Determination of the in situ growth rate of Microcystis based on carbon and nitrogen stable isotope fractionation
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ABSTRACT
Stable isotope fractionation of carbon and nitrogen in algal cells can be affected by photosynthesis, temperature, nutrient and CO2 concentrations, and cell size. As a consequence, carbon and nitrogen stable isotope techniques are not popular for determining algal growth rates. To counter these issues, this study used BG11 medium to cultivate Microcystis in the laboratory. First, the carbon and nitrogen stable isotope values of the culture medium and the algae are determined. Then, based on changes in isotope fractionation before and after cell division, a function \( \mu = 1.32(1 + x)^{-0.52} \) relating growth rate and stable isotope fractionation is established. By substituting stable isotope values from Taihu Lake water and Microcystis into this function, the growth rate of the Microcystis in Taihu Lake is calculated to be 0.64 d\(^{-1}\) in May and 0.12 d\(^{-1}\) in September, with an average growth rate of 0.42 d\(^{-1}\).

By incorporating most of the above-mentioned factors influencing isotope fractionation, this method can determine the growth rate of algae based directly on the stable isotope fractionation relationship, enabling simple and practical determination of algae growth rates.

Key words | algae growth rate, carbon and nitrogen stable isotopes, function fitting, Microcystis

INTRODUCTION

In situ growth rate is a key metric used to measure algal growth and evaluate algal blooms. It is also used to explore the mechanisms of algal succession and elucidate algal bloom formation processes (Lewis & Diaz-Pulido 2017). Therefore, the determination of in situ algal growth rate plays an important role in studies of algal blooms. At present, common methods to determine in situ growth rate can be classified into two categories. The first includes calculations based on the physiological activity or schizogenesis characteristics of algae cells, such as the photosynthetic rate calculation method (Sutherland et al. 2015), the cell division frequency method (Bothwell & Kilroy 2011), the radioisotope method (Zhang & Mei 2015), the RNA/cell method (Nagai et al. 2011), and the black and white bottle method (Tortell et al. 2013). The second includes methods based on changes in the apparent biomass (or cell density), such as the in situ culture method (Mahdavi et al. 2015), the flow cytometry method (Faber et al. 2010), and the in situ enclosure or water column method (Mhlanga et al. 2014). Of these methods, the cell division frequency method is difficult to apply in practice (and has only been applied in Microcystis to date; Hlavová et al. 2015), the photosynthetic rate calculation is complicated (Shin et al. 2016), and the flow cytometry method cannot be applied in the field. In comparison, the in situ culture method cultivates algae cells in a container, after removing all predators, and determines the apparent growth rate of algae based on changes in the number of cells, biological volume, or other biomass indicators. This method has been widely used for determining the in situ growth rate of algae (Jazzar et al. 2015). This study aims to: (1) determine the growth rate of Microcystis in Taihu Lake by applying the stable isotope and in situ culture techniques in the laboratory; and (2) evaluate the feasibility of using this method to determine the in situ
growth rate. The purpose of this study is to find a simple and
feasible approach for determining algal growth rate using
the stable isotope technique.

There are two methods to determine the plant growth
rate ($\mu$) based on carbon isotopes: the $^{14}$C radioactive iso-
tope method and the $^{13}$C stable isotope method. The
former uses radioactive $^{14}$C to label chlorophyll, caroten-
oids, or proteins in phytoplankton to estimate $\mu$. The
chlorophyll labeling method, which is also known as the
Redalje–Laws chlorophyll method after the scientists who
first used it (Redalje & Laws 1981), is most commonly
used. Other researchers have made subsequent improve-
ments to this method, such as estimating $\mu$ by labeling
carotenoids or proteins, but the principle remains the
same. The Redalje–Laws chlorophyll labeling method is
among the most widely accepted and commonly used
methods for determining phytoplankton $\mu$ (Pei & Laws
2013, 2014). However, this method can underestimate $\mu$ due
to decreasing C:Chl-a ratios in shade phytoplankton under
intense light conditions, or, conversely, overestimate it due
to increasing C:Chl-a ratios in sun phytoplankton under
dark conditions (Mcgehee 2014). In view of these limita-
tions, scholars have continued to modify the Redalje–Laws
method. For example, Gieskes et al. estimated the $\mu$ of
diatoms and Cyanobacteria by labeling and determining the
isotopic ratios of various carotenoids (Gieskes & Kraay
1989). DiTullio and Laws calculated the value of $\mu$ by measur-
ing radioactive absorption by protein components in
phytoplankton cells (DiTullio & Laws 1986). Pei Shaofeng
and Laws et al. studied diurnal cycle cultures for two repre-
sentative marine algae species (Isochrysis galbana and
Chlorella kessleri) with different respiratory patterns for
carbon, and then used nitrogen as a limiting factor for
growth rate control to examine the difference between pri-
mary productivity and actual productivity in the ocean;
productivity was determined using the $^{14}$C tracing technique
(Pei et al. 2014). Xu et al. used the Redalje–Laws and caroten-
oid-labeling methods to calculate the growth rate of various
phytoplankton species in the South China Sea, with results
ranging from 0.07 d$^{-1}$ to 1.68 d$^{-1}$ (Xu & Huang 2010).

Japan and some other countries restrict the application of
$^{14}$C isotope methods due to the radioactivity of $^{14}$C. As
the $^{14}$C and $^{13}$C methods are consistent in principle and
result, a number of scholars have turned to the stable isotope
$^{13}$C, which does not pose a radioactive hazard, to determine $\mu$.
Laws and Popp presented the first study on the use of the
stable isotope $^{13}$C to determine growth rate (Laws et al.
1995). In order to calculate $\mu$, the relationships between $\mu$,
CO$_2$, and $\delta^{13}$C(CO$_2$ and plant) are established according
to known photosynthetic processes. Parameters related
to CO$_2$ may be affected by the coefficient describing CO$_2$
diffusion into the plant, the CO$_2$ fixation coefficient, the
intracellular and extracellular CO$_2$ concentrations, and the
cell volume or surface area, all of which are difficult to deter-
mine. Therefore, this method is impractical and unpopular,
and has only been used in a limited body of research.
Many scholars have explored the impacts of characteristics
such as pH value, temperature, nutrient salt concentration,
plant shape, plant size, and cell surface area on carbon
and nitrogen fractionation. Laws and Popp determined the $\delta^{13}$C (for both aqueous CO$_2$ and the phytoplankton),
CO$_2$ concentrations, and $\mu$ of Phaeodactylum tricornutum
under laboratory culture conditions, and established
relationships between these factors; however, because it is
difficult to measure the $\delta^{13}$C of CO$_2$ in water, the practical
applications of this method are limited (Laws et al. 1997).

Popp formulated an equation relating stable carbon isotope
fractionation, CO$_2$, and $\mu$. Since this equation involves vari-
ables such as fixation, flow, and diffusion of CO$_2$ in a plant,
calculating $\mu$ remains a challenge (Popp et al. 1998). In 2006,
Popp proposed a method for estimating plant $\mu$ that, while
similar to earlier methods, involves ketone labeling with $^{13}$C
instead of chlorophyll labeling with $^{14}$C (Popp et al. 2006).
Tanaka found that, for every change of $-1.7 \pm 0.2$‰ in
$\Delta \delta^{13}$C$_{\text{DIC}}$, $\mu$ decreased by 1% according to a linear relation-
ship between $\mu$ and $\delta^{13}$C$_{\text{DIC}}$. The greatest achievement of
this study lies in its quantification of the relationship between
$\mu$ and $\delta^{13}$C$_{\text{DIC}}$, which simplifies the determination of $\mu$ using
the $^{13}$C method. However, the simple linear fit con-
bstructed between $\Delta \delta^{13}$C$_{\text{DIC}}$ and $\mu$ had a correlation
coefficient of only $r^2 = 0.39$, suggesting a low correlation
(Tanaka et al. 2008). Therefore, the results obtained using
this method may not reflect actual growth rates.

In summary, because $^{14}$C is radioactive, its application
has been restricted. In view of the consistency between
$^{14}$C and $^{13}$C methods in principle and result, scholars have
adopted the non-radioactive stable isotope $^{13}$C to measure
plant $\mu$, but issues with this method include the large
number of factors to be calculated, the complexity of the calculations, and the difficulty of quantifying growth rate. Carbon and nitrogen stable isotope fractionation relationships determined in previous works are used herein to calculate fractionation relationships before and after algal cell division using laboratory-cultured Microcystis aeruginosa at the logarithmic growth phase. Then, linear fits are used to obtain a function describing the relationship between growth rate and the carbon-nitrogen fractionation. This function is used to calculate the growth rate of Microcystis in different growth phases during an algal bloom in Taihu Lake. This method greatly simplifies the calculation and quantification processes, and can be used with field stable isotope measurements to rapidly calculate the growth rate of algae in various phases.

MATERIALS AND METHODS

Microcystis laboratory culture and counting

The algae samples were purchased from the Institute of Hydrobiology, Chinese Academy of Sciences (FACHB 469), and cultured in the BG11 medium. First, 150 mL of BG11 medium, which was previously filtered through a 0.45 μm membrane and sterilized under high temperature, was placed in a 250 mL Erlenmeyer flask. The initial algae density was 6.7 × 10^5 cells/L, culture temperature was 25 °C, light-dark ratio was 12:12 hours, and light intensity was 30 μmol·photos·m^{-2}·s^{-1}. In order to prevent the adhesion of Microcystis to the inner wall of the conical flask during the culture process, the conical flask was gently shaken three times a day.

To avoid the impact of cell population on counting accuracy, 10 mL of algae solution was extracted after thorough agitation and transferred into a 50 mL centrifuge tube. A water bath oscillator was used to sonicate the tube for 5 min below 100 °C at 120 rad·min^{-1} in order to separate the cell population into individual cells. Next, a blood cell counting chamber was used under a microscope (Olympus CX31, 400×) to count the cells; three replicates were performed. If the replicate error was below 10%, the average value was taken as the cell density of Microcystis; otherwise, the counting process was repeated until three groups of results with errors below 10% were achieved, after which the average value was used (Wang et al. 2015).

The growth rate is calculated using the equation μ = ln (D_t/D_0)/t, where μ is the growth rate, and D_t and D_0 are the cell density at time t and initial time, respectively.

Sampling points and frequency

Field sampling was conducted at Meiliang Bay in Taihu Lake. Taihu Lake, which is China’s third largest freshwater lake, has suffered frequent large-scale Microcystis blooms, often spanning hundreds of square kilometers, in recent years. Eutrophication is severe in Meiliang Bay, which has been impacted by urbanization and agricultural production in the surrounding area, and Microcystis blooms occur yearly (Tang et al. 2016). Six samples in total were collected once per month from April to September. A map of the sampling locations is shown in Figure 1.

Carbon and nitrogen isotope analysis

Stable isotope analysis was performed for the experimental samples at the Isotope Analysis Laboratory at Hohai University, using an Integra 2 carbon and nitrogen isotope mass spectrometer. In order to ensure the accuracy of the results and the stability of the instruments, a standard sample was measured before testing experimental samples and again after every 10 samples. Each sample was tested in triplicate; the average of data points with relative errors within ±5% was used as the measured value of this sample. After preheating, 50 mL of culture medium was extracted and filtered through a 0.45 μm glass membrane. Then, 5 mL of alkaline
potassium persulfate solution was added to the solution. After digestion at 120 °C for 30 min, the solution was dried at 70 °C, and the resulting powder was collected, packaged in capsules, and analyzed to determine the stable nitrogen isotope content of the lake water. Excess BaCl₂ solution was added to a second sample after filtering. The sample was dried at 70 °C, and an appropriate amount of the residual solid was collected from the wall of the conical flask, encapsulated, and analyzed to determine the stable carbon isotope content. The glass membrane containing Microcystis residue from filtration was freeze-dried. Then, an appropriate amount of dry Microcystis was collected, encapsulated, and analyzed to determine the carbon and nitrogen stable isotope content of the given Microcystis sample.

The two-source linear model

In order to meet growth and reproduction requirements, Microcystis absorbs carbon- and nitrogen-containing nutrients from the culture medium during logarithmic propagation; Microcystis cells also inherit carbon and nitrogen nutrients from the parent body during proliferation. To evaluate the ratio of nutrients absorbed from the culture medium to those inherited from the parent body, a two-source mixed model is introduced. Taking the carbon stable isotope as an example, the equation is as follows:

\[ K_{\text{carbon}} = 1 - \frac{\delta^{13}C_a - \delta^{13}C_{\text{consumer}} + \Delta \delta^{13}C}{\delta^{13}C_a - \delta^{13}C_b} \]  

where \( K_{\text{carbon}} \) denotes the proportional contribution of food ‘a’ to the consumer; \( \delta^{13}C_a \) and \( \delta^{13}C_b \) represent the isotope ratios of foods ‘a’ and ‘b,’ respectively; \( \Delta \delta^{13}C \) denotes the preconcentration of the carbon isotope (usually considered to be equal to 0‰); and \( \delta^{13}C_{\text{consumer}} \) is the isotope ratio of the consumer.

RESULTS

The density and growth rate of laboratory-cultured Microcystis aeruginosa

The density and growth rate of Microcystis aeruginosa are shown in Figures 2 and 3, respectively. The algal density reaches a maximum value on day 13 of culture, after which the density tends to stabilize (Figure 2). The growth rate decreases to 0.01 d⁻¹ from an initial value of 0.79 d⁻¹.

Changes in total nitrogen and total inorganic carbon concentrations in culture medium and TN and total carbon in concentrations in Microcystis aeruginosa

Changes in total nitrogen (TN) and total inorganic carbon (DIC) concentrations in the culture medium are shown in Figure 4, while changes in TN and total carbon (TC) concentrations in Microcystis aeruginosa are shown in Figure 5. TN concentrations in the culture medium decrease gradually with increasing culture time, while DIC concentrations
decrease gradually at first and then begin to increase. DIC concentrations reach a minimum between 6–8 d of culture time, during which the growth rate is maintained at $\sim 0.3 \text{ d}^{-1}$ (Figure 3). With increasing culture time, the TN and TC contents of Microcystis aeruginosa first increase and then decrease. They both stabilize at their maximum values during the 7–9 d interval.

Changes in $\delta^{13}$C and $\delta^{15}$N in Microcystis aeruginosa and the culture medium

$\delta^{15}$N values for Microcystis aeruginosa reach a maximum of 11.28‰ between 6–7 d of culture time, while the $\delta^{15}$N value for the culture medium reaches a maximum of 16.36‰ at 11 d. Changes in the Microcystis aeruginosa $\delta^{15}$N occur prior to those in the culture medium.

Carbon and nitrogen isotope fractionation relationships in laboratory-cultured Microcystis aeruginosa

Microcystis aeruginosa proliferate rapidly in laboratory culture environments due to favorable growth conditions including high temperature, ample light, and plentiful...
nutrients. During the proliferation process, newly-generated *Microcystis aeruginosa* inherit some of their carbon and nitrogen nutrients from their parent body and absorb more from the external environment to maintain their growth. Carbon and nitrogen fractionation relationships for *Microcystis aeruginosa* before and after proliferation are shown in Table 1.

### Statistical fitting between $\mu$ and the carbon fractionation ratio

As shown in Table 1, the carbon isotope exhibits continuous changes in fractionation with increasing culture time, but the nitrogen isotope does not. Thus, the fitting process is conducted for $\mu$ and the carbon fractionation relationship, and the results are shown in Figure 8.

The relationship between $\mu$ and carbon fractionation takes the form of the power function $\mu = 1.32(1 + x)^{-0.52}$, where $\mu$ is the growth rate and $x$ is the carbon fractionation ratio of the offspring inherited from the parent.

### $\delta^{13}C$ values for lake water and *Microcystis* from Meiliang Bay in Taihu Lake

Carbon isotope data for lake water and *Microcystis* collected between April and September are shown in Table 2.

### Calculation of the *Microcystis* growth rate in Taihu Lake

Substituting the carbon isotope values from Table 2 into the fitting function shown in Figure 8 yields the growth rate of *Microcystis* in Taihu Lake between April and September. The results of this calculation are shown in Table 3. The growth rate of *Microcystis* in Taihu Lake reached a maximum of 0.64 d$^{-1}$ in May and a minimum of 0.12 d$^{-1}$ in September. The average growth rate is 0.42 d$^{-1}$.

### DISCUSSION

The natural growth rate of lake algae

This study determined the *in situ* growth rate of laboratory-cultured *Microcystis aeruginosa*, and the carbon and nitrogen stable isotope content of the culture medium and the algae. The carbon fractionation ratio inherited from the *Microcystis* parent was calculated using the carbon isotope content of the culture medium and *Microcystis*. Then, the functional relationship between the *in situ* growth rate and the carbon fractionation ratio inherited from the parent was obtained by statistical analysis. The latter was calculated for the period between April and September using carbon isotope values from water and *Microcystis* collected at Meiliang Bay in Taihu Lake. The results indicate that the growth rate of the *Microcystis* at Meiliang Bay ranged from 0.12 d$^{-1}$ to 0.64 d$^{-1}$ and that it varied over time, reaching the maximum value in May and the minimum value in September.

Since the growth of *Microcystis* is affected by water quality, water flow, predation, competition, and other factors...
in the natural environment, *Microcystis* cells propagate relatively slowly. As the effects of the factors mentioned above are difficult to quantify, it is challenging to obtain the actual net growth rate of *Microcystis* in field conditions. In September 1998, Tsujimura (2003) established five sampling points in Nagahama Bay in Lake Biwa, Japan, between the shore and center of the lake, determining that the near-shore growth rates of *Microcystis aeruginosa* and *Microcystis wesenbergii* were 0.36 d\(^{-1}\) and 0.49 d\(^{-1}\), respectively. Yamamoto measured the *in situ* growth rate of three types of algae in a small eutrophic pond in Japan between July and October 2005 using the frequency of dividing cells (FDC) method (Yamamoto & Tsukada 2009). In that study, the growth rate of *Microcystis aeruginosa* ranged from 0.15 d\(^{-1}\) to 0.38 d\(^{-1}\), the growth rate of *Microcystis viridis* ranged from 0.14 d\(^{-1}\) to 0.63 d\(^{-1}\), and the growth rate of *Microcystis wesenbergii* ranged from 0.18 d\(^{-1}\) to 0.61 d\(^{-1}\). Cao *et al.* measured the *in situ* growth rate at Meiliang Bay in Taihu Lake between March and June 2005 using the *in situ* water column culture method (Cao *et al.* 2006), finding that the *in situ* growth rate of *Microcystis* at Meiliang Bay ranged from 0.008 d\(^{-1}\) to 0.18 d\(^{-1}\). Wu *et al.* measured the *in situ* growth rate of *Microcystis* at Meiliang Bay in August 2007 using the FDC method (Wu & Kong 2008) and a modified formula based on indoor culture conditions. They calculated the growth rates at four sampling points between the bay and the lake center to be 0.23, 0.19, 0.37, and 0.26 d\(^{-1}\). The Meiliang Bay growth rates determined in this study are similar to those measured by Tsujimura, Yamamoto, and Wu *et al.* In particular, the growth rate of 0.37 d\(^{-1}\) (the third sampling point) in Wu *et al.* is extremely similar to the growth rates determined herein, and these similarities support the credibility of results obtained using the method presented here. The results of this study are inconsistent with those of Cao *et al.*; however, their results also differ from the studies concerning natural water bodies presented in the discussion above. The low growth rate reported in Cao *et al.* may derive from the time and method of measurement. As the *Microcystis* in Taihu Lake are just beginning to recover (i.e., are still in the adaption phase) in March, it is difficult to measure growth rate at this time. In addition, the *in situ* water column culture method isolates the interior water column from the external environment, hindering the exchange of matter. When algal propagation begins, this isolation leads to nutrient deficiencies inside the main body, which in turn affects algal growth and reproduction. As a result, the measured growth rate may be lower than the natural growth rate.

### The relationship between carbon and nitrogen isotope fractionation and the growth rate

During the logarithmic growth phase, *Microcystis aeruginosa* requires a large amount of CO\(_2\) for photosynthesis in order to maintain a high growth rate. However, the only external carbon source in the culture medium is CO\(_2\) dissolved from air, of which the concentration is quite low. In addition, algal propagation further decreases the carbon concentration in the culture medium, causing lower-than-normal dissolved CO\(_2\) concentrations. As a result, *Microcystis aeruginosa* respiration is enhanced and the organic matter originally produced by photosynthesis decomposes into CO\(_2\), which is released into the medium. Thus, carbon isotope fractionation is accelerated, leading to the loss of \(\delta^{13}C\) in the algae cells; in other words, the \(\delta^{13}C\) value of *Microcystis aeruginosa* becomes more negative (Figure 5). These results support the conclusions of Popp *et al.* (1998).
During the process of converting absorbed carbon and nitrogen into organic matter, plants will make preferential use of light isotopes (such as $^{14}$N), leaving heavy isotopes (such as $^{15}$N) behind (Hungate et al. 2015). During the logarithmic growth phase, Microcystis aeruginosa requires large amounts of carbon and nitrogen nutrients to maintain rapid growth and propagation. The lack of external nitrogen sources means there is a shortage of nitrogen nutrients in the culture medium and, with decreasing nitrogen availability and exhaustion of the light isotope, the rate of utilization of the heavy nitrogen isotope gradually increases. This process leads to increasing $\delta^{15}$N values in both the algae and the culture medium. During algal growth and reproduction processes, some cells will decay and release nitrogen. Thus, the initially insufficient nitrogen concentration in the culture medium will recover to a certain degree, and as a result the $\delta^{15}$N values of the algae and the culture medium gradually decrease. The $\delta^{15}$N values of the algae and the culture medium will reach a maximum value when Microcystis aeruginosa is in the logarithmic growth phase. Although Microcystis aeruginosa decay releases nitrogen into the culture medium continuously, gradual increases in algae cell nitrogen absorption lead to decreasing nitrogen concentrations in the culture medium, often below the initial concentration (Figure 6). While the $\delta^{15}$N value for Microcystis aeruginosa reaches a maximum during the logarithmic growth phase, the $\delta^{14}$N value of the culture medium minimizes during the stabilized phase, indicating that extensive algal proliferation can lead to decreasing $\delta^{15}$N. This is consistent with the findings of Peterson et al. (2001), which suggest that Cyanobacteria blooms can reduce the $\delta^{15}$N value of water. Algal growth and decay control the process of nitrogen isotope absorption from, and release into, the culture medium; thus, the $\delta^{15}$N value of the culture medium changes drastically during the culture process. Therefore, it is impossible to use laboratory culture data, such as $\delta^{15}$N and $\mu$, to establish a functional relationship for Microcystis aeruginosa.

**The feasibility of using the stable isotope fractionation fitting method to determine the growth rate**

During algal growth, algal propagation, or changes in the external environment, the scale of stable isotope fractionation can be affected by a variety of factors including photosynthesis, plant size, temperature, nutrient concentration, pH value that affects CO$_2$ or HCO$_3$ concentration, and dissolved oxygen. When the pH value is relatively low, the CO$_2$ concentration in the water is high. During the photosynthesis process, there are sufficient C sources to be used by the algae. In accordance with the rules of isotope utilization, Microcystis will give priority to the use of light isotopes, resulting in the decrease of $^{13}$C content in Microcystis and the increase of $^{13}$C content in the water (culture medium). If the pH value is relatively high, the C sources in the water body are mainly CO$_2^+$, and the photosynthesis of algae will be restricted. As a consequence, the available C sources will be reduced (Wu et al. 2012), while $^{13}$C will accumulate in the algae. Therefore, the variation in the pH value will lead to the changes of C fractionation and affect the growth rate of algae. Unreasonably low content of dissolved oxygen will lead to algae death, while unreasonably high content of dissolved oxygen will inhibit algae photosynthesis. Both conditions can change the growth rate of algae. A variety of external conditions, such as ultrasonic treatment and algaecide, can lead to algae death directly, resulting in the changes of algae growth rate (Emery et al. 2015). Both the changes of the photosynthesis of algae itself and the changes of environmental factors, including temperature, nutrient concentration, and pH value, as well as some external factors (ultrasonic treatment and algaecide) may lead to the variation in algae growth rate, and in turn change the C fractionation. Because of the reasons above, all these factors must be carefully considered, and the stable isotope technique for determining algal growth rates is not as simple or convenient as the radioactive isotope technique. These difficulties have contributed to the slow application of the stable isotope technique in this field. In the method used here, the process of calculating the algal growth rate is similar to the determination of TN and TP via spectrophotometry; the functional relationship obtained is equivalent to the standard curve, while the field data are equivalent to the absorbance. Therefore, the method described in this paper is theoretically feasible. It incorporates various factors that affect stable isotope fractionation into the fitting function and avoids a number of interferences, greatly simplifying the determination of algae growth rate using the stable.
isotope technique. The application of this method therefore becomes feasible.

In this study, the BG11 culture medium was used for the in situ laboratory culture of Microcystis. The composition of this culture medium differs from that of Taihu Lake water; thus, the functional fitting curve obtained in this study may not accurately reflect the relationship between algal isotope content and growth rate in Taihu Lake. However, the main objectives of this study were to (1) examine the feasibility of the stable isotope technique for the determination of growth rate and (2) determine whether this method can be popularized. Therefore, the BG11 medium was not replaced by Taihu Lake water. At the same time, the method described in this paper does not apply to extreme environmental conditions, such as a water body with discharges of untreated sewage and the calculation of growth rate after algal removal treatment. Hence, the use of this method is subject to certain restrictions. Future studies may improve the laboratory culture process to obtain the actual functional relationship between isotopic content and growth rate, thereby enabling the accurate determination of algal growth rates in natural environments.

CONCLUSIONS

(1) Carbon and nitrogen fractionation differs among Microcystis cyanobacteria. It is possible to establish a functional relationship between carbon fractionation and growth rate, but not between nitrogen fractionation and growth rate.

(2) During the algal proliferation period, when the carbon fractionation of the offspring inherited from the parent is between 0–100%, the algae undergo a period of logarithmic growth. The relationship between carbon fractionation ($x$) and growth rate ($\mu$) in the logarithmic growth phase is $\mu = 1.32(1 + x)^{0.52}$.

(3) Considering the regularity of the isotope fractionation in laboratory-cultured Microcystis cyanobacteria, the functional relationship between algal growth rate and isotope fractionation can be used to calculate Microcystis growth rates in Taihu Lake. Calculated growth rates are 0.64 d$^{-1}$ in May and 0.12 d$^{-1}$ in September, with an average growth rate of 0.42 d$^{-1}$.

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