Effects of Iron on Growth and Intracellular Chemical Contents of *Microcystis aeruginosa*[1]

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Objective To investigate the effect of iron on the growth, physiology and photosynthesis of cyanobacteria. **Methods** A gradient of iron concentrations was employed to investigate the growth, photo-pigments (chlorophyll A and phycocyanin), and cell chemical contents (C, N, P) of *Microcystis aeruginosa* in response to different iron additions. **Results** The specific growth rate during the exponential growth phase, as well as the cell chlorophyll A and the phycocyanin content, was limited by iron below 12.3 μmol $Fe{\cdot}L^{-1}$. The growth was inhibited when the iron concentration was at 24.6 μmol $Fe{\cdot}L^{-1}$. The cell chlorophyll A and the phycocyanin content were saturated when the iron concentration was above 12.3 μmol $Fe{\cdot}L^{-1}$ and declined slightly at 24.6 μmol $Fe{\cdot}L^{-1}$. At a low iron concentration (about 6.15 μmol $Fe{\cdot}L^{-1}$ and less), the cell nitrogen and carbohydrate content were iron limited, and the variation of the cell phosphorus content was similar to that of the nitrogen and carbohydrate, with a transition point of 12.3 μmol $Fe{\cdot}L^{-1}$. **Conclusion** The variation of cynobacteria growth is synchronous with that of the photo-pigments or the cell chemical content, and there exist relationships among photosynthesis, growth and internal chemical content, which could be useful for the growth estimation from the cell characteristics.

Key words: Iron; Growth; Photo-pigments; Carbohydrate; Phosphorus; Nitrogen

INTRODUCTION

Virtually all organisms except lactobacilli have an absolute requirement for iron, and since iron-containing compounds are ubiquitous in the cell, it has been suggested that the reactions catalyzed by iron may have constituted the first step in the origin of life[1-2]. Iron is essential for cell growth, for many iron-containing proteins catalyze key reactions involved in photosynthesis, respiration, nitrogen (specifically nitrate and nitrite) assimilation, nitrogen fixation (in the case of cyanobacteria), photo-pigment synthesis, DNA synthesis, and a number of other biosynthetic or degrading reactions[3-5]. Because of its importance to phytoplankton growth, iron can play the role as a limiting nutrient in some waters.

The potential importance of iron as a limiting factor for primary production in the sea has long been emphasized[4]. Enrichment experiments in bottles and in mesoscale patches of surface water have shown that iron limits algal growth in major regions of the ocean[6-8]. Even though the concentrations of iron in limnetic systems are often much higher than those in the oceans[9], iron limitation of phytoplankton or the stimulation by iron enrichment in lakes has been suggested for a long time[4-5, 10-11].

On the other hand, cyanobacterial blooms are threatening many aquatic ecosystems, including the lakes such as the Taihu[12] Lake, the Chaohu Lake, and the Dianchi Lake in China. Lake eutrophication has become a serious environmental problem in this country. Recent data have shown that 57.5% of the 40 surveyed lakes are in eutrophic or hypertrophic status[13].

Ferric sulphate is used as a phosphate stripper in eutrophic water-bodies to reduce phosphorus concentration, for phosphorus is often the primary factor for lake eutrophication. It is also likely that in some sewage treatment plants more iron is used than is required for optimal phosphorus removal[14]. Thus, the iron concentrations in freshwaters could be increased in these ways. The iron concentrations in the waters of the Taihu Lake, the Chaohu Lake, and the Dianchi

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Lake vary from 0.134 to 35 μmol L^{-1}[15-17].

Because of the importance of iron on phytoplankton growth and success, it may play a critical role in cyanobacterial blooms. The aim of the present study was to investigate the effects of iron on the growth, physiology, and photosynthesis of cyanobacteria. A gradient of iron concentrations was employed; the response of the cell of *Microcystis aeruginosa* to different iron additions was investigated. Therefore, this research is relevant to a better understanding of the potential effects of iron loading on cyanobacterial blooms.

MATERIALS AND METHODS

The bloom-forming *Microcystis aeruginosa* (FACHB 912), provided by Freshwater Algae Culture Collection, Institute of Hydrobiology, Chinese Academy of Sciences, was used for the experiments. *M. aeruginosa* is commonly considered as a bloom-forming cyanobacterium. The culture used in this study (FACHB 912) was isolated from Lake Taihu; it was grown in BG-11 medium at a constant temperature of 25±2 ℃ and an irradiance of 25 μmol m^{-2} s^{-1} with a 14:10 light: dark cycle, without aeration but with daily stirring.

The uni-algal *M. aeruginosa* culture was grown to an exponential phase in the complete BG-11 medium. Prior to inoculation, the culture was centrifugated. The supernatant was dropped, and the cells were washed with the iron-free BG-11 medium for three times, then resuspended with the iron-free medium. The resuspended cells were inoculated at an initial cell density of ~1×10^5 cells mL^{-1} into capped sterilized Erlenmeyer Flasks containing the BG-11 medium of different iron concentrations. Four iron concentrations of 3.07, 6.15, 12.3, and 24.6 μmol Fe·L^{-1} in the medium and a control (without iron) were employed. The concentration of iron in the medium was adjusted by adding different amounts of ammonium ferric citrate. The cultures were illuminated with cool fluorescent white lamps (CDMTP150W942, Philips). Experiments were carried out at the temperature of 25±2 ℃ and the irradiance of 25 μmol photons m^{-2} s^{-1} with a 14:10 light: dark cycle, and the cultures were stirred four times each day.

Growth of *M. aeruginosa* was determined by taking 0.1 mL culture samples from the different treatments on the third day, and every day thereafter. The cells were counted twice by using a blood-cell counting chamber, and the relative errors were below 0.10. The specific growth rate (μ/d^{-1}) during the exponential growth phase was calculated by Eq. (1):

$$\mu = (\ln(N_t) - \ln(N_0)) / t \quad (1)$$

where N_0 is the initial cell density (cells mL^{-1}), and N_t is the cell density (cells mL^{-1}) after incubation for t days.

For the determination of cell chemical content, cyanobacterial cells were filtered through 0.45 μm pore size cellulose-acetate membrane filters, and then washed with BG-11 medium. It should be noted that they were washed with P free medium for P content determination, while washed with N free medium for N content determination. The P content was measured as the total P by the molybdenum blue method after digestion by H_2SO_4 and $K_2S_2O_8$ (121 ℃, 30 min), and the N content was measured as total N by ultraviolet spectrophotometric screening method after digestion by $K_2S_2O_8$ and NaOH (121 ℃, 30 min)[18]. The carbohydrate was spectrophotometrically determined at 620 nm by Anthrone Reagent[19]. The cell P, N, and carbohydrate content were calculated by subtracting the content in the filtrate from that in the culture solution.

Chlorophyll A was measured spectrophotometrically after the extraction with acetone (90% V/V) at 4 ℃ in the dark for 24 hours, as required by the USEPA standard method[20].

The *in vivo* fluorescence intensity at 25 ℃ was measured using a fluorospectrophotometer (RF-5301PC, Shimadzu, Japan) with 10 nm excitation and emission slits. The fluorescence ($F_{620\text{-}645}$) was excited using a wavelength of 620 nm and its emission was detected at 645 nm.

RESULTS

Effects of Iron on Growth

Fig. 1 shows the yield of *M. aeruginosa* at five iron concentrations over time. There was no obvious difference in biomass yield between the control without iron and the treatment group with 3.07, 6.15, or 12.3 μmol Fe·L^{-1} in the first 6 culture days (Paired-Samples T test, P>0.05). The yields varied in the following 6 days, and it was greatest at 3.07 μmol Fe·L^{-1}. At this concentration, the yield was peaked on the 11th culture day and then decreased. Since the culture was grown in the complete BG-11 medium with 12.3 μmol Fe·L^{-1} before inoculation, the intracellular storage of Fe could support their demand in the first 6 days. However, the yield of the treatment with 24.6 μmol Fe·L^{-1} was below the control from the initial day (Paired-Samples T test, P=0.049<0.05). It indicated that the growth was obviously inhibited at the concentration of 24.6 μmol Fe·L^{-1}, which was similar to the results of Lv *et al.*[21] that inhibition was observed at the concentration of 30 μmol Fe·L^{-1}.

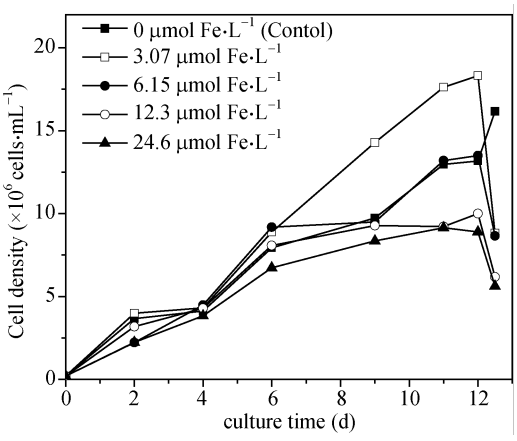

FIG. 1. Growth curves of *microcystis aeruginosa* cultures at different iron concentrations.

The effect of iron on the specific growth rate of *M. aeruginosa* is shown in Fig. 2. The specific growth rate during the exponential growth phase went up with the increase of iron concentration below 12.3 μmol Fe·L^{-1}. However, it declined when the iron concentration was increased to 24.6 μmol Fe·L^{-1} (Fig. 2). Therefore, the growth of *M. aeruginosa* was limited by iron when the iron concentration was below 12.3 μmol Fe·L^{-1}, and it was inhibited when the iron concentration was 24.6 μmol Fe·L^{-1}.

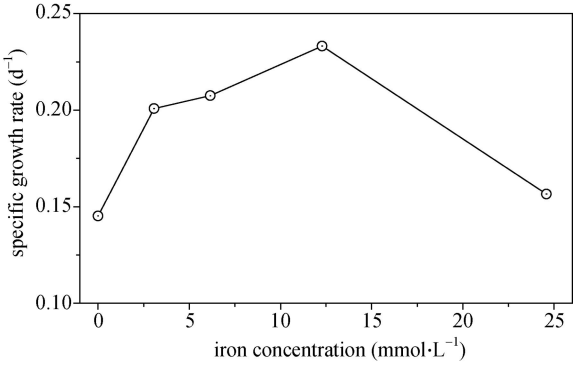

FIG. 2. Effect of iron on specific growth rate of *microcystis aeruginosa*.

Effects of Iron on Photo-pigments

The effect of iron on the cell chlorophyll A content (Q_{Chla}) of *M. aeruginosa* is shown in Fig. 3(A). The Q_{Chla} was limited by the iron concentration below 12.3 μmol Fe·L^{-1}. However, it was saturated when the iron concentration was above 12.3 μmol Fe·L^{-1}.

The *in vivo* fluorescence (excitation at 620 nm and emission at 645 nm, $F_{620\text{-}645}$) was detected to evaluate the cell phycocyanin content of *M. aeruginosa*. It was found that the main source of the *in vivo* chlorophyll fluorescence was Photosystem II (PS II) antenna system, which consisted of an evolutionary conserved chlorophyll A-containing core and species-dependent peripheral antenna. Cyanobacteria possess phycobilisomes which contained phycocyanin and function as peripheral antenna[22]. The *in vivo* fluorescence of the phycocyanin is always assessed at an excitation wavelength of 620 nm and an emission wavelength of 645 nm[23], and is almost independent of the photosynthetic reaction[24].

The $F_{620\text{-}645}$ per cell at different iron concentrations are shown in Fig. 3(B). The variation of phycocyanin shows a similar pattern with that of chlorophyll A. The iron concentration of 12.3 μmol Fe·L^{-1} is a transition point. The cell phycocyanin content was iron limited when the iron concentration was below 12.3 μmol Fe·L^{-1}, however, it declined slightly when the iron concentration was higher than the transition point.

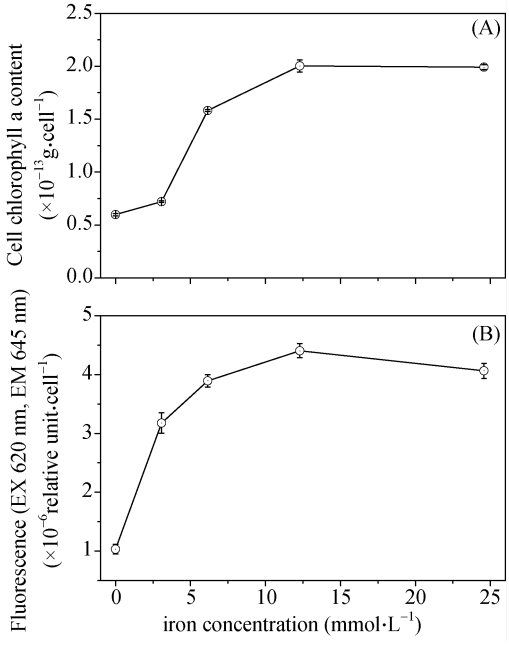

FIG. 3. Effects of iron on cell photo-pigment content of *microcystis aeruginosa*.

Effects of Iron on Cell Carbohydrate, N and P Content

The effects of iron on cell carbohydrate, nitrogen and phosphorus content of *M. aeruginosa* are shown in Fig. 4, suggesting a similar variation pattern. At a low iron concentration (about 6.15 μmol Fe·L^{-1} and less), the cell carbohydrate content (Q_C) increased in

response to the increase of iron concentration (Fig. 4A). However, the effect of iron on Q_C was not obvious above 6.15 μmol Fe·L^{-1}. The variation of cell nitrogen content (Q_N) was nearly the same as that of Q_C (Fig. 4B). The variation of cell phosphorus content (Q_P) is a little different from Q_C or Q_N, with a transition point of 12.3 μmol Fe·L^{-1}(Fig. 4C).

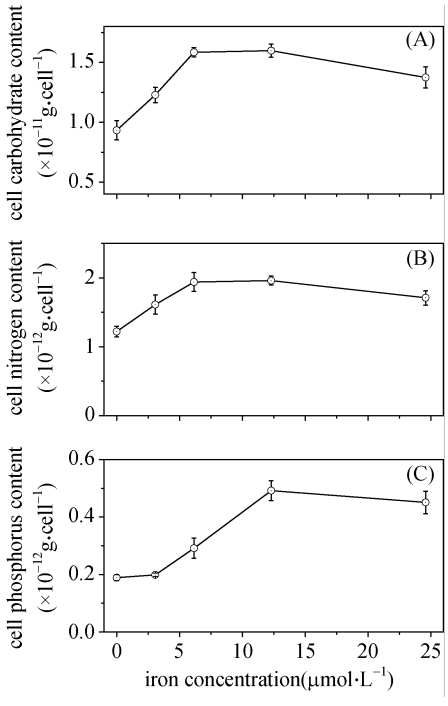

FIG. 4. Effects of iron on cell carbohydrate, N and P content of *Microcystis aeruginosa*.

DISSCUSSIONS

In the photosynthetic cell, there is a requirement of iron in photosynthetic and respiratory electron transport, nitrate assimilation and nitrogen fixation[3-5]. In photosynthesis, iron is required for iron-containing compounds in the electron transport chain, for the biosynthesis of pigments, and for the assembly of the photosynthetic apparatus. The stimulating effect of iron on photosynthesis of plankton has been reported in numerous experiments with additions of iron to marine and freshwater algal communities[25-26]. Iron is a key component of chromophore synthesis, and biosynthesis of chlorophyll and phycobilin pigments have iron dependent steps even though neither of them contain iron[3]. Iron affects chlorophyll synthesis indirectly by affecting its precursor δ-aminolevulinic acid (ALA)[27]. The decrease of cell chlorophyll A or phycocyanin content in cyanobacterial cells[28] and other photosynthetic cells grown under iron-deficient conditions was widely observed[29]. Thus iron limitation generally causes decreased synthesis of pigments (both chlorophyll A and phycobilin pigments, Fig. 3).

The decrease of pigment synthesis leads to fewer photons captured, causing a severe decrease in the net photosynthesis (in terms of the total carbon fixed). This could partially explain why the carbohydrate accumulated in the *M. aeruginosa* cell was limited by iron at low concentration (below 6.15 μmol Fe·L^{-1} (Fig. 4A).

Iron affects the activity of nitrate reductase and nitrogenase, and is directly involved in nitrate and nitrite reduction[3, 5, 27, 29]. The two most energy-demanding systems in the cell, photosynthetic carbon reduction and nitrogen reduction, are both highly dependent on iron-containing compounds[3]. Nitrogen metabolism is closely connected with carbon fixation, as both processes compete for energy and reductant generated by the light reactions of the photosynthesis. The interaction with carbon metabolism is further enhanced by the need for carbon skeletons to incorporate nitrogen into protein. In nutrient replete cells carbohydrate stores are small and assimilation of combined inorganic nitrogen is strongly dependent on recent CO_2 fixation[30]. The parallel variations of Q_N and Q_C (Fig. 4A and B) should be related to the interaction of carbon metabolism and nitrogen reduction.

The loss of pigments is paralleled by a decrease in thylakoid membranes[31]. The synthesis of pigment-protein complexes demand not only nitrogen, but also P for the phospholipid synthesis[32]. This should contribute to the parallel variations of photo-pigments and Q_N or Q_P (Figs. 3, 4B and 4C).

Growth of cyanobacteria is obviously affected by iron concentration[14, 21]. The findings of the study have shown that the specific growth rate during the exponential growth phase is limited by the iron below 12.3 μmol Fe·L^{-1} and is inhibited when the iron concentration is 24.6 μmol Fe·L^{-1}. Though the concentrations of iron in limnetic systems are often low, the accumulation of iron in lakes by load from nonpoint pollution sources and industrial pollution sources may greatly simulate the growth of cyanobacteria and consequently cause blooms. Thus the study on the effect of iron on cyanobacteria may be useful to cyanobacterial bloom control and early-warning.

The variation of growth is synchronous with that of photo-pigments or cell chemical content. It indicates that there exist relationships among photosynthesis, growth and internal chemical content, which could be useful for growth estimation from cell

characteristics.

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