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# Chapter

# Cyanobacteria Growth Kinetics

Leda Giannuzzi

#### **Abstract**

Harmful cyanobacterial blooms are a global problem for freshwater ecosystems used for drinking water supply and recreational purposes. Cyanobacteria also produce a wide variety of toxic secondary metabolites, called cyanotoxins. High water temperatures have been known to lead to cyanobacterial bloom development in temperate and semiarid regions. Increased temperatures as a result of climate change could therefore favor the growth of cyanobacteria, thus augmenting the risks associated with the blooms. Though temperature is the main factor affecting the growth kinetics of bacteria, the availability of nutrients such as nitrogen and phosphorus also plays a significant role. This chapter studies the growth kinetics of toxin-producing *Microcystis aeruginosa* and evaluates potential risks to the population in scenarios of climate change and the presence of nutrients. The most suitable control methods for mitigation are also evaluated.

**Keywords:** modeling of cyanobacterial, harmful cyanobacterial, *Microcystis aeruginosa*, growth kinetics, control of harmful cyanobacterial

#### 1. Introduction

1

Eutrophication resulting from harmful cyanobacterial blooms is a frequent nuisance phenomenon in freshwater lakes and estuaries around the world, posing a serious threat to aquatic ecosystems and human health [1, 2]. Cyanobacteria thus constitute a global problem in freshwater ecosystems used for drinking water and recreational purposes [3]. The potential damage to water supplies, recreation, tourism, aquaculture, and agriculture could have a substantial economic and social impact. The most commonly occurring genera of cyanobacteria include *Microcystis*, *Oscillatoria*, *Anabaena*, and *Aphanizomenon*.

For well over a century, many animal and human poisonings have been associated with Cyanobacteria and their toxins; the death of livestock, wildlife, and pets due to ingestion of water containing toxic cyanobacterial cells or toxins released by the cells has been extensively documented. Human poisonings have also been reported [4]. The occurrence of toxic cyanobacteria has become a worldwide problem [5, 6].

One group of toxic compounds synthesized by several cyanobacteria (*Microcystis, Anabaena, Planktothrix,* and *Nostoc*) comprises numerous hepatotoxic cyclic heptapeptide microcystins [7, 8].

In Argentina, in recent decades, blooms have been recorded in rivers, lakes, coastal lagoons, and estuaries throughout the country, demonstrating the geographical extent of the problem. An increase has been detected both in the number of responsible species and in the frequency and intensity of the harmful events. The genera most commonly associated with the development of toxic blooms are *Microcystis* and *Dolichospermum* (ex *Anabaena*), and the most cited cyanotoxins are

microcystins [9–11]. In 2014, a series of harmful episodes caused by cyanobacteria blooms associated with water treatment systems in different parts of Argentina occurred. The presence of cyanobacteria and cyanotoxins has been reported in several sources of drinking water. *Microcystis* colony cells and microcystins were detected in water in the cities of La Plata and Ensenada, Buenos Aires, Argentina, in 2006 [12], evidencing the inefficiency of the water treatment plant.

Giannuzzi et al. [13] reported an acute case of cyanobacterial poisoning in the Salto Grande dam, Argentina, which occurred in January 2007. A young man accidentally became immersed in an intense bloom of *Microcystis* spp. with 48.6  $\mu$ g L<sup>-1</sup> of microcystin-LR in water samples. The patient was hospitalized in intensive care and diagnosed with an atypical pneumonia. A week after exposure, the patient developed hepatotoxicosis with a significant increase in hepatic damage biomarkers (ALT, AST, and  $\gamma$ GT). Complete recovery took 20 days. It is not known whether there was an eventual chronic intoxication after the acute poisoning. In the year 2000 in Bahía Blanca (Buenos Aires, Argentina), alterations were detected in the organoleptic characteristics of the water network (unpleasant odor and taste), product of the liberation of geosmin by *Dolichospermum circinalis* blooms. This episode coincided with the appearance of dermal and respiratory problems in the population [14].

The duration of cyanobacterial blooms in temperate zones can last 2–4 months during the summer period, whereas in tropical and subtropical regions of Australia, China, and Brazil, they can sometimes persist all year round [15].

The major factors that influence the growth of cyanobacteria are light, temperature, and the nutrients composition of the suspending medium.

High water temperatures have been known to lead to cyanobacterial bloom development in temperate [16–18] and semiarid regions [19]. Increasing air and water temperatures as a result of climate change are likely to promote a faster algal growth rate [20, 21].

Nitrogen (N) and/or phosphorus (P) levels can also positively affect cyanobacterial growth in lakes and river. The absolute and relative concentrations of these nutrients affect the growth rate, abundance, and composition of phytoplankton in lake water [22] as commonly measured in terms of their trophic state, defined as the total weight of biomass in a given water body at the time of measurement [23]. Many studies show that phosphorus is the limiting nutrient in freshwater bodies [24, 25], and other studies show the relationship between cyanobacterial abundance and phosphorus concentrations in lakes [26, 27].

The trophic state of a lake generally increases with increases in total nitrogen (TN) and total phosphorus (TP) concentrations. Resolving lake or river eutrophication problems calls for a better understanding of the water and air temperature-dependence of algal blooms.

A high P concentration is considered to be the main cause of *Microcystis* blooms in the Nakdong River of Korea [28, 29]. Schindler [30, 31] report that N is unlikely to be the limiting factor for blooms because of the presence of  $N_2$ -fixing cyanobacterium in water bodies. Phosphate ( $PO_4^{3-}$ ) is released from the sediment during summer, absorbed by *Microcystis* and stored in the bottom layer [32, 33]; using its gas vacuole, the *Microcystis* then moves toward the high-intensity light at the surface to generate blooms [34–36].

Provided factors such as illumination and nutrients remain saturating, and the photosynthetic and specific maximal growth rate responses of different algal species to temperature can be compared [34].

Physiological properties within a single species, including photosynthetic response, can change according to the growth conditions [37]. Photoperiodicity-and light intensity-dependent changes in photosynthetic parameters and different pigments such as chlorophyll a and phycocyanin are to be expected.

The general consensus is that the optimum growth temperature for cyanobacteria is higher than that for most algae. Paerl [38] reported the optimum temperature to be higher than 25°C, overlapping with that of green algae (27–32.8°C) but clearly differing from that of dinoflagellates (17–27°C) and diatoms (17–22°C). Crettaz Minaglia [39] reported the optimum growth temperature for native M. aeruginosa to be 33.39  $\pm$  0.55°C.

Lürling [40] found similar optimal temperatures for two strains of *M. aeruginosa* (30.0–32.5°C). These data suggest that the native strain of *M. aeruginosa* is able to compete favorably with other phytoplankton species, producing more frequent blooming events in scenarios of climate change.

Paerl [41] reported that higher temperatures (up to 25°C) due to climate change may lead to increased cyanobacterial growth rates and thus higher cyanobacterial dominance in temperate water bodies [17, 20]. This trend would be further facilitated by cyanobacterial buoyancy, which aids their proliferation in increasingly stratified conditions because decreasing water viscosity at higher temperatures results in higher flotation velocities of buoyant cyanobacteria [19, 42].

Many authors describe an inverse relationship between temperature and microcystin production.

Crettaz Minaglia [39] found that the production of MC-LR decreased with increasing temperature, coinciding with the findings of [43–50].

van der Westhuizen [51] reported that optimal growth conditions do not coincide with the production of toxins. Similarly, Gorham [43] affirmed that the optimum temperature for growth (30–35°C) differed from that for optimal toxicity (25°C).

In an interesting paper, Mowe et al. [52] suggest that higher mean water temperatures resulting from climate change will generally not affect *Microcystis* spp. growth rates in Singapore, except for increases in *M. ichthyoblabe* strains. However, depending on the species, the toxin cell ratio may increase under moderate warming scenarios.

Further studies on the temperature dependency of the different physiological processes affecting growth (e.g., carbon fixation, photorespiration, and respiration) are required in order to better understand the differences in temperature sensitivity between *Microcystis* growth and toxins production.

# 2. Modeling M. aeruginosa growth

An evaluation of microbiological cyanobacterial processes calls for kinetics studies examining the rates of production of cells and their metabolites and the effects of various factors on these rates.

One of the basic tools in microbiology is growth kinetics, defined as the relationship between a specific growth rate and parameters such as temperature, pH, light intensity, short wavelength radiations, pH, and nutrients.

A convenient way to evaluate laboratory-based bacteria growth systems under different abiotic factors is to examine the parameters characterizing the three phases of bacterial growth: the lag phase, the exponential phase, and the stationary phase.

During the lag phase, which can last from 1 hour up to several days, there is very little change in the number of bacteria cells because while they are adapting to the growth conditions, they are still immature and unable to reproduce. This is the period when the synthesis of RNA, enzymes, and other molecules occurs.

The exponential phase is characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. With no limitations in place, doubling continues at a constant rate, leading to a doubling of the number of cells and the rate of population increase with each consecutive time period. Plotting the logarithm of cell number against time produces a straight

line, the slope of which indicates the specific growth rate of the organism, which is a measure of the number of divisions per cell per unit time. The actual rate of this growth depends on the growth conditions, which affect the frequency of cell division events and the probability of both daughter cells surviving. Under controlled conditions, the cyanobacteria population can be doubled four times a day and then tripled. However, this exponential growth eventually comes to an end when the medium becomes depleted of nutrients and enriched with waste.

The stationary phase results when the death rate is equal to the growth rate, often because of the depletion of an essential nutrient and/or the formation of an inhibitory product such as an organic acid, giving rise to a "smooth," horizontal line on the curve.

The final phase is the death phase. Bacterial death can be the result of lack of nutrients, environmental temperature above or below the tolerance band for the species, or other deleterious conditions.

Modeling a cyanobacterial growth curve allows one to reduce recorded data to a limited number of parameters of interest such as the specific growth rate, lag phase duration, and maximum population density.

The growth models found in the literature describe only the number of organisms and do not include substrate consumption as would a model based on the Monod equation. However, the substrate level is not of interest in our application since we assume there to be sufficient substrate to allow cyanobacterial growth.

An assessment of natural populations of *Microcystis aeruginosa* requires data on pure culture growth under well-defined conditions.

Studying the growth kinetics of *Microcystis* in relation to nutrient concentrations is very important for management purposes [53].

In batch culture methods, the culture is not maintained at a specific growth stage with constant addition and removal of culture medium and cells [54].

This makes it an appropriate method for our *M. aeruginosa* study since the natural ecosystem is not steady state either: weather-related factors cause changes in nutrient loading, resulting in varying nutrient concentrations, with no expected resupply of nutrients to the water column. *Microcystis* habitats are therefore more like batch experiments than continuous cultures able to reach a steady state [55].

The basic batch culture growth model emphasizes aspects of bacterial growth, which may differ from the growth of other organisms. Plotting an experimentally determined cell number or cell mass concentration (or their logarithms) against time gives rise to a characteristic curve.

For the purpose of modeling the growth of *M. aeruginosa*, three primary continuous population models can be used.

The simplest is a linear function based on the exponential or Malthus model, called a simple exponential growth model. This model assumes that the growth rate of the population is proportional to its density

$$lnN = \ln(N_0) + \mu * t \tag{1}$$

where N(t) is the population at time t,  $N_0$  is the initial population,  $\mu$  is a constant indicating the rate of growth, and t is time. The parameter  $\mu$  is called the specific growth rate and is expressed in reciprocal time units.

This model is widely used in microorganisms and is also very useful for describing the population growth of many organisms over short periods of time, there being no space or resource limitations.

During a batch cultivation, the specific growth rate changes continuously from zero to a maximum value,  $\mu_{max}$ . The value of the maximum specific growth rate depends on the type of microorganism and on physical and chemical cultivation

conditions (temperature, pH, medium composition, light, etc.). Under given culture conditions, it is constant and represents an important characteristic of the process.

The specific growth rate  $(\mu)$  can be calculated between successive sampling points from a simple first-order rate law using the equation

$$\mu = \frac{ln\frac{N_1}{N_0}}{t_1 - t_0} \tag{2}$$

where  $N_0$  is the cell number per mL culture at time  $t_0$  and  $N_1$  is the cell number at time  $t_1$ .

The main parameter characterizing the growth rate is the specific growth rate, which can be used to express other growth parameters given below.

The relationship between the specific growth rate ( $\mu$ ) and cell number doubling time ( $t_d$ ) can be obtained by inserting into the equation  $N=2N_0$  and  $t=t_d$ .

$$t_d = \frac{\ln 2}{\mu} = \frac{0.693}{\mu} \tag{3}$$

Yet another model is the logistic or Verhulst model (Eq. (4)), a quadratic function based on the previous model under the assumption that the population cannot grow indefinitely and faster. In this model,  $\mu$  is not a constant, but is a linearly increasing function of population density. This model has two equilibrium points defined as N values where the growth velocity is zero. These points correspond to N = 0 and N = K (load capacity). The load capacity refers to the maximum population that its environment can sustain in terms of resource or space availability.

$$N(t) = \frac{K}{1 + \frac{K - N_0}{N_0} e^{-\mu . t}}$$
 (4)

where N(t) is the final population,  $N_0$  is the initial population,  $\mu$  is a constant that indicates the growth rate, t is time, and K is the load capacity.

Growth rate  $(\mu)$  is commonly expressed in the literature as a function of light, nutrient, pH, ionic conditions, and temperature. Modeling the growth rate is based on simply multiplying the functions upon which growth is dependent:

$$\mu = f(N) * f(I) * f(T)$$
 (5)

where  $\mu$  (time<sup>-1</sup>) is the cyanobacterial growth rate and f(I), f(N), and f(T) are the effects of irradiance, nutrients, and temperature on the growth rate, respectively.

Another method to calculate the three parameters of the three phases of bacterial growth mentioned earlier is using the modified Gompertz equation, a double exponential function based on four parameters, which describes an asymmetric sigmoid curve Eq. (6) [56].

The Gompertz model is one of the most widely used and recommended models from which lag time, maximum growth rate, and maximum population density (stationary phase) can be obtained directly from nonlinear regression of the cell numbers versus time data [57, 58].

A Gompertz model describing the growth of *M. aeruginosa* would be a good fit for analyzing the effect of temperature, irradiance, and nutrients on the parameters of kinetic growth curves in batch culture.

$$log(N) = a + c \times exp(-exp(-b \times (t - m)))$$
 (6)

where log(N) is the decimal logarithm of the cell counts ( $log(cell mL^{-1})$ ), t is time (days), and a is the logarithm of the asymptotic counts when time decreases indefinitely (roughly equivalent to the logarithm of the initial levels of cyanobacteria ( $log(cell mL^{-1})$ ), c is the logarithm of the asymptotic counts when time is increased indefinitely (the number of  $log(cell mL^{-1})$ ), b is the growth rate relative to time ( $log(cell mL^{-1})$ ), and m is the required time to reach the maximum growth rate ( $log(cell mL^{-1})$ ).

The maximum or specific growth rate ( $\mu$ ) value is defined as the tangent in the inflection point and was calculated as  $\mu$  = b.c/e with e = 2.7182 (days<sup>-1</sup>). The lag phase duration (LPD) is defined as the t-axis intercept of this tangent, the asymptote, and was calculated as LPD = m - 1/b, (days); the maximum population density MPD = a + c (log (cell mL<sup>-1</sup>) was derived from these parameters [59].

The value of modeling has been recognized for a number of years. Accurate and well-validated models are able to predict the behavior of dynamically changing systems and provide data and insights that would be difficult or impossible to obtain by conventional field.

**Figure 1** shows a detail of *M. aeruginosa* isolated from the environment. When these cells are grown in culture medium, they lose their capacity to colonies form. In this review, the Gompertz equation was applied to *M. aeruginosa* growth in culture media (BG11) at different temperatures. The cells mL<sup>-1</sup> data were fitted to the Gompertz equation by nonlinear regression using the program Systat (Systat Inc., version 5.0). The selected algorithm calculates the set of parameters with the lowest residual sum of squares and a 95% confidence interval for *M. aeruginosa* growth.

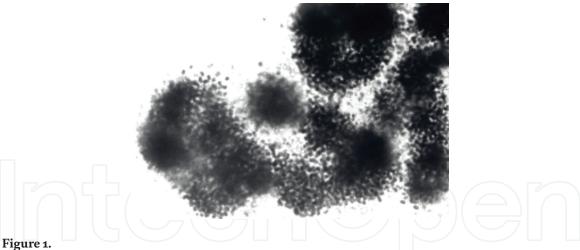
In a previous work, we informed two additional kinetic parameters: generation time (GT) and the relative lag phase duration (RLPD). Generation time was defined as the time for the bacterial population to double in cell numbers and was calculated by dividing  $\mu$  values by 0.301 (equivalent to  $\log_{10} 2$ ); GT is thus a measure of the metabolic rate in a new environment. The RLPD, defined by the amount of work to be done in adjusting to a new environment and the rate at which that work is done, was calculated by dividing LPD by GT [39].

For *M. aeruginosa*, the growth rate shows a lag phase followed by an exponential phase and finally a decreasing growth rate down to zero, resulting in a maximum value of the number of cells (**Figure 2**). During the experiment, the number of cells mL<sup>-1</sup> increased exponentially in all cultures after a lag phase. **Figure 2** shows *M. aeruginosa* counts growing in BG11 modified medium at 26, 28, 30, and 35°C.

By examining these parameters, the blooming behavior of *M. aeruginosa* can be predicted and early warning signs recognized in time to take preventive action. Although some authors have performed laboratory experiments using *M. aeruginosa* under different temperature conditions, irradiance and N:P ratio [51, 60–64], and modeled their growth, they only applied the linear growth model to the exponential phase of the curve.

The Gompertz parameters of these curves were reported by Crettaz Minaglia et al. [39]. It can be seen that as the temperature increases, the value of  $\mu$  increases and LPD decreases. Thus, when the temperature changed from 26 to 35°C, the  $\mu$  values increased from 0.18 to 0.24 days<sup>-1</sup>, and LPD decreased from 4.10 to 0.75 days, nonsignificant differences were found for the MPD values (7.25–7.10 log CFU mL<sup>-1</sup>). The GT parameter ranged from 1.67 to 1.25 days, and RLPD from 2.40 to 0.60.

The ratio of the specific growth rate to the lag time duration was approximately constant, suggesting a linear relationship between lag phase and the reciprocal of the growth rate. This finding was corroborated by Crettaz Minaglia [39], who reported that the lag phase showed a linear behavior with the reciprocal specific growth rate for *M. aeruginosa*. The correlation coefficient (R<sup>2</sup>) was 0.86.



Light microscopy image of a Microcystis aeruginosa colony isolated from the environment (kindness of Ricardo Echenique).

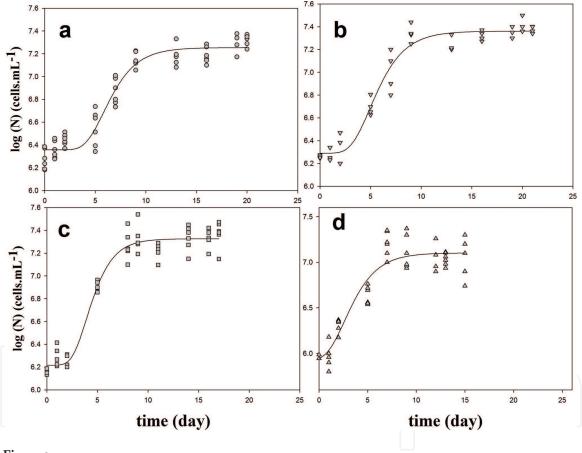


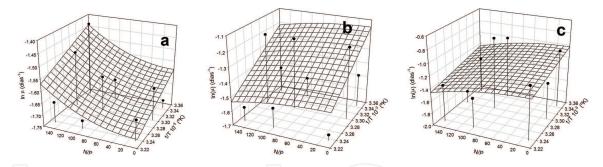
Figure 2.

Effect of temperature on M. aeruginosa growth in culture media: (a) 25, (b) 28, (c) 30, and (d) 36°C.

Lyck [61] reported specific growth rate values ranging from 0.52 to 0.54 day<sup>-1</sup> calculated between successive sampling times according to a simple first-order rate function using cell concentration (cells mL<sup>-1</sup>).

In reviewing available literature on the effects of temperature on growth rates, Canale and Vogel [65] concluded that as temperature increased, the highest growth rates for broad phytoplankton groups changed from diatoms, via green algae to cyanobacteria (blue-green algae). Species-specific responses are, however, highly variable [66].

The specific growth rate and lag phase duration are known to be affected by many variables, and the cyanobacterial responses to changes in the environment are complex and difficult to characterize. **Figure 3** shows an example of the variation



**Figure 3.** Surface response plots showing the dependence of specific growth rate parameters of M. aeruginosa on temperature and N/P ratio: (a) 30, (b) 50, and (c) 70  $\mu$ mol photons  $m^{-2}$  s<sup>-1</sup>.

in the specific growth rate with changes in temperature (26, 30, and 36°C), nutrient (N/P 10, 100, and 150), and irradiance (30, 50, 70  $\mu$ mol photon m<sup>-2</sup> s<sup>-1</sup>) conditions for *M. aeruginosa* growing in culture medium. For combined temperature and the N/P ratio, we chose the Arrhenius-type temperature dependence model for each irradiance as the starting point for developing a model including both temperature and N/P effect. After fitting to different models, those with the highest correlation coefficients and the lowest errors in the estimated parameters were selected. The following Eq. (7) was obtained by stepwise analysis with statistical SYSTAT software and describes both the inverse absolute temperature effect (25, 30, and 36°C) and N/P ratio dependence (10, 100, and 150) on the specific growth rate.

$$ln\mu = K1 + K2 * \frac{1}{T} + K3 * \left(\frac{N^2}{P}\right)$$
 (7)

The percent variance was very high at 98.8%, indicating a very good fit of the model to the data. The parameter  $K_2$  was 0.89, 2.25, and 1.56 for 30, 50, and 70  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup>, respectively. The values of  $K_2 = E_a/R$  where  $E_a$  is the activation energy of  $\mu$  (KJ mol<sup>-1</sup>) applied Eq. (7) and R is the gas constant (8.31 KJ K<sup>-1</sup> mol<sup>-1</sup>). In the present study,  $E_a$  was 7.40, 18.69, and 12.96 KJ K<sup>-1</sup> mol<sup>-1</sup> for 30, 50, and 70  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup>, respectively.

**Figure 3a–c** shows examples of a surface response plot corresponding to Eq. (7) obtained by fitting  $\ln \mu$  of *M. aeruginosa* versus temperature and N/P ratio.

Using the model reported here, we determined the combined effects of the N/P ratio and temperature on specific growth rates in controlled laboratory assays, thus enabling us to predict *M. aeruginosa* growth under different conditions from those tested experimentally in this work, but within the studied range of temperatures and N/P ratio.

The Gompertz model was successfully tested with the experimental data for *M. aeruginosa* at different temperature, ration N/P, and light intensity (data not show). It is very important to test the model under different conditions or to verify the model for other species of cyanobacteria and diatoms.

However, many open questions remain concerning the validity of applying laboratory-observed growth kinetics to environmental growth conditions, with diverging data being reported for pure cultures growing with single substrates.

Although our model only takes into account temperature and N/P ratio, it would be important to extend the modeling to other factors such as pH and elements such as metals (Fe, Mn, etc.). Further studies are required to gain deeper insight into the factors that influence growth in order to better predict aspects related to *M. aeruginosa* blooms.

# 3. Cyanobacterial control

Cyanobacterial blooms can lead to the accumulation of cyanotoxins in aquatic animals, eventually posing a high risk to human health as well.

In the current scenario of growing problems associated with cyanobacterial blooms and their toxins, an environmentally compatible control strategy is urgently required. The removal of harmful cyanobacterial blooms is a crucial step for the adequate maintenance of water supplies and for the safety of food and aquatic products. Controlling cyanobacterial blooms is likely to become an even more challenging task in the future due to global warming effects.

Despite the availability of control methods for cyanobacterial blooms, it has not yet been possible to prevent the excessive proliferation of these organisms, which have adapted so successfully to water surfaces. The effectiveness of control methods naturally varies according to the circumstances (type and size of the lake, retention time, degree of alteration, quantity of nutrient load, quality and quantity of sediments, season, amount of aquatic life, etc.); they are not universal and their use may be restricted to particular circumstances.

The preferred method for preventing these blooms is to reduce the availability of nutrients, especially phosphorus, the main cause of the massive presence of cyanobacteria. This implies the rehabilitation of point and nonpoint sources of nutrients (discharge of effluents, drift of chemical substances from agriculture, and erosion of urban and forest areas) [67]. In those cases where nutrient reduction is not possible, more drastic, short-term action has been proposed in the form of chemical, physical, and biological approaches [68], each with its advantages and disadvantages for application to the control of harmful algal blooms.

A widely adopted chemical approach is the addition of algaecide (copper sulfate), oxidants (chlorine, potassium permanganate), and flocculants (FeCl<sub>3</sub>, AlCl<sub>3</sub>, polyaluminum chloride) etc., all of which have proven to be efficient in removing cyanobacteria cells. However, though chemical approaches can take rapid effect in removing algal blooms, they can cause secondary pollution of aquatic environments [69]. Their main disadvantage is that they do not selectively target harmful cyanobacteria and can lead to the elimination or damage to nonharmful algae or beneficial organisms. Depending on the oxidant and cyanotoxin type, some oxidants can cause the release of toxins, and the subsequent rapid oxidation of the toxins must therefore be assured [70].

The application of chemical agents to lakes and water bodies often leads to the collapse of aquatic ecosystems.

Hydrogen peroxide (HP) is selective for cyanobacteria (vs. eukaryotic algae and higher plants) and poses no serious long-term threats to the system because of its rapid decomposition without producing persistent toxic chemicals or by-products that cause esthetic odor or color issues. It has been reported that HP has potential for removing *Microcystis* sp. and microcystins in different environments. Lakes dominated by *M. aeruginosa*, *Aphanizomenon*, and *Dolichospermum* (formerly called Anabaena) have been successfully treated with HP [71]. It is important to assess the impact of HP on elements of the ecosystem such as larval fish, macroinvertebrates, and zooplankton.

Physical approaches, such as mixing lake waters using an air compressor, ultrasonic damage to algal cells and pressure devices to collapse cyanobacterial gas vesicles, have also been proposed to control algal blooms. Other treatments such as the mechanical removal of cyanobacterial biomass and sediments and hypolimnetic aeration and oxygenation have also been described.

The most apparent merit of physical approaches for the removal of algae as opposed to chemical manipulations is that they are less likely to give rise to secondary pollution. However, the physical removal of algae is energy intensive and tends to be of low efficiency. Moreover, injury to nontarget organisms by energy-intensive treatments also limits the field application on a large scale [72].

Though biological approaches to controlling toxic cyanobacteria and harmful cyanobacterial blooms tend to be environmentally friendly, their efficiency is determined by many biotic and abiotic factors in the environment. It is well known that MCs can be degraded by local bacterial communities frequently exposed to cyanobacterial blooms.

The removal of MCs has been reported by a group of microorganisms generically referred to as a consortium [73].

Furthermore, a large group of bacteria able to degrade MCs has been isolated, Sphingomonadaceae being the most studied family. Most of these organisms have been identified as *Sphingomonas* [74] and *Sphingopyxis* [75].

Some biologically derived bioactive substances inhibit the growth of aquatic bloom-forming cyanobacteria [76–78], including plant extracts and identified natural chemicals from plants and microorganisms.

Aquatic plants such as Stratiotes aloides [79] Myriophyllum spicatum [80], Phragmites communis [81], Ceratophyllum demersum and Najas marina spp., Intermedia [82], and extracts of Ephedra equisetina root have been reported to inhibit the growth of cyanobacteria. Most of these substances are biodegraded in natural environments. However, actual field applications to control harmful cyanobacteria are currently very limited owing to the high cost of algicide preparations and low algae-removal efficiency compared to chemical algicides.

In view of the paucity of studies on the ecological and public health risks associated with most antialgal substances, their application should be very carefully evaluated. Only ecologically safe and easily applicable substances should be used for cyanobacterial growth control.

#### 4. Conclusions

Mathematical modeling applied to *M. aeruginosa* growth is an efficient tool to predict the effect of different variable as temperature, irradiance, and nutrients on the kinetic parameters. The current study provides quantitative evidence of the effects of temperature, irradiance, and nutrients on *M. aeruginosa* growth. The above data suggest that the native strain of *M. aeruginosa* is able to compete favorably with other phytoplankton species, producing more frequent blooming events in scenarios of climate change. In the current scenario of growing problems associated with cyanobacterial blooms and their toxins, an environmentally compatible control strategy is urgently required. However, the use of control agents, whether physical, chemical, and biological, is not yet sufficiently safe due to certain harmful effects on the environment. Only ecologically safe and easily applicable substances should be used for cyanobacterial growth control.

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## **Conflict of interest**

No conflict of interest.





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#### References

- [1] Hudnell HK. The state of U.S. freshwater harmful algal blooms assessments, policy and legislation. Toxicon. 2010;55:1024-1034. DOI: 10.1016/j.toxicon.2009.07.021
- [2] Carey CC, Ibelings BW, Hoffmann EP, Hamilton DP, Brookes JD. Ecophysiological adaptations that favour freshwater cyanobacteria in a changing climate. Water Research. 2012;**46**:1394-1407. DOI: 10.1016/j.watres.2011.12.016
- [3] Paerl HW, Fulton RS, Moisander PH, Dyble J. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. The Scientific World. 2001;1:76-113. DOI: 10.1100/tsw.2001.16
- [4] Azevedo S, Carmichael WW, Jochimsen E, Rinehart K, Lau S, Shaw G, et al. Human intoxication by microcystins during renal dialysis treatment in Caruaru/Brazil. Toxicology. 2002;**181/182**:441-446
- [5] Codd GA. Cyanobacterial toxins: Occurrence, properties and biological significance. Water Science and Technology. 1995;**32**:149-156
- [6] Namikoshi M, Rinehart KL. Bioactive compounds produced by cyanobacteria. Journal of Industrial Microbiology. 1996;17:373-384. DOI: 10.1007/bf01574768
- [7] Carmichael WW. Cyanobacteria secondary metabolites-the cyanotoxins. A review. The Journal of Applied Bacteriology. 1992;72:445-459. DOI: 10.1111/j.1365-2672.1992.tb01858.x
- [8] Carmichael WW. The cyanotoxins. Advances in Botanical Research. 1997;27:211-256. DOI: 10.1016/s0065-2296(08)60282-7
- [9] Echenique RO, Aguilera A. Cyanobacteria toxígenas: aspectos generales para su identificación

- taxonómica. In: Giannuzzi L, editor. Cianobacterias y cianotoxinas: identificación, toxicología, monitoreo y evaluación de riesgo. Corrientes: Moglia Impresiones; 2009. pp. 37-51
- [10] Azevedo S. South and Central America: Toxic cyanobacterial. In: Codd GA, Azevedo SMFO, Bagchi SN, Burch MD, Carmichael WW, Harding WR, Kaya K, Utkilen HC, editors. CYANONET. A Global Network for Cyanobacterial Bloom and Toxin Risk Management. Initial Situation Assessment and Recommendations, Technical documents in Hydrology PHI-VI. 2005. pp. 115-126
- [11] Otaño S, Salerno G, Ruiz M, Aguilera A, Echenique RO. ARGENTINA: Cyanobacteria and cyanotoxins: Identification, toxicology, monitoring and risk assessment. In: Chorus I, editor. Current Approaches to Cyanotoxin Risk Assessment, Risk Management and Regulations in Different Countries. Wörlitzer Platz 1, 06844 Dessau-Roßlau, Germany: Federal Environment Agency (Umweltbundesamt); 2012. pp. 16-20
- [12] Echenique RO, Aguilera A, Giannuzzi L. Problems on drinking water related to toxigenic cyanobacteria: Some cases studied in Argentina. In: Tell G, Izaguirre I, O'Farrel I, editors. Freshwater phytoplankton from Argentina. Advances in Limnology. Vol. 65. 2014. pp. 431-444. DOI: 10.1127/1612-166X/2014/0065-0055
- [13] Giannuzzi L, Sedan D, Echenique R, Andrinolo D. An acute case of intoxication with cyanobacteria and cyanotoxins in recreational water in Salto Grande dam, Argentina. Marine Drugs. 2011;9:2164-2175. DOI: 10.3390/md9112164
- [14] Echenique R, Giannuzzi L, Ferrari L. Drinking water: Problems related

- to water supply in bahía Blanca, Argentina. Acta Toxicológica Argentina. 2006;**14**(2):2-9
- [15] Sivonen K, Jones G. Cyanobacterial toxins. In: Chorus I, Bartram J, editors. Toxic Cyanobacteria in Water. A Guide to their Public Health Consequences, Monitoring and Management. London: E & FN Spon, WHO; 1999. pp. 41-112. DOI: 10.4324/9780203478073
- [16] Robarts RD, Zohary T. Temperature effects on photosynthetic capacity, respiration, and growth rates of bloomforming cyanobacteria. New Zealand Journal of Marine and Freshwater Research. 1987;21:391-399. DOI: 10.1080/00288330.1987.9516235
- [17] Jöhnk KD, Huisman J, Sharples J, Sommeijer B, Visser PM, Stroom JM. Summer heatwaves promote blooms of harmful cyanobacteria. Global Change Biology. 2008;**14**:495-512. DOI: 10.1111/j.1365-2486.2007.01510.x
- [18] Wu Y, Li L, Gan N, Zheng L, Ma H, Shan K, et al. Seasonal dynamics of water bloom-forming *Microcystis* morphospecies and the associated extracellular microcystin concentrations in large, shallow, eutrophic Dianchi Lake. Journal of Environmental Sciences. 2014:1921-1929. DOI: 10.1016/j. jes.2014.06.031
- [19] Mitrovic SM, Oliver RL, Rees C. Critical flow velocities for the growth and dominance of *Anabaena circinalis* in some turbid freshwater rivers. Freshwater Biology. 2003;**48**:164-174. DOI: 10.1046/j.1365-2427.2003.00957.x
- [20] Davis TW, Berry DB, Boyer GL, Gobler CJ. The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms. Harmful Algae. 2009;8:715-725. DOI: 10.1139/f05-239
- [21] Jones ML, Shuter BJ, Zhao YM, Stockwell JD. Forecasting effects

- of climate change on Great Lakes fisheries: Models that link habitat supply to population dynamics can help. Canadian Journal of Fisheries and Aquatic Sciences. 2006;63:457-468. DOI: 10.1139/f05-239
- [22] Bergström A. The use of TN:TP and DIN:TP ratios as indicators for phytoplankton nutrient limitation in oligotrophic lakes affected by N deposition. Aquatic Sciences. 2010;72:277-281. DOI: 10.1007/s00027-010-0132-0
- [23] Carlson RE. A trophic state index for lakes. Limnology and Oceanography. 1977;**22**:361-369. DOI: 10.4319/lo.1977.22.2.0361
- [24] Schindler DW, Hecky RE, Findlay DL, Stainton MP, Parker BR, Paterson MJ, et al. Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. Proceedings of the National Academy of Sciences. 2008;105(32):11254-11258. DOI: 10.1073/pnas.0805108105
- [25] Saxton MA, Arnold RJ, Bourbonniere RA, McKay RML, Wilhelm SW. Plasticity of total and intracellular phosphorus quotas in *Microcystis aeruginosa* cultures and Lake Erie algal assemblages. Frontiers in Microbiology. 2012;3:19. DOI: 10.3389/ fmicb.2012.00003
- [26] Chaffin JD, Bridgeman TB, Heckathorn SA, Mishra S. Assessment of *Microcystis* growth rate potential and nutrient status across a trophic gradient in western Lake Erie. Journal of Great Lakes Research. 2011;37(1):92-100. DOI: 10.1016/j.jglr.2010.11.016
- [27] Downing JA, Watson SB, McCauley E. Predicting cyanobacteria dominance in lakes. Canadian Journal of Fisheries and Aquatic Sciences. 2001;58(10):1905-1908. DOI: 10.1139/f01-143

- [28] Kim EH, Kang SK. The effect of heavy metal ions on the growth of *Microcystis aeruginosa*. Journal of Korean Society on Water Quality. 1993;**9**:193-200. DOI: 10.1016/0041-0101(91)90254-0
- [29] Lee TG, Park SW, Yu TS, Kim J. The growth and coagulation characteristics of *Microcystis aeruginosa* during water treatment processes. Journal of the Korean Society of Water and Wastewater. 1998;6:33-42
- [30] Schindler DW. The dilemma of controlling cultural eutrophication of lakes. Proceedings of the Biological Sciences. 2012;279:4322-4333. DOI: 10.1098/rspb.2012.1032
- [31] Schindler DW, Hecky RE, Findlay DL, Stainton MP, Parker BR, Paterson MJ, et al. Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. Proceedings of the National Academy of Sciences of the United States of America. 2008;105:11254-11258. DOI: 10.1073/pnas.0805108105
- [32] Jacobson L, Halmann M. Polyphosphate metabolism in the blue-green alga, *Microcystis aeruginosa*. Journal of Plankton Research. 1982;4:481-488. DOI: 10.1093/plankt/4.3.481
- [33] Jung H-Y, Cho K-J. Environmental conditions of sediment and bottom waters near sediment in the downstream of the Nagdong River. Korean Journal of Limnology. 2003;36:311-321
- [34] Reynolds CS, Jaworski GHM, Cmiech HA, Leedale GF. On the annual cycle of the blue-green alga *Microcystis aeruginosa* Kütz. Emend Elenkin. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. 1981;293:419-476. DOI: 10.1098/rstb.1981.0081. Available from: http://www.jstor.org/stable/2395592

- [35] Conley DJ, Paerl HW, Howarth RW, Boesch DF, Seitzinger SP, Havens KE, et al. Controlling eutrophication: Nitrogen and phosphorus. Science. 2009;**323**:1014-1015. DOI: 10.1126/science.1167755
- [36] Ahn C-Y, Lee CS, Choi JW, Lee S, Oh H-M. Global occurrence of harmful cyanobacterial blooms and N, P-limitation strategy for bloom control. Korean Journal of Environmental Biology. 2015;33:1-6. DOI: 10.11626/KJEB.2015.33.1.001
- [37] Tempest D, Neijssel O, Zevenboom W. Properties and performance of microorganisms in laboratory culture; their relevance to growth in natural ecosystems. Symposium of the Society for General Microbiology. 1983;34:119-152
- [38] Paerl HW. Mitigating harmful cyanobacterial blooms in a human-and climatically-impacted world. Life. 2014;4(4):988-1012. DOI: 10.3390/life4040988
- [39] Crettaz Minaglia M, Rosso L, Aranda J, Goñi S, Sedan D, Andrinolo D, et al. Mathematical modeling of *Microcystis aeruginosa* growth and [D-Leu1] microcystin-LR production in culture media at different temperatures. Harmful Algae. 2017;67:13-25. DOI: 10.1016/j.hal.2017.05.006
- [40] Lürling M, Eshetu F, Faassen EJ, Kosten S, Huszar VLM. Comparison of cyanobacterial and green algal growth rates at different temperatures. Freshwater Biology. 2013;58(3):552-559. DOI: 10.1111/j.1365-2427.2012.02866.x
- [41] Paerl HW, Huisman J. Mini review: Climate change: A catalyst for global expansion of harmful cyanobacterial blooms. Environmental Microbiology Reports. 2009;**1**(1):27-37. DOI: 10.1111/j.1758-2229.2008.00004.x
- [42] O'Neil JM, Davis TW, Burford MA, Gobler CJ. The rise of harmful

- cyanobacteria blooms: The potential roles of eutrophication and climate change. Harmful Algae. 2012;**14**:313-334. DOI: 10.1016/j.hal.2011.10.027
- [43] Gorham PR. Toxic algae. In: Jackson DF, editor. Algae and Man. New York, N.Y: Plenum Press; 1964. pp. 307-336. DOI: 10.1007/978-1-4684-1719-7\_15
- [44] Runnegar MTC, Falconer IR, Jackson ARB, McInnes A. Toxin production by *Microcystis aeruginosa* cultures. Toxicon. 1983;**21**(Suppl 3):377-380. DOI: 10.1016/0041-0101(83)90233-7
- [45] Codd GA, Poon GK. Cyanobacterial toxins. Proceedings of the Phytochemical Society of Europe. 1988;28:283-296
- [46] Sivonen K. Effects of light, temperature, nitrate orthophosphate, and bacteria on growth and hepatotoxin production by *Oscillatoria agardhii* strains. Applied and Environmental Microbiology. 1990;**56**:2658-2666
- [47] Rapala J, Sivonen K, Lyra C, Niemela SI. Variation of microcystins, cyanobacterial hepatoxins, in *Anabaena* spp. as a function of growth stimuli. Applied and Environmental Microbiology. 1997;63:2206-2212
- [48] Lehman PW, Boyer G, Satchwell M, Waller S. The influence of environmental conditions on the seasonal variation of *Microcystis* cell density and microcystins concentration in San Francisco estuary. Hydrobiologia. 2008;**600**:187-204. DOI: 10.1007/s10750-007-9231-x
- [49] Wang Q, Niu Y, Xie P, Chen J, Ma ZM, Tao M, et al. Factors affecting temporal and spatial variations of microcystins in Gonghu Bay of Lake Taihu, with potential risk of microcystin contamination to human health. Scientific World Journal. 2010;10:1795-1809. DOI: 10.1100/tsw.2010.172

- [50] Giannuzzi L, Krockc B, Crettaz Minaglia MC, Rosso L, Houghton C, Sedan D, et al. Growth, toxin production, active oxygen species and catalase activity of *Microcystis aeruginosa* (Cyanophyceae) exposed to temperature stress. Comparative Biochemistry and Physiology, Part C. 2016;**189**:22-30. DOI: 10.1016/j.cbpc.2016.07.001
- [51] van der Westhuizen AJ, Eloff JN, Krüger GHJ. Effect of temperature and light (fluence rate) on the composition of the toxin of the cyanobacterium *Microcystis aeruginosa* (UV-006). Archiv für Hydrobiologie. 1986;**108**(2):145-154. DOI: 10.1007/BF00395897
- [52] Mowe M, Porojan C, Abbas F, Mitrovic S, Lim R, Furey A, et al. Rising temperatures may increase growth rates and microcystin production in tropical *Microcystis* species. Harmful Algae. 2015;**50**:88-98. DOI: 10.1016/j. hal.2015.10.011
- [53] Tsukada H, Tsujimura S, Nakahara H. Effect of nutrient availability on the C, N, and P elemental ratios in the cyanobacterium *Microcystis aeruginosa*. Limnology. 2006;7(3):185, 17-192. DOI: 10.1007/s10201-006-0188-7
- [54] Seviour RJ, Nielsen PH, editors. Microbial Ecology of Activated Sludge. London, UK: IWA Publishing; 2010. DOI: 10.2166/9781780401645
- [55] Ghaffar S, Stevenson RJ, Khan Z. Effect of phosphorus stress on *Microcystis aeruginosa* growth and phosphorus uptake. PLoS One. 2017;**12**(3):e0174349. DOI: 10.1371/journal.pone.0174349
- [56] Zwietering MH, de Koos JT, Hasenack BE, de Wit JC, van't Riet K. Modelling of the bacterial growth as a function of temperature. Applied and Environmental Microbiology. 1991;57:1094-1101
- [57] Zwietering MH, Jongenburger FM, Roumbouts M, Van't Riet K. Modelling

- of the bacterial growth curve. Applied and Environmental Microbiology. 1990;56:1875-1881
- [58] Whiting RC. Microbial modeling in foods. Critical Reviews in Food Science and Nutrition. 1995;35:467-494. DOI: 10.1080/10408399509527711
- [59] Giannuzzi L, Pinotti A, Zaritzky N. Mathematical modelling of microbial growth in packaged refrigerated beef stored at different temperatures. International Journal of Food Microbiology. 1998;**39**:101-110. DOI: 10.1016/s0168-1605(97)00127-x
- [60] Fujimoto N, Sudo R, Sugiura N, Inamori Y. Nutrient-limited growth of *Microcystis aeruginosa* and *Phomzidium tenue* and competition under various N:P supply ratios and temperatures. Limnology and Oceanography. 1997;42:250-256. DOI: 10.4319/lo.1997.42.2.0250
- [61] Lyck S. Simultaneous changes in cell quotas of microcystin, chlorophyll a, protein and carbohydrate during different growth phases of a batch culture experiment with *Microcystis aeruginosa*. Journal of Plankton Research. 2004;**26**(7):727-736. DOI: 10.1093/plankt/fbh071
- [62] Jiang Y, Ji B, Wong RNS, Wong NH. Statistical study on the effects of environmental factors on the growth and microcystins production of bloomforming cyanobacterium—*Microcystis aeruginosa*. Harmful Algae. 2008;7:127-136. DOI: 10.1016/j.hal.2007.05.012
- [63] Jähnichen S, Long BM, Petzoldt T. Microcystin production by *Microcystis aeruginosa*: Direct regulation by multiple environmental factors. Harmful Algae. 2001;**12**:95-104. DOI: 10.1016/j. hal.2011.09.002
- [64] Bortoli S, Oliveira-Silva D, KrügerdT, Dörra FA, Colepicolo P, VolmerD, et al. Growth and microcystin

- production of a Brazilian *Microcystis aeruginosa* strain (LTPNA 02) under different nutrient conditions. Revista Brasileira de Farmacognosia. 2014;**24**:389-398. DOI: 10.1016/j. bjp.2014.07.019
- [65] Canale RP, Vogel AH. Effects of temperature on phytoplankton growth. Journal of the Environmental Engineering Division, American Society of Civil Engineers. 1974;**100**:229-241
- [66] Reynolds CS. The Ecology of Freshwater Phytoplankton. Cambridge: Cambridge University Press; 1984. DOI: 10.1046/j.1365-2427.2002.00888.x
- [67] Shao J, Li R, Lepo J, Gu D. Review potential for control of harmful cyanobacterial blooms using biologically derived substances: Problems and prospects. Journal of Environmental Management. 2013;125:149-155. DOI: 10.1016/j.jenvman.2013.04.001
- [68] Anderson DM. Turning back the harmful red tides. Nature. 1997;**38**:513-514. DOI: 10.1038/41415
- [69] Jančula D, Marsálek B. Critical review of actually available chemical compounds for prevention and management of cyanobacterial blooms. Chemosphere. 2011;85:1415-1422. DOI: 10.1016/j.chemosphere.2011.08.036
- [70] Zamyadi A, Dorner S, Sauve S, Ellis D, Bolduc A, Bastien C, et al. Species-dependence of cyanobacteria removal efficiency by different drinking water treatment processes. Water Research. 2013;47:2689-2700. DOI: 10.1016/j. watres.2013.02.040
- [71] Matthijs HCP, Jančula D, Visser PM, Maršálek B. Existing and emerging cyanocidal compounds: New perspectives for cyanobacterial bloom mitigation. Aquatic Ecology. 2016;50:443-460. DOI: 10.1007/s10452-016-9577-0

[72] Shao J, Li R, Lepo J, Gu J. Potential for control of harmful cyanobacterial blooms using biologically derived substances: Problems and prospects. Journal of Environmental Management. 2013;125:149-155. DOI: 10.1016/j. jenvman.2013.04.001

[73] Li J, Shimizu K, Maseda H, Lu Z, Utsumi M, Zhang Z, et al. Investigations into the biodegradation of microcystin-lr mediated by the biofilm in wintertime from a biological treatment facility in a drinkingwater treatment plant. Bioresource Technology. 2012;**106**:27-35. DOI: 10.1016/j.biortech.2011.11.099

[74] Ame MV, Galanti LN, Menone ML, Gerpe MS, Moreno VJ, Wunderlin DA. Microcystin–LR,–RR,–YR and–LA in water samples and fishes from a shallow lake in Argentina. Harmful Algae. 2010;**9**(1):66-73. DOI: 10.1016/j. hal.2009.08.001

[75] Okano K, Shimizu K, Kawauchi Y, Maseda H, Utsumi M, Zhang Z, et al. Characteristics of a microcystin-degrading bacterium under alkaline environmental conditions. Journal of Toxicology. 2009:1-8. DOI: 10.1155/2009/954291

[76] Ridge I, Barrett PRF. Algal control with barley straw. Aspects of Applied Biology. 1992;**29**:457-462

[77] Schrader KK, de Regt MQ, Tidwell PR, Tucker CS, Duke SO. Selective growth inhibition of the musty-odor producing cyanobacterium *Oscillatoria* cf. chalybea by natural compounds. Bulletin of Environmental Contamination and Toxicology. 1998;**60**:651-658. DOI: 10.1007/s001289900676

[78] Nakai S, Inoue Y, Hosomi M, Murakami A. *Myriophyllum spicatum* released allelopathic polyphenols inhibiting growth of blue-green algae *Microcystis aeruginosa*. Water Research. 2000;**34**:3026-3032. DOI: 10.1016/s0043-1354(00)00039-7

[79] Mulderij G, Smolders Alfons JP, van Donk E. Allelopathic effect of the aquatic macrophyte, *Stratiotes aloides*, on natural phytoplankton. Freshwater Biology. 2006;**51**:554-561. DOI: 10.1111/j.1365-2427.2006.01510.x

[80] Planas D, Sarhan F, Dube L, Godmaire H, Cadieux C. Ecological significance of phenolic compounds of *Myriophyllum spicatum*. Verhandlungen des Internationalen Verein Limnologie. 1981;**21**:1492-1496. DOI: 10.1080/03680770.1980.11897219

[81] Li FM, Hu HY. Isolation and characterization of a novel antialgal allelochemical from *Phragmites communis*. Applied and Environmental Microbiology. 2005;**71**:6545-6553. DOI: 10.1128/aem.71.11.6545-6553.2005

[82] Gross EM, Meyer H, Schilling G. Release and ecological impact of algicidal hydrolysable polyphenols in *Myriophyllum spicatum*. Phytochemistry. 1996;**41**:133-138. DOI: 10.1016/0031-9422(95)00598-6

