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# **Experimental Study of the Quantitative Impact of Flow Turbulence on Algal Growth**

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Abstract: Flow turbulence has been widely accepted as one of the essential factors affecting phytoplankton growth. In this study, laboratory cultures of Microcystis aeruginosa in beakers were carried out under different turbulent conditions to identify the quantitative relationship between the algal growth rate and the turbulent intensity. The turbulent intensity (represented by energy dissipation rate,  $\varepsilon$ ) was simulated with the software FLUENT. Daily measurement of the two parameters (algal biomass and chlorophyll-a concentration) was carried out during the experimental period to represent the algal growth rate. Meanwhile, the rates of photosynthetic oxygen evolution and chlorophyll fluorescence intensity were calculated to investigate the photosynthetic efficiency. The results indicated that the growth rate of Microcystis aeruginosa became higher in the turbulent environment than in the still water environment under the designed experimental conditions. The peak growth rate of *Microcystis aeruginosa* occurred when  $\varepsilon$  was  $6.44 \times 10^{-2} \text{ m}^2/\text{s}^3$ , over which the rate declined, probably due to unfavorable impacts of strong turbulence. In comparison, the maximum rate of photosynthetic oxygen evolution occurred when  $\varepsilon$  was 0.19 m<sup>2</sup>/s<sup>3</sup>. Based on the findings of this study, an exponential function was proposed in order to incorporate the effect of flow turbulence into the existing algal growth models, which usually just consider the impacts of nutrient availability, illumination, and temperature.

Keywords: flow turbulence; Microcystis aeruginosa; algal biomass; chlorophyll-a; growth rate

# 1. Introduction

Rapid industrialization has resulted in severe environmental pollution, and bluegreen algal blooms have become a global phenomenon with increasingly remarkable intensity and frequency throughout the world in recent decades. Microcystis aeruginosa is the most typical bloom-forming freshwater cyanobacteria in freshwater all over the world [1]. Microcystis blooms can initiate severe environmental and ecological events, causing blockage of drinking water supply systems, producing unpleasant odors, reducing water clarity, removing dissolved oxygen during decomposition, and so on [2]. Some species of Microcystis aeruginosa are potentially toxic and can produce microcystins, which may pose severe health risks to humans and other mammals [3]. Many studies have been conducted to explore the influence mechanism to solve algal blooms [4,5]. It is generally believed that significant influence factors of water eutrophication are climate [6] (illumination, temperature, etc.), nutrients [7,8], and hydrodynamic conditions [9–11]. The effect of hydrodynamics on algae is reflected in two aspects: hydraulic flush and the effect of small-scale turbulence on algal growth. Some researchers have argued about the influence of hydrodynamic force on algae's physiological and ecological characteristics, such as cell division, cell volume, cell morphology, and photosynthetic characteristics [12–14].



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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). The turbulent intensity is characterized by many parameters, such as energy dissipation rate  $\varepsilon$  [15,16], Reynolds number Re [17], shear stress  $\tau$  [18], and so on, among which the most common parameter is turbulent energy dissipation rate. It was reported that the turbulent energy dissipation rate was an important variable in studying the effects of small-scale fluid flow on microbial physiology at the cellular level [19], which reflected the energy transfer in the turbulent environment, from large-scale to small-scale and ultimate dissipation due to molecular adhesion. In addition, hydrodynamic influences would be covered up by other factors due to complicated influence factors in natural water bodies. Therefore, a single-factor method was adopted in the laboratory experiments in most research studies. Sullivan et al. reported that the division rate of *Lingulodinium polyedrum* cells increased with the increase of  $\varepsilon$  from  $10^{-8}$  m<sup>2</sup>/s<sup>3</sup> to  $10^{-4}$ m<sup>2</sup>/s<sup>3</sup> [12]. Missaghi found that the cell biomass of *Microcystis aeruginosa* reached the maximum at  $\varepsilon$ ~8.0 ×  $10^{-5}$  m<sup>2</sup>/s<sup>3</sup> [20].

Apart from field observation and laboratory experiments, many researchers are devoted to the development of mathematical models. The flow velocity influence functions of algal growth were proposed by analyzing algal data of different areas [21–23]. However, the research results showed poor comparability due to different parameters selected to characterize the turbulent intensity in laboratory experiments and mathematical models.

Though many scholars have investigated the impact of small-scale hydrodynamicor wind-induced turbulence on algal growth to unveil the natural phenomena through observations and tests, the influence mechanism still remains unclear. In addition, there are few mathematical functions available to describe the effect of flow turbulence on *Microcystis aeruginosa* growth. In this paper, laboratory experiments were conducted to simulate the growth of *Microcystis aeruginosa* under different turbulent conditions. The influence of hydrodynamics characterized by turbulent intensity on the growth of *Microcystis aeruginosa* was analyzed, and a mathematical function to quantify the impact of flow turbulence on *Microcystis aeruginosa* growth was proposed.

# 2. Materials and Methods

# 2.1. Algae

*Microcystis aeruginosa* FACHB 905 was purchased from the Freshwater Algae Culture Collection of the Institute of Hydrobiology (FACHB), Chinese Academy of Sciences, Wuhan, China. Before the experiment, algae cells were cultured in BG11 (Table 1) medium under light intensity 4000 lx and temperature 26 °C for one week until the logarithmic growth phase.

	Component	Content
1	NaNO <sub>3</sub>	1.5 g/L
2	$K_2HPO_4$	0.04 g/L
3	MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.075 g/L
4	CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.036 g/L
5	Citric acid	0.006 g/L
6	Ferric ammonium citrate	0.006 g/L
7	EDTANa <sub>2</sub>	0.001 g/L
8	Na <sub>2</sub> CO <sub>3</sub>	0.02 g/L
9	A5 (Trace metal solution)	1  mL/L

Table 1. The medium formula of BG11.

#### 2.2. Experimental Apparatus and Methods

In the experiment, 2000 mL beakers were placed in an artificial climate chamber with a magnetic stirrer to control the turbulent intensity by adjusting the rotor speed. Turbulent intensity was set in the range of 100–200 RPM due to the magnetic stirrer's limitation, and was conducted at 0, 100, 150, and 200 RPM. The experiments were carried out in an artificial climate chamber under constant temperature (25.8 °C), light intensity (4000 lx), and light/dark ratio (12 h/12 h). A certain amount of medium and algae in the logarithmic

growth phase were added to the 2000 mL beakers. From the day of inoculation, samples were taken simultaneously every day, and then the medium was supplemented to 1800 mL. Cell density, chlorophyll-a concentration, and the rate of photosynthetic oxygen evolution were measured every day at the same time. The test process continued until the stable phase. The triplicate experiments were conducted at constant temperature and light. The medium and vessels were sterilized with an autoclave before the investigation. In addition, the experiment was conducted under axenic conditions.

The cell density (cells/mL) was measured with a hemocytometer and optical microscope (CX21, Olympus America Inc., Melville, NY, USA), while the chlorophyll-a concentration ( $\mu$ g/L) and the chlorophyll fluorescence intensity (Chla-f) were measured using PHYTO-PAM (Heinz Walz GmbH, Eichenring, Germany). A liquid oxygen electrode (CHLOROLAB 2, Hansatech, Norfolk, UK) was used to determine the rate of photosynthetic oxygen evolution ( $\mu$ mol O<sub>2</sub>/(mg Chl-a × h)).

#### 2.3. Statistical Analysis and Software

The data were presented as mean  $\pm$  standard deviation, with one-way ANOVA to analyze the differences in the growth rate among all groups, with *p* < 0.05 as the level of significance. Origin (OriginLab, Northampton, MA, USA) and MATLAB (MATLAB, R2018a, MathWorks) were adopted for data analyses. The commercial computational fluid dynamics (CFD) simulation software FLUENT (Ansys Fluent Inc., Lebanon, NH, USA) was used to compute the turbulent energy dissipation rate  $\varepsilon$ .

#### 3. Results

## 3.1. The Distribution of Turbulent Intensity

The energy dissipation rates ( $\epsilon$ ) of the magnetic rotor under different rotor speeds were estimated by FLUENT (Table 2). CFD software Fluent was used for 1:1 3D-modelling, and the number of model mesh was  $2 \times 10^6$ , with default settings for boundary conditions. Figure 1 shows the turbulent intensity distribution in a beaker with the magnetic stirrer speed at 200 RPM.

	The Energy Dissipation Rate $\varepsilon/(m^2/s^3)$		
Rotor Speed n/RPM	Average	Maximum	Minimum
100	$7.40  imes 10^{-3}$	4.58	$3.78 imes10^{-5}$
150	$6.44 imes10^{-2}$	15.82	$4.36 imes10^{-5}$
200	0.19	44.31	$3.29 imes10^{-4}$
300	0.62	155.51	$1.74 imes10^{-3}$
400	1.50	377.35	$4.18 imes10^{-3}$

**Table 2.** Rotor speeds (n) and energy dissipation rates ( $\varepsilon$ ).

As shown in Figure 1, the turbulent intensity is distributed uniformly in the beaker's upper part at 200 RPM. The mean, maximum, and minimum values are 0.19 m<sup>2</sup>/s<sup>3</sup>, 44.31 m<sup>2</sup>/s<sup>3</sup>, and 3.29 × 10<sup>-4</sup> m<sup>2</sup>/s<sup>3</sup>, respectively. It was reported that the range of oceanic turbulence energy dissipation rate was  $2.8 \times 10^{-11}$ – $47 \times 10^{-3}$  m<sup>2</sup>/s<sup>3</sup>, plain rivers  $10^{-6}$ – $10^{-3}$  m<sup>2</sup>/s<sup>3</sup>, and lakes  $10^{-9}$ – $10^{-6}$  m<sup>2</sup>/s<sup>3</sup> [24–26]. According to Table 2, the range of  $\varepsilon$  at 7.4 × 10<sup>-3</sup>–0.19 m<sup>2</sup>/s<sup>3</sup> in the experiment covers the turbulent intensity range of natural water, which implies that the test turbulence range set in the investigation is reasonable.



Figure 1. Turbulent intensity distribution in a beaker at 200 RPM.

#### 3.2. The Effect of Turbulence on Algal Growth

Figure 2a shows that the algal growth process is approximately the same, with algal cells' lag growth phase 1, followed by logarithmic growth phase 2, and the final stationary growth phase 3. During the first five days post-inoculation, there was no noticeable difference in growth curves irrespective of various turbulent intensities. Figure 2b shows that the experimental groups are significantly higher than the control groups from the 6th day onwards (p < 0.05). Furthermore, in the range of <150 RPM, cell density gradually increased with turbulent intensity, and algal growth decreased when turbulent intensity was higher than 150 RPM. When turbulent intensity was 150 RPM, algal growth was in the optimum state. Figures 3 and 4 show a similar result: the average specific growth rate and chlorophyll-a concentration under 150 PRM are higher than other groups. It could be concluded that different turbulent intensities have positive effects on algal growth, with 150 RPM ( $\varepsilon = 6.44 \times 10^{-2} \text{ m}^2/\text{s}^3$ ) corresponding to the peak algal growth rate.

Growth rate  $\mu = \frac{ln\left(\frac{x_n}{x_{n-1}}\right)}{t_n - t_{n-1}}$ , where  $x_n$  is algal biomass for the day;  $x_{n-1}$  is biomass for the last day; and  $t_{n-1}$  is cultivation time corresponding to  $x_n$  and  $x_{n-1}$ . Error bars represent the SD of triplicate samples. Different letters indicate significance between different treatments.

Figure 4a shows that the changing trend of chlorophyll-a concentration is roughly consistent with algal biomass. At the initial stage of culture, due to *Microcystis aeruginosa* in the adaptation phase, only a small amount of chlorophyll-a was synthesized. After six days of culture, all experimental groups entered the logarithmic growth phase, with a logarithmical increase of chlorophyll-a concentration. Moreover, Figure 4b shows that the chlorophyll-a concentration of experimental groups was significantly higher than the control group (p < 0.05). In addition, the Chl-a concentration variation was different from that of the algae number. We assume that algal cells still accumulate materials such as chlorophyll in the stable phase, but algae cell numbers no longer increase due to the limitation of environmental conditions.

## 3.3. The Effect of Turbulence on Physiological Characteristics

Figure 5 shows that the photosynthetic oxygen evolution rate has a trend of increasing first and then decreasing. Algae cells were in the lag phase on the 3rd day, and the rate of the control group was lower significantly (p < 0.05) than the experimental groups. Compared

with the control group, the photosynthetic oxygen evolution of each experimental group increased to varying degrees. The photosynthetic oxygen evolution rate increased from  $135.51 \pm 15$  to  $384.40 \pm 55 \ \mu mol \ O_2/(mg \ Chl-a \times h)$  and increased by 175.53% under 200 RPM, which indicated that the algae photosynthetic rate at the lag growth phase would be remarkably promoted by turbulence. On the 6th day, algae entered the logarithmic growth phase. All of the groups' photosynthetic rates increased. When algal cells were at the logarithmic growth middle phase on the 9th day, experimental groups' photosynthetic rates decreased a lot. Moreover, the under-200-RPM group's rate was a little higher than other groups. This is probably because algal biomass increased rapidly with chlorophyll synthesis, which resulted in severe mutual shading in the culture medium, chlorophyll unavailable to illumination, and chlorophyll's low activity [27]. Figure 6 shows the ratio of variable to maximal chlorophyll fluorescence (Fv/Fm) versus time as a reflection of photosystem II activity. Overall, the control group and experimental groups' chlorophyll fluorescence intensities showed a tendency to rise, plateau, decrease, then remain steady. There was no apparent difference between the control group and the experimental groups within the first eight days; however, the experimental groups' chlorophyll fluorescence intensities became significantly higher than the control group starting from the 12th day (p < 0.05), with little difference between the experimental groups, illustrating the beneficial effect of turbulence for the photosystem II activity of Microcystis aeruginosa.



**Figure 2.** Cell density (**a**) and the 6th-day density ranges (**b**) under different turbulent intensities. Error bars represent the standard deviation (SD) of triplicate samples. Some error bars are too small to show. Different letters indicate significance between different treatments (p < 0.05).



**Figure 3.** *Microcystis aeruginosa*'s growth rate under different turbulent intensities. Different letters indicate significance between different treatments (p < 0.05).



**Figure 4.** Chl-a concentration of *Microcystis aeruginosa* (**a**) and its value ranges (**b**) over the span of 18 days of incubation under different turbulence conditions. Error bars represent the SD of triplicate samples. Some error bars are too small to show. Different letters indicate significance between different treatments (p < 0.05).



**Figure 5.** The rate of photosynthetic oxygen evolution of *Microcystis aeruginosa* under different turbulence conditions. Error bars represent the SD of triplicate samples. Different letters indicate significance between different treatments (p < 0.05).



**Figure 6.** The chlorophyll fluorescence intensity of *Microcystis aeruginosa* under different turbulence conditions. Error bars represent the SD of triplicate samples.

# 3.4. The Turbulence Effect Exponential Function

Flow turbulence has important effects on algal growth [15,17,24,26] and must be considered in the study of water eutrophication. In the current dynamic ecological model, the effect of turbulence on algal growth is usually not considered. In this study, an

exponential function for algae growth was developed to analyze the effects of turbulence on algae growth. The turbulent intensity was represented by the energy dissipation rate,  $\varepsilon$ :

$$f(\varepsilon) = c e^{\frac{-(\varepsilon-a)^2}{b}}$$
(1)

where  $\varepsilon$  is the turbulence energy dissipation rate; *a* is optimum  $\varepsilon$  for algal growth; *b* is an adjustment coefficient of turbulence's effect on algal growth; and *c* is the turbulence coefficient at the optimal turbulence, which generally is 1. The flow turbulence promoted the growth of *Microcystis aeruginosa* compared with the controlled groups (0 RPM) from experimental results. In addition, when the turbulent intensity was  $6.44 \times 10^{-2} \text{ m}^2/\text{s}^3$ (150 RPM), the algal growth rate reached the maximum. As shown in Figure 7, the function fits well with the results:  $f(\varepsilon)$  reaches the maximum when  $\varepsilon = a$ . The value of  $f(\varepsilon)$  also increases with the increase of  $\varepsilon$  when  $\varepsilon < a$ , and decreases when  $\varepsilon > a$ . Furthermore, the parameters of the function were calculated and validated with independent experimental data:  $a = 6.44 \times 10^{-2} \text{ m}^2/\text{s}^3$ ,  $b = 9.774 \times 10^{-3} \text{ m}^4/\text{s}^6$ , and c = 1. Some researchers found that low turbulent intensity promoted algal growth and high turbulent intensity decreased algal growth [16,18,28]. The function may also describe these trends of algal growth with turbulent intensity.



**Figure 7.** Turbulence influence function diagram (the red circles are the values obtained from the current study).

## 4. Discussion

The results suggest different effects on *Microcystis aeruginosa* growth corresponding to various turbulence conditions, in particular the scenario of slight turbulence, which is consistent with Hondzo's findings of a nearly 2-fold increase in the growth rate of *Selenastrum capricornutum* achieved for an energy dissipation rate  $10^{-7}$  m<sup>2</sup>/s<sup>3</sup> [16]. Such promotion is probably due to two mechanisms: the augmentation of both nutrient absorption and algal photosynthetic efficiency.

Karp-Boss defined an area around algal cells as the diffusion boundary layer, where the nutrient concentration is less than 90% of the surrounding concentration. Uniform flow and shear flow could distort the diffusion boundary layer and make the concentration gradient steeper [29]. Moreover, Hondzo reported that flow turbulence could cause algal extracellular diffusion layer thinning and could facilitate the transport of nutrients to algal cells [16]. In addition, it was reported that turbulence affected algal growth through algal photosynthesis efficiency. Mitsuhashi found that turbulence shear effectively reduced *Chlorella's* photosynthesis efficiency [18]. Li reported that different turbulent intensities promoted the photosynthetic activity of algal cells. In this study, the rate of photosynthetic oxygen evolution was larger under turbulence conditions than the control group (0 RPM) in both the lag growth and logarithmic growth phases. However, there was little difference in algal cell photosystem II activity with the increase of turbulent intensity. It is therefore indicated that strong turbulence may have no adverse effect on the photosynthetic activity of *Microcystis aeruginosa*, which means the decrease of *Microcystis aeruginosa*'s growth could not be explained from the view of photosynthesis.

Some studies have also suggested turbulence has an effect on the division and proliferation of algal cells. Sullivan discovered that the cell division rate of *Lingulodinium polyedrum* tended to present as a straight climb when  $\varepsilon$  increased from  $10^{-8} \text{ m}^2/\text{s}^3$  to  $10^{-4} \text{ m}^2/\text{s}^3$ . When  $\varepsilon$  reached  $10^{-3} \text{ m}^2/\text{s}^3$ , the rate decreased noticeably [12]. Bolli held that strong turbulence ( $\varepsilon = 2.7 \times 10^{-3} \text{ m}^2/\text{s}^3$ ) had an inhibitory effect on the cyst proliferation of *Alexandrium minimum* and *A. catenella* [30]. *Microcystis aeruginosa* cells were unicellular cells that undergo cell division to produce daughter cells [31]. In the experiment, the average growth rate of *Microcystis aeruginosa* first increased and then decreased with the increase of turbulent intensity. The peak value occurred when the turbulent intensity was  $6.44 \times 10^{-2} \text{ m}^2/\text{s}^3$  (150 RPM), over which the increasing rate was curbed instead. It is therefore inferred that the turbulence may slow down the growth of *Microcystis aeruginosa* by inhibiting cell division.

Laboratory simulations and microcosms can provide a more controlled analysis and evaluation of phenomena in the natural environment that may not be possible in situ. However, lab simulations may introduce conditions and circumstances with an inevitable discrepancy from the natural environment. For example, the vortices in a fluid-filled beaker generated by a stirring bar may not be equivalent to more straight-line currents in a larger volume in the natural environment. Some laboratory-generated vortices may partially simulate natural spiral eddies that are set up in a natural aquatic environment. However, other experimental lab apparatuses (such as the impact forces of the impeller stir bar on the suspended algae) would not necessarily be present in the natural environment. Some of these aspects of the laboratory experiment should be considered in the future. The current results are based on the experiments of *Microcystis aeruginosa*, and the effect of flow turbulence on other algae species remains to be explored.

# 5. Conclusions

Laboratory cultures of *Microcystis aeruginosa* in beakers were carried out under different turbulent conditions to identify the quantitative relationship between the algal growth rate and the turbulent intensity. The results indicated that flow turbulence could promote the growth of *Microcystis aeruginosa* and algal photosynthetic activity. Both the chlorophyll-a concentration and algal biomass increased under the designed turbulent condition (energy dissipation rate  $\varepsilon$  of 7.40 × 10<sup>-3</sup>, 6.44 × 10<sup>-2</sup>, and 0.19 m<sup>2</sup>/s<sup>3</sup>) compared with the control group. However, the peak growth rate of *Microcystis aeruginosa* occurred when  $\varepsilon$  was 6.44 × 10<sup>-2</sup> m<sup>2</sup>/s<sup>3</sup>, over which the rate declined, probably due to unfavorable impacts of strong turbulence. In comparison, the maximum rate of photosynthetic oxygen evolution occurred when  $\varepsilon$  was 0.19 m<sup>2</sup>/s<sup>3</sup>. Based on the results, an exponential function was proposed to represent the effect of flow turbulence on *Microcystis aeruginosa* growth. Three parameters were included in the function to define the algal growth pattern in the turbulent environment. The parameter values for defining the growth pattern of *Microcystis aeruginosa* were further calibrated with the experiment results in the study.

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