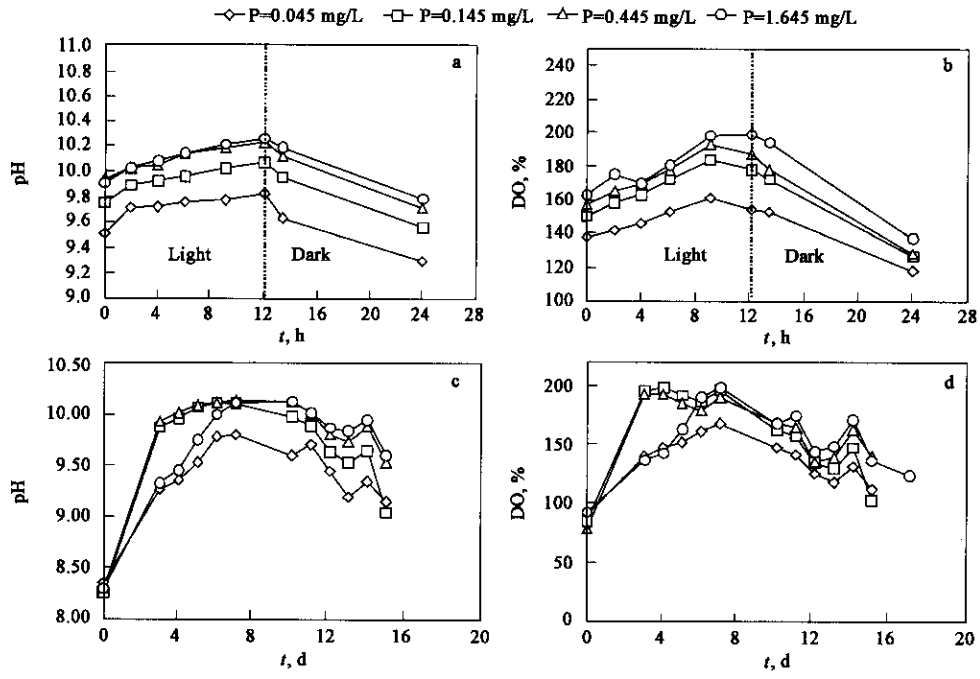

Fig.5 The diurnal change of pH and DO(on the day 10) and their change during the growth process at different phosphorus concentration in microcosm experiments

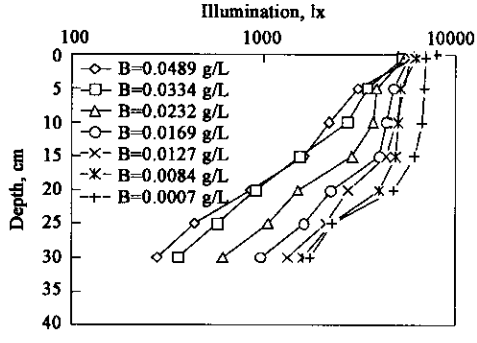

Fig. 6 Light attenuation in microcosm experiments at different algal biomass (B is biomass, dw mg)

In the microcosm experiments, pH and DO increased with the increase of phosphorus concentration from 0.045 mg/L to 0.445 mg/L whereas IC, DIN and I decreased simultaneously. Studies suggested that *Microcystis* grew well at pH from 7.0—10, and 8.0—9.0 was the best condition (Coleman, 1981). The initial pH of the simulated Taihu Lake water was about 8.3 when it was in equilibrium with air and the sediment, and it increased to about 10 during the growth processes. Therefore, the growth is suppressed with the increase of pH. The decrease of light intensity underwater has similar effect on *Microcystis* growth as pH does because the light gradients became steeper and the vertical zone appropriate for *Microcystis* growth became smaller with the

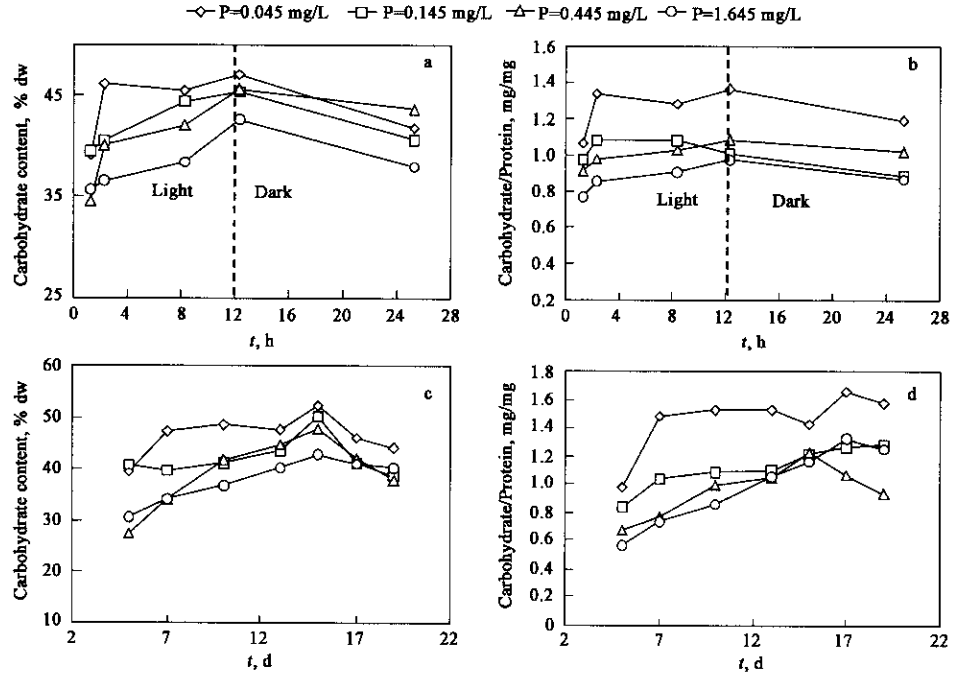

Fig.7 Diurnal change of cellular carbohydrate and cellular carbohydrate/protein(a and b; on the day 10) of *Microcystis* and their changing during the algal growth processes(c and d) in microcosm experiments at different phosphorus concentrations

biomass increasing. The nitrogen and carbon resource consumption also has inverse effect on *Microcystis* growth. Consequently, the changes of environmental factors mentioned above with increasing phosphorous concentration from 0.045 mg/L to 0.445 mg/L generally have inverse effects on *Microcystis* growth in microcosm experiments.

Once the phosphorus concentration was higher than 0.445 mg/L, the biomass in the microcosm experiments did not increase any more with the increase of phosphorus concentrations. However, the biomass in the flask experiment kept on increasing when the phosphorus concentrations were ranging from 0.045 to 1.645 mg/L. It is likely that the key factor of controlling algal growth has changed from phosphorus concentration to the "environmental factors" mentioned above. Because the simulated lake water in flask and microcosm experiments had the same composition, the most probable primary limited "environmental factor" in microcosms at high phosphorus concentration is light. Due to the "depth" of simulated lake water in microcosms(35 cm) is much greater than those in flasks(less than 2 cm) and light attenuation exponentially with depth, *Microcystis* will induce much more significant self-shading for algal growth in microcosms than in flasks. Furthermore, as the microcosm experiments have smaller surface/volume ratio for carbon dioxide exchanging, pH may play more important role in microcosm than in flask experiments.

Paradoxically, high pH, low IC and I in microcosms of higher phosphorus concentration simulated water have inverse effect on *Microcystis* growth, and they may favor the dominance of *Microcystis*. As blue-green algae usually have the advantageous adaptation to high pH, high affinity for carbon dioxide(Shapiro, 1984; 1990; 1997) and low light energy requirement (Zevenboom, 1980), the changed environmental factors, i.e., high pH, low IC and I may also have more significantly inverse effects on the eukaryotic algal growth than on the blue-green algal growth.

Responding to the changed environmental factors, buoyancy regulation may favor cell buoyancy and make *Microcystis* a superior competitor for light. Carbohydrate is the most important cell ballast for *Microcystis* as it has high density (1550 kg/m^3), and its content can be varied significantly within one day. Diurnal buoyancy regulation of *Microcystis* is predominantly controlled by the changes of stored carbohydrate (Kromkamp, 1984; Thomas, 1986). Wallace's studies(Wallace, 1999; 2000) suggested that the carbohydrate change was influenced by light intensity, exposure time, response time, light history and so on. The values C_c and C_c/C_p decreased with the increase of phosphorus concentrations, which might resulte from low carbohydrate synthesis(normalized to biomass) caused by low light intensity, high pH and low IC in high phosphorus concentrations. Furthermore, the negative ballast, gas vesicle of the blue-green algae may also be influenced by environmental conditions. Gas vesicle synthesis may also be stimulated by low light intensity(reviewed by Walsby, 1994) and thus favors cell buoyancy. Contrary to the effect of environmental condition variation on *Microcystis* growth, physiological and biochemical responses of buoyancy regulation seems to favor algal growth.

The main hypotheses that cause the dominance of blue-green algae in lakes are high temperature, low N:P ratio, low light, buoyant regulation, high pH/low IC and colony forming to avoid preying. Sharpiro (Sharpiro, 1990) suggested that each hypothesis above just fit to some instances at some time and at some locations. It can be concluded from this study that low N:P ratio, low light, buoyant regulation, and high pH/low IC could occur at the same time in the simulated lake water of high phosphorus level, and phosphorus has complex interactions with the "environmental factors" in microcosm experiments. Efforts to prevent water bloom formation in eutrophic lakes should consider not only the phosphorous concentration, but also the complex interactions between phosphorus and environmental factors.

References:

Bulgakov N G, Levich A P, 1999. The nitrogen: phosphorus ratio as a factor regulating phytoplankton community structure[J]. Archiv Fur Hydrobiologie, 146(1): 3—22.

Coleman J R, Colman B, 1981. Inorganic carbon accumulation and photosynthesis in a blue-green Alga as a function of external pH[J]. Plant Physiology, 67: 912—917.

Cai Q, Gao X, Chen Y *et al*., 1994. Dynamic variations of water quality in Lake Tai Hu and multivariate analysis of its influential factors [C]. In: Environmental Protection and Lake Ecosystem(Sund, H. H., Stabel H., Geller W., *et al*. ed.). Proc. Int. Symp. Wuxi March 27-April 1, 1993, Nanjing: China Science and Technology Press. 217—230.

Dokulil M T, Teubner K, 2000. Cyanobacterial dominance in lakes [J]. Hydrobiologia, 438: 1—12.

USEPA, 1971. No. 600/4—79—020, method 0365.2, 0351.2 [S]. US Environmental Protection Agency

Hebert D P J, Phipps P J, Strange R W, 1971. Chemical analysis of microbial cells[M]. In: Method in microbiology(Norris J.R., Ribbons D.W. ed.). V. 5B, Academic Press. 209—344.

Kalff J, 2001. Limnology[M]. N J: Prentice Hall, Upper Saddle River. 309—348.

Kromkamp J, Mur L R, 1984. Buoyant density changes in the cyanobacterium *Microcystis aeruginosadue* to changes in the cellular carbohydrate content[J]. FEMS Microbiology Letter, 25: 105—109.

Oliver R L, Ganf G G, 2000. Freshwater blooms[M]. In: The ecology of cyanobacteria(Whitten B.A., Potts M. ed.). Netherlands: Kluwer Academic Publishers. 149—194.

Okada M, Sudo R, 1980. Microcosm system study of algal bloom formation[J]. Journal WPCF, 52: 1029—1033.

Reynolds C S, Oliver R L, Walsby A E, 1987. Cyanobacterial dominance: the role of buoyancy regulation in dynamic lake environments[J]. New Zealand J mar Freshwat Res, 21: 379—390.

Smith V H, 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton[J]. Science, 221: 669—671.

Shapiro J, 1984. Blue-green dominance in lakes: the role and management significance of pH and CO_2[J]. Int Rev Ges Hydrobiol, 69: 765—780.

Shapiro J, 1990. Current beliefs regarding dominance of bluegreens: the case for the importance of CO_2 and pH[J]. Verh Int Ver Limnol, 24: 38—54.

Shapiro J, 1997. The role of carbon dioxide in the initiation and maintenance of blue-green dominance in lakes[J]. Freshwater Biology, 37: 307—323.

Thomas R H, Walsby A E, 1986. Buoyancy regulation in a strain of *Microcystis* [J]. J Gen Microbiol, 131: 799—809.

Wallace B B, Hamilton D P, 1999. The effect of variations in irradiance on buoyancy regulation in *Microcystis aeruginosa* [J]. Limnology and Oceanography, 44(2): 273—281.

Wallace B B, Hamilton D P, 2000. Simulation of water-bloom formation in the cyanobacterium *Microcystis aeruginosa* [J]. Journal of Plankton Research, 22: 1127—1138.

Walsby A E, 1994. Gas vesicles[J]. Microbiological Reviews, 58: 94—144.

Zevenboom W, Mur L R, 1980. N_2-fixing cyanobacteria: why they do not become dominant in Dutch hypertrophic lakes [M]. In: Hypertrophic ecosystems (Barica J., Mur L. R. ed.). Junk: The Hague. 123—130.

(Received for review March 8, 2005. Accepted May 18, 2005)