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Mechanism of the influence of hydrodynamics on *Microcystis aeruginosa*, a dominant bloom species in reservoirs

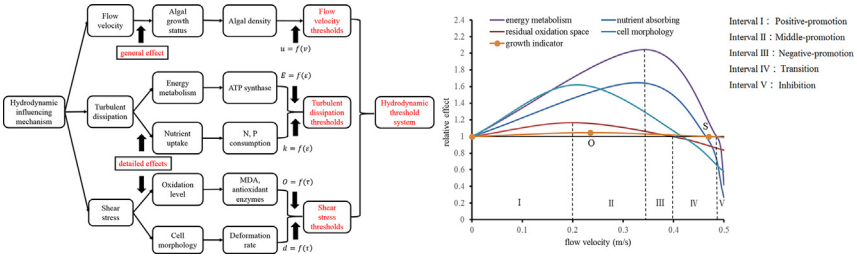
Yang Song, Ling-Lei Zhang *, Jia Li, Min Chen, Yao-Wen Zhang

Institute of Ecology and Environment, State Key Laboratory of Hydraulics and Mountain River Engineering, College of Water Resource & Hydropower, Sichuan University, Chengdu, Sichuan, China

HIGHLIGHTS

- Experiments and numerical simulations were combined to study the effects of hydrodynamics on algal growth.
- Algal growth was promoted by moderate hydrodynamic conditions but was inhibited by high hydrodynamic conditions.
- High shear stress can damage algal cell morphology.
- This study contributes to the development of an algal cell morphology evaluation system.
- The hydrodynamic threshold system for *M. aeruginosa* was obtained.

GRAPHICAL ABSTRACT



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ABSTRACT

Hydrodynamic conditions play a key role in algal blooms, which have become an increasing threat to aquatic environments, especially reservoirs. *Microcystis aeruginosa* is a dominant species in algal blooms in reservoirs and releases large amounts of algal toxins during algal bloom events. The algal growth characteristics and the corresponding mechanism of the influence of hydrodynamic conditions were explored using custom hydraulic rotating devices. The long-term experimental results were as follows: (1) a moderate flow velocity increased the algal growth rate and prolonged algal lifetime relative to static water; (2) moderate water turbulence promoted energy metabolism and nutrient absorbance in algal cells; (3) moderate shear stress reduced oxidation levels in algal cells and improved algal cell morphology; (4) under hydrodynamic treatment, algal cell deformation was confirmed by scanning electron microscopy (SEM), and a high shear stress of 0.0104 Pa induced by a flow of 0.5 m/s may have destroyed cell morphology and disturbed reactive oxygen species (ROS) metabolism; (5) algal cell morphology evaluation (including circle ratio, eccentricity, diameter increasing rate, and deformation rate) was established; (6) based on algal growth status and specific effects, five independent intervals (including 'positive-promotion', 'middle-promotion', 'negative-promotion', 'transition', and 'inhibition') and the hydrodynamic threshold system (including flow velocity, turbulent dissipation, and shear stress) were established; and (7) for *M. aeruginosa*, the optimum flow velocity was 0.24 m/s, and the static-equivalent flow velocity was 0.47 m/s. These results provide a basic summary of the hydrodynamic effects on algal growth and a useful reference for the control of *M. aeruginosa* blooms in reservoirs.

1. Introduction

Harmful algal blooms have become an increasingly serious threat to aquatic environments and water resources (Gardner et al., 2017;

* Corresponding author.
E-mail address: zhanglinglei@hotmail.com. (L.-L. Zhang).

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Watson et al., 2016; Catherine et al., 2013). Blooms are more likely to occur in lakes and reservoirs because of their low flow velocity, long flushing time and low self-cleaning capacity (Herman et al., 2017; Mao et al., 2015). Algal blooms mainly depend on nutrients, light, temperature and hydrodynamic conditions (Li et al., 2014; Long et al., 2011). Among these factors, hydrodynamic conditions are often the easiest to regulate and control, especially in reservoirs. Therefore, many scholars have concentrated on hydrodynamic conditions (Ruiz-Villarreal et al., 2016; King, 2014), which might be a more dominant and controllable factor than external factors, such as nutrients. From previous studies, it can generally be concluded that hydrodynamic conditions promote algal growth in three ways: 1) low flow velocity promotes algal growth (Špoljar et al., 2017); 2) appropriate shear stress increases algal productivity and stimulates metabolism (Hadiyanto et al., 2013); and 3) moderate water turbulence increases nutrient uptake in algal cells (Xiao et al., 2016). These studies concentrated mostly on the growth of algal biomass, whereas the effects of hydrodynamics on the algal lifecycle, especially the decline of algal biomass, were less explored. In addition, physiological changes in algal cells and specific effects, such as cell deformation caused by hydrodynamic conditions, have rarely been evaluated in these reports. Thus, the hydrodynamic thresholds for specific growth characteristics remain unclear. Moreover, the mechanisms by which hydrodynamic conditions influence algal cells remain to be explored, especially from the perspectives of physiology and morphology.

As a widely distributed and common toxic cyanobacteria, *Microcystis aeruginosa* is a high-profile species and has been studied by many researchers. Specifically, when algal blooms occur, *M. aeruginosa* can produce a green, foul-smelling floating membrane, and it often releases toxins (Liang et al., 2017). Previous studies on the control of algal blooms have mainly focused on physical injury, biochemical methods and UV irradiation (Barrado-Moreno et al., 2017; Visser et al., 2016; He et al., 2016; Sun et al., 2015; Chen et al., 2009). These methods typically have limited scopes and high costs. Hydrodynamic operation is a convenient, effective and economic in situ method of controlling algal blooms in reservoirs. However, research on the use of hydrodynamic operation for preventing and controlling algal blooms is still in its infancy. Ji et al. (2017) and Lian et al. (2014) reported that increasing the fluctuation frequency of water levels to create an adverse hydraulic environment could effectively control algal blooms. According to their results, current reservoir operations are more similar to conceptual approaches and lack operational principles. The main reason is that the details of the influencing mechanism of hydrodynamic conditions for algae are uncertain and insufficient. In addition, hydrodynamic thresholds, such as the optimum turbulent dissipation and harmful shear stress, are not clear, and this lack of information presents obstacles for formulating operational principles. Therefore, understanding the influencing mechanisms and establishing a hydrodynamic threshold system can provide a theoretical basis for reservoir operations to control algal blooms.

In this study, *M. aeruginosa*, the dominant species of algal blooms in reservoirs, was chosen as the research object. We examined its internal physiology and cell morphology as well as its external environmental indicators to explore the specific effects and influencing mechanism of hydrodynamic conditions on algal growth. The goals were (1) identify changes in algal biomass, physiology and morphology caused by hydrodynamic treatment; (2) explore specific effects (i.e., energy metabolism, nutrient uptake, oxidation level and cell morphology) of hydrodynamic conditions on algal cells; and (3) determine the influencing mechanism of hydrodynamic conditions and develop a hydrodynamic threshold system for *M. aeruginosa*.

2. Materials and methods

2.1. Algae and culture conditions

Microcystis aeruginosa FACHB-315 was obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology (Wuhan, China). Healthy algal cultures were selected, and equal amounts were transferred into six individual custom hydraulic rotating apparatuses that were filled with 10 L of BG-11 medium. The temperature of the medium was 25 °C, and the light intensity was 2000 Lux for the 90-day experiment. The experimental apparatus contained an inner cylinder and an outer cylinder, which created a specific angular velocity when the inner cylinder and its four blades rotated. The angular velocities were 0, 5, 8, 11, 14, and 17 rad/s for the six experimental apparatuses, respectively. Algae under 0 rad/s was regarded as the control group.

The numerical model of the experimental apparatus and the simulated flow field are shown in Fig. 1, which also adequately describes the experimental apparatus under real conditions. The real apparatus was constructed of polymethyl methacrylate and contained both a rotating inner cylinder and an outer cylinder. A speed-regulating motor was connected to the inner cylinder and was used to create a specific hydrodynamic condition for each apparatus. The diameter of the inner cylinder was 11 cm with four blades (1.3 cm × 0.5 cm) and was 2.7 cm from the bottom. The diameter of the outer cylinder was 28 cm with a height of 35 cm. Algal biomass was determined following the method of P. Li et al. (2015). The initial algal density for each experimental group was controlled at 2–3 × 10^8 cells/L.

2.2. Experimental design and analytical methods

A sample of 50 mL of algae solution was obtained from each experimental group every six days and was used for further testing. External water quality parameters, including chlorophyll *a* (Chl *a*), total nitrogen (TN) and total phosphorus (TP), were determined following the method of China's national standard (Wei et al., 2002). Turbidity (TU) was estimated by a portable device (HACH-2100Q). The malondialdehyde (MDA) content and the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and adenosine triphosphate (ATP) synthase in algal cells were measured by using commercial kits purchased from NanJing JianChen Bioengineering Institute in China. The MDA content was expressed as nmol/10^8 cells, and each enzyme activity listed above was expressed as unit/10^8 cells. For the purpose of exploring algal growth characteristics, we defined several parameters.

1) The algal growth rate was defined as

$$u = \frac{\ln X_t - \ln X_{t_0}}{t}, \tag{1}$$

where X_t is the maximum algal density of logarithmic growth phase (cells/L), X_{t_0} is the initial algal density at the beginning of the logarithmic growth phase (cells/L), and t is the duration of the logarithmic growth phase (d).

2) Energy metabolism rate was defined as

$$E = \overline{A}, \tag{2}$$

where $\overline{A}$ is the average of ATP synthase activity (unit/10^8 cells) in each group during the 90-day experiment.

3) Nutrient consumption rate in the medium effectively reflected the nutrient uptake rate of algae and was defined as

$$k = \frac{1}{2}\left(\frac{N_{90} - N_0}{N_0} + \frac{P_{90} - P_0}{P_0}\right), \tag{3}$$

where N_{90} and P_{90} are the TN and TP concentrations (mg/L), respectively, in the medium at the end of the experiment; and N_0 and P_0 are