BIOMEDICAL AND ENVIRONMENTAL SCIENCES **19**, 399-404 (2006)

Effects of Iron and Phosphorus on *Microcystis* Physiological Reactions

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Objective To observe the effects of iron and phosphorus on *Microcystis* physiological reactions. **Methods** The experimental conditions were chosen as the light dark cycles of 16 h 8 h, 12 h 12 h, and 8 h 16 h. The cell change of morphology and life history, cell number, cell color, and cell area of *Microcystis* were analyzed quantitatively. According to the resource competition and Monod equation, *Microcystis* kinetics of phosphorus and iron were also examined. **Results** The longer light time caused more special cell division, slower growth rate, and easier change of bigger cell area. The color of alga was changed from green to brown. K_s and μ_{max} of phosphorus absorption were 0.0352 μmol·$L^{-1}$and 0.493 d^{-1}, respectively. Those of iron absorption were 0.00323 μmol·$L^{-1}$and 0.483 d^{-1}. **Conclusion** *Microcystis* bloom is more dominant than other algae.

Key words: *Microcystis*; Morphology; Light: Dark; Dynamics; Iron; Phosphorus

INTRODUCTION

Cyanobacteria are probably the best-studied group of phytoplanktonic microorganisms, because of their success and ubiquity in freshwater systems[1]. For example, *Microcystis* blooms often occur in summer in eutrophic temperate lakes and cause various problems, such as reduced transparency, decreased biodiversity, potential occurrence of oxygen depletion, odor and taste compounds, as well as production of toxins hazardous to animals and humans[2-3].

Besides the morphology of lakes, elevated temperatures, low light-energy requirements of *Microcystis* as the steering factor for bloom formation, light dark cycles, might be also of decisive importance for *Microcystis* dominance. A single factor is rarely responsible for the appearance of the species, and the reasons for such outbreaks remain largely unclear though considerable researches have been undertaken.

Among various cyanobacteria, Nostocalean blue-green algae such as Anabaena, Aphanizomenon, and Gloeotrichia form akinetes acting as seeds for the next generation[4-5], can survive on sediments of water bodies in the whole winter. Although the toxic blue-green alga *Microcystis* does not produce any specialized dormant cells like akinetes under adverse growth conditions, it was reported that there is the next water bloom in an area[6]. Some observations lead us to hypothesize that intensive growth of *Microcystis* greatly contributes to the formation of *Microcystis* bloom. In contrast, others considered that water bloom forms in the overwintering benthic populations[7]. Consequently, the importance of recruitment for the initiation of *Microcystis* bloom is still unclear.

In the present study, the characteristics of *Microcystis* cells and special reproductive style were observed under the different light dark cycles. The growth dynamics of *Microcystis* were measured with the concentration gradients of phosphorus and iron based on Tilman's mechanistic theory of resource competition[8]. The aims were to get some evidence of the *Microcystis* dominance forming blooms in some conditions, to deepen our understanding of the ecology and resource competition energy of the species, and to cut the life cycle or control the nutrient resource as a potential management strategy for the prevention of the next *Microcystis* bloom.

MATERIALS AND METHODS

Strains and Culture Conditions

Algal strain used was *Microcystis aeruginosa* which was kindly provided by FACHB in Institute of

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0895-3988/2006
CN 11-2816/Q

Hydrobiology. The cells were grown axenically in BG-11 growth medium at 26℃. Illumination was provided by cool-white fluorescent light at 60 μmol photons $m^{-2}·s^{-1}$ on the water surface. Then the alga was put into 1000 mL flasks containing 400 mL medium, the initial algal density was about 10^5 cells·mL^{-1}.

Total Cell Number and Cell Morphology Analyses Method

Samples were taken at indicated times, and the growth parameters were measured immediately. The population of *Microcystis aeruginosa* cells was counted and the varieties of cell division were investigated under a compound microscope (Nikon 400) using a hemocytometer after stained with Lugol's iodine solution. The chlorophyll-a content was calculated as previously described[9]. Samples were filtered through GF/C filter paper and the chlorophyll-a was extracted using ethanol (95%). The optical densities of the extracts at 665 and 750 nm were determined using a spectrophotometer. The cell area was photographed and analyzed with software Image-Pro Plus (Media Cybernetics Inc).

Experimental Designs

The groups were placed on three light dark cycles, respectively as the experimental design I (16 hours 8 hours, 12 hours 12 hours, and 8 hours 16 hours). The experimental design Ⅱ is shown in Table 1. The compositions of different P and Fe concentration media, except for the components of P and Fe, were the same as those of BG-11 medium. The nutrients P and Fe were placed as the concentration gradients (Table 1).

TABLE 1

Experimental Design Ⅱ

Nutrient Element	Group 1	Group 2	Group 3	Group 4	Group 5
Fe (mol/L)	No	1.23×10^{-8}	4.305×10^{-8}	8.61×10^{-8}	1.23×10^{-7}
P (mol/L)	No	1.75×10^{-7}	6.125×10^{-7}	1.225×10^{-6}	1.75×10^{-6}

In the experimental design I, the cell density and cell area were measured and the reproductive style was investigated. Growth dynamics of *Microcystis aeruginosa* competition for phosphorus and iron, half-saturated constants and the maximal specific growth rate, were calculated in design Ⅱ. Then its growth trends could be predicted by the modification of Monod equation in some concentration ranges of P and Fe.

Dynamic Model

Under steady state conditions, we used the equation of Monod model

$$\mu= (\mu_{max}\cdot C_s)/(K_s + C_s) \rightarrow C_s/\mu= K_s/\mu_{max} + C_s/\mu_{max} \quad (1)$$

to calculate the growth kinetics for the micro-alga. The specific growth rate, reported here as the percentage increase in biomass per day, was calculated by the equation

$$\mu(d^{-1})= (\ln N_t - \ln N_0) / t \quad (2)$$

Data were fitted to the treated Monod model.

Tilman has provided the R-rule hypothesis as fundamental of resource competition theory in 1982[8]. A single species could deplete the external concentration of the limiting nutrient to the corresponding level at a given equilibrium turnover rate (reproduction rate= loss rate in chemostat culture given by the dilution rate):

$$R= D\cdot K_s/ (\mu_{max} - D) \quad (3)$$

μ: the per capita reproduction rate, d^{-1}; μ_{max}: the maximal growth rate, d^{-1}; K_s: the half-saturation constant, μmol/L; C_s: the concentration of the limiting nutrient, μmol/L; N_0: the initial cell number; N_t: the cell number on the day t; R: the residual equilibrium nutrient concentration for a species, μmol/L; D: the dilution rate for a species.

RESULTS

Under the laboratory conditions, *Microcystis* was grown under the white light fluorescent lamps, and intensity was chosen so as to keep an optimal and stable growth. But the light dark cycles were treated as 16 h : 8 h, 12 h : 12 h, and 8 h : 16 h. In the three cycles the number of algal cells was different as shown in Fig. 1. Groups 1 and 2 revealed the *Microcystis* growth in the complete nutrient media, in which the population of *Microcystis* under the light dark cycles of 16 h : 8 h and 12 h : 12 h was more than that under the light dark cycle of 8 h : 16 h during the former ten days, but it did not hold on during the following days and the *Microcystis* in the cycle of 8 h : 16 h increased the growth rate quicker than the other two cultures. Moreover, the trends of chlorophyll-a content change in different light dark cycles were almost similar to those of total cell number change (Fig. 2). The macroscopic observation

also revealed that the culture color varied with the chlorophyll-a content changes. The longer the light dark cycles, the easier the changes of alga from green to brown in the cultures. With the extension of culture time, the average cell area in the culture of 16 h : 8 h cycle became the largest during the three light dark cycles, while in the 12 h : 12 h cycle culture it took second place on the 20th day. Although the total cell number in the 8 h : 16 h cycle increased, the cell area changed least up to the 20th culture day (Figs. 1 and 3).

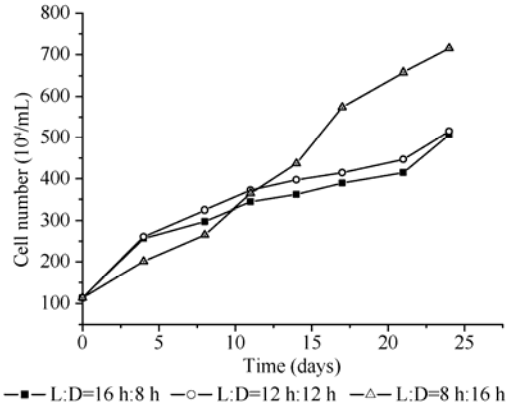

FIG. 1. Total cell number curves in three light dark cycles.

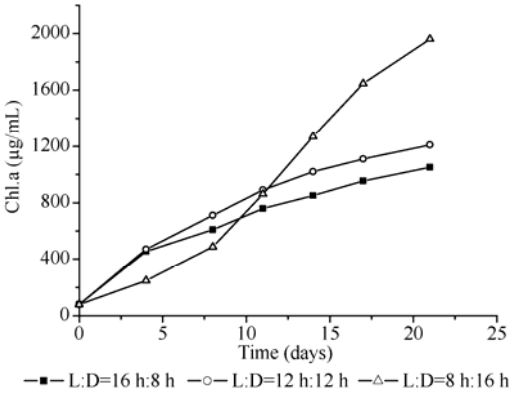

FIG. 2. Chlorophyll-a contents in three light dark cycles.

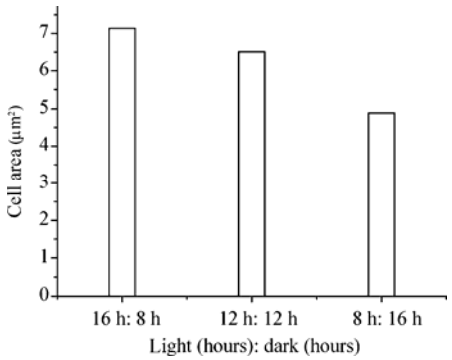

FIG. 3. Cell areas in three light dark cycles on the 20th day.

The distinct phenomena induced by the light dark cycle, such as special cell divisions, also appeared in these cultures. The varieties of cell division were investigated in the three light dark cycles. Normal *Microcystis* cells increased their size and then divided into the next generation (Figs. 4 a-c). In Figs. 4 d-g, there were cell divisions of *Microcystis* in the light dark cycles of 16 h for 8 h and 12 h for 12 h, which were extremely different from Fig. 4 b.

There were three kinds of possibly special division. The first was that the materials in the cell membrane concentrated into one point that came out of the membrane (Figs. 4-e, i) and then grew into vegetative cells. The second (Figs. 4-f, Fig. 4-g) was that two points were respectively formed in a dividing cell including two connecting bodies. After the two bodies separated from each other, the point acted as the first type. If not, the point departed from the old membrane before the cell divided completely. We called it as the third (Fig. 4-h). In Fig. 5, the results were the cell number of special division production and common cell number in the three light dark cycles. The longer the light time the more the special cell division. During the former ten culture days the special cell division in the 8 h for 16 h cycle medium was more than that in other cultures. But the privilege gradually disappeared during the later culture period. The special cell division in the 12 h for 12 h cycle medium was always less than that in the 16 h for 8 h cycle culture.

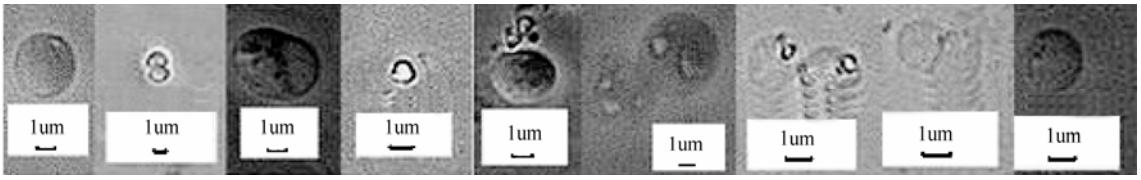

FIG. 4. Diagram of the life cycle of *Microcystis* (on the microscope): from the left to the right is Figs. 4-a, b, c, d, e, f, g, h, and i respectively. Figs. 4-a, c: normal cells. Fig. 4-b: normal cell division. Fig. 4-d: special division production "the point". Fig. 4-e: dividing cell with one special cell. Figs. 4-f, g: dividing cell with two special points respectively. Figs. 4-h, i: empty membrane without the point.

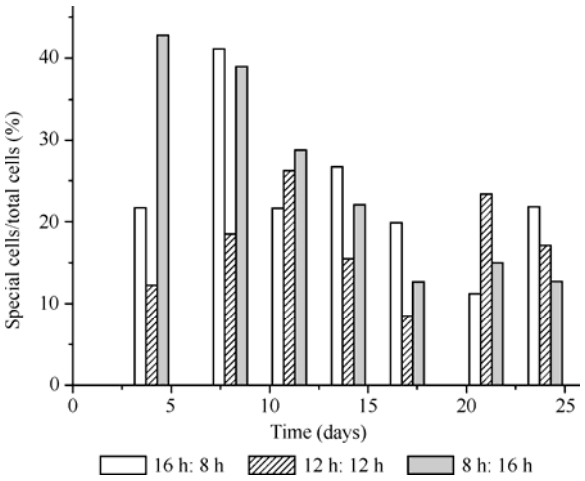

FIG. 5. Proportion of special cells and total cells in three light dark cycles.

The experiments of growth dynamics for phosphorus and iron were made sequentially. Fig. 6 shows the results of the single-species Monod growth experiments for *Microcystis* under P and Fe per limitation respectively. The estimated maximal per capita growth rate of *Microcystis* on the P limitation respectirely. The estimated maximal limitation medium was appreciably greater than that under Fe limitation condition, being 0.493 d^{-1} and 0.483 d^{-1} respectively. The phosphorus and iron half-saturation constants for *Microcystis* were 0.0352 μmol/L and 0.00323 μmol/L, being significantly different. Although the initial algal population densities of *Microcystis* might affect the growth rate and certain nutrient half-saturation constants, there was no experiment investigating its impact. Comparison of the parameters indicated that the result of competition was affected by the incubation conditions including the limited-nutrient concentration range and the physical environment, independent of the initial algal population densities.

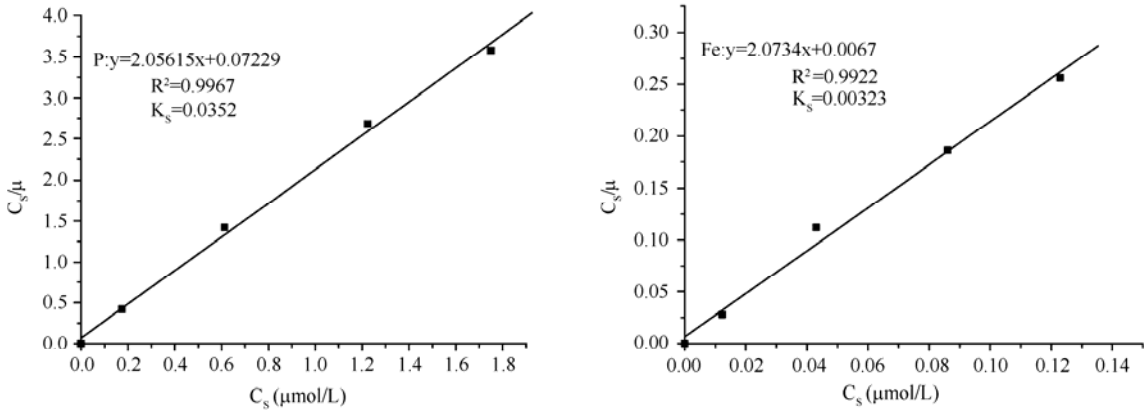

FIG. 6. *Microcystis* Monod growth kinetic experiments performed under conditions of limiting phosphorus and iron element respectively.

DISCUSSION

The present study showed that the effects of light dark cycles on the alga *Microcystis* cell number and the chlorophyll-a content during the earlier culture stage were 8 h for 16 h >12 h for 12 h >16 h for 8 h, but during the later stage were 16 h for 8 h >12 h for 12 h >8 h for 16 h. The results indicate that the longer the light time is, the easier the change of alga from green to brown is. The effects on the cell area on the 20th culture day were 16 h for 8 h >12 h for 12 h >8 h for 16 h. The light dark cycle imitating the natural change of daytime night time cycle, did not alter the light quality or abandon light energy. Synchronous growth and cell division of algae can be achieved by alternating light and dark cycles[10]. But all the results emphasize that longer light time does not accelerate the growth efficiently. Light controls the life of alga. In natural habitats, algal photosynthesis is performed with the aid of adaptive mechanisms under a characteristic light gradient. Many workers have found various adaptive strategies against the biased light regime. The short light dark cycle maintains and promotes the *Microcystis* living, which maybe a reason for its easy dominance in lakes or ponds and overwintering, though it does not produce any specialized dormant cells to put up with adverse growth conditions. Accordingly, cell structure and

components may be influenced. Enzyme activity can be a good indicator of stress due to the lack of phosphorus and iron element. Further works, such as the mechanism of composition and function alterations caused by phosphorus or iron deficiency will be carried out in our laboratory.

Exposed to differently treated cultures, the biochemical morphological difference of *Microcystis* will be brought out but the nature of the transponder converting mechanical stimulus into biochemical activity is unknown. *Microcystis* is highly adaptable prokaryotes. In general, *Microcystis* vegetative cells possess typical vegetative cycles, circular shape and normal cyanobacteria cell components, such as only one form of chlorophyll, characteristic biliprotein and pigment phycobilins. However, the life cycle of *Microcystis* does not act as seeds for the next generation like other algae. For example, Anabaena among various cyanobacteria forms a filamentous body called a trichome, consisting most of vegetative cells and differentiating into heterocycts and akinetes according to their environmental conditions[11]. Researches also indicate that *Microcystis* cells do not produce any specialized dormant cells like akinetes when environmental conditions become unfavorable. In our studies, the results were strengthened and the special phenomena not reported were observed.

The per capita reproduction rate (μ) of algal population is related to the ambient concentration of the limiting nutrient (C_s) according to the Monod equation that has become one of the most influential and widespread models to describe the growth behavior of nutrient-limited cultures under steady state or under non-steady state-conditions[12]. If several species are competing for a nutrient and if their loss rates are known, the outcome of competition can be predicted by calculating R for each species, and the species with the lowest R will win the competition. There are extensive tests with microalgae competing in laboratory microcosms near equilibrium[13], higher plants[14] and zooplankton communities[15]. The effects of dilution rates and concentrations of phosphorus[16] and ammonium[17] on the competition in a chemostat between *Microcystis* and other algal organisms have also been tested. However, no such studies are available for comparison using the concentrations of iron so far.

With the stated D resource competition theory and the Monod equation predicion the species with higher μ_{max}, lower K_s and R should be dominant[18]. If the two species have almost the same R calculated from K_s and μ_{max}, they should therefore coexist under nutrient-limited conditions. There are K_s and μ_{max} of P being 0.412 μmol/L and 1.098 d^{-1} respectively in the phosphorus concentration range of 0.136-16.950 μmol/L[16], and 0.548 μmol/L and 1.143 d^{-1} in the range of 1.613-11.613 μmol/L[19]. However, the present study showed that the two parameters of 0.0352 μmol/L and 0.493 d^{-1} of P were much lower than the above outcomes. K_s and μ_{max} of *Microcystis* were 0.00323 μmol/L and 0.483 d^{-1}, being lower than most of other algae, suggesting that *Microcystis* Monod kinetics of P and Fe in the experiments further approves its competitive privilege in the natural environment, especially in the low concentration range. With the known data about the physical condition, the Monod model may be the best predictor for the competition between *Microcystis* and other microorganisms.

In conclusion, the light dark cycle of 16 h : 8 h causes more special cell division, easier change of bigger cell area, easier color changes of alga from green to brown, shorter cell life cycle, slower growth rate than the light dark cycles of 12 h : 12 h and 8 h : 16 h. Combined with the lower *Microcystis* Monod kinetics of P and Fe, *Microcystis* further displays its competitive dominance in lakes and ponds.

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(*Received June* 3, 2004 *Accepted July* 24, 2005)