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Experimental investigation on the mechanism of the effect of flow velocity on *Cyclotella meneghiniana*

Jianwei Zhang[1], Hongdong Ma[1], Gangfu Song[2]✉, Yitong Ding[1] & Sitong Guo[2]

To investigate the growth patterns and influencing mechanisms of algal cells in the Henan section of the Middle route of South-to-North Water Transfer project under varying flow velocities, we focused on studying the dominant diatom species in this region. Utilizing self-designed experimental devices, a flow rate range of 0 to 1.0 m s^{-1} was established, and the growth conditions of *Cyclotella meneghiniana* were recorded for each group. The findings revealed that under different flow rates, *C. meneghiniana* exhibited a critical flow rate threshold at 0.4 m s^{-1}, demonstrating an overall trend characterized by 'when the flow velocity is relatively low, an increase in flow velocity will promote the growth of *C. meneghiniana*. However, when the flow velocity is relatively high, an increase in flow velocity will instead inhibit the growth of *C. meneghiniana*.' By combining these experimental results with theoretical analysis, we explored the underlying mechanism behind the influence of flow velocity on algal cells. Our experiments demonstrated that below the critical flow rate, increased fluid velocity enhanced nutrient absorption by promoting contact between algal cells and nutrients, thereby facilitating algal cell growth. However, as fluid shear stress intensified with higher flow velocities, it eventually caused mechanical damage to cell structures leading to a critical threshold being reached. These research outcomes provide valuable insights into understanding how water dynamics impact algae cell growth while offering technical support for controlling algae proliferation based on principles derived from water dynamics within the Henan section of South-to-North Water Transfer project.

Keywords Hydrodynamics, Velocity gradient, *Cyclotella meneghiniana*, Flow velocity-mechanism of algal cell influence

The Middle route of the South-to-North Water Diversion Project constitutes a significant component within China's "four horizontal and three vertical" water network, traversing the provinces of Henan and Hebei, as well as the municipalities of Beijing and Tianjin[1]. The Middle Route project has successfully delivered approximately 55 billion cubic meters of water directly to over 85 million individuals up until this point[2]. The resolution of water resource imbalance in China and the promotion of sustainable national economic development constitute a pivotal strategic fundamental project, which plays a crucial role in facilitating the optimal allocation of water resources within the country[3]. With the exacerbation of global climate change, issues such as significant disparities in ground temperature, frequent occurrences of extreme weather events, and impaired water flow due to drought will augment the frequency of algal blooms[4,5]. In recent years, the phenomenon of algae growth has garnered significant attention from both domestic and international scholars, leading to a plethora of studies investigating the frequency and fundamental conditions underlying algal bloom occurrences[6–8]. However, there is a paucity of research on the correlation between algae and hydrodynamics in large-scale water diversion projects. Currently, the excessive proliferation of algae organisms in the middle route project significantly impacts the water quality of the South-to-North Water Diversion Project. The Middle Route project spans 731 km within Henan Province, constituting approximately half of its total length (Fig. 1). Hence, it is imperative to investigate the governing principles and mechanisms underlying dominant algae species' response to flow velocity gradients in the Henan section for effective inhibition of algal growth based on hydrodynamic principles during later stages of engineering.

Previous studies have primarily focused on investigating the impact of inorganic salts, temperature, and light on algae growth. However, recent attention from scholars has gradually shifted towards exploring the relationship between hydrodynamics and algae growth. It has been demonstrated that flow rate plays a significant role in

[1]School of Water Resources, North China University of Water Resources and Electric Power, Zhengzhou 450046, China. [2]School of Environmental and Municipal Engineering, North China University of Water Resources and Electric Power, Zhengzhou 450046, China. ✉email: 2468083230@qq.com

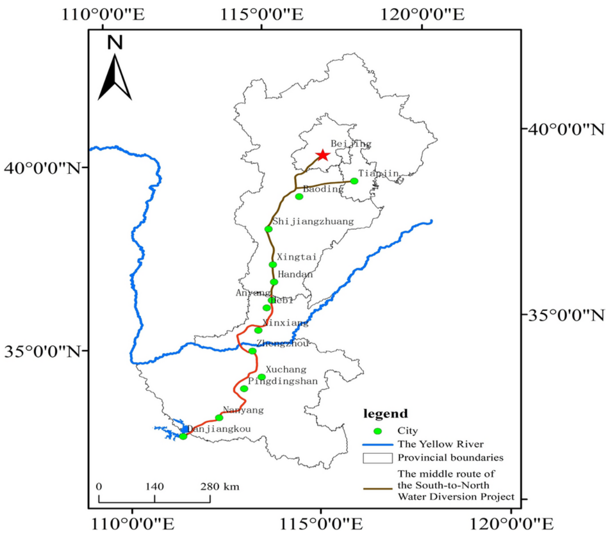

Fig. 1. Schematic diagram of the middle route of the South-to-North Water Diversion project. This map was created using ArcGIS 10.4 (https://www.esri.com) based on data from Natural Earth Data (https://www.naturalearthdata.com/).

influencing algae growth. For instance, some researchers have conducted indoor simulation experiments to examine the effect of velocity on algal cell metabolism and have identified the optimal growth velocity for algal cells under experimental conditions[9]. Through simulation experiments, researchers have discovered that a lower flow rate promotes the growth of algae, whereas a higher flow rate inhibits it; however, the precise critical flow rate required to effectively control algae growth remains uncertain[10,11]. Chinese scholars with expertise in the field have observed through systematic monitoring that alterations in hydrodynamic conditions resulting from the diversion of water along the middle route of the South-to-North Water Transfer project lead to discernible changes in the composition of algae communities[12]. The photosynthetic intensity of *Cyclotella meneghiniana* cells exhibited significant variations under different flow velocity conditions, as observed by several scholars[13]. By simulating water disturbance, experts in the relevant fields have discovered significant variations in the stress response of different algae to identical changes in flow velocity[14]. Furthermore, a comprehensive hydrodynamic and algal ecological model was developed by eminent scholars to accurately simulate the dynamics of alkaline lake conditions[15]. The influence of hydrodynamic forces on algal cell growth has been investigated by scholars in emerging disciplines, utilizing optical physical instruments[16]. Furthermore, experts in related fields have also conducted studies on the approach of modulating hydrodynamic conditions during reservoir operation to effectively control the exponential proliferation of algae[17].

Overseas, hydrodynamic principles have been employed to inhibit algae growth, such as the management of critical flow rates for controlling bloom outbreaks in Australia[11,18]. However, due to the unclear influence mechanism and variations in the influence laws among different algae species and flow rate gradients, the current application scope of algal inhibition based on hydrodynamic principles is relatively limited. Moreover, there is a lack of research on algae in the Henan section of the Middle route project of South-to-North Water Transfer project, resulting in an unclear understanding of the influence law of actual flow velocity on dominant species within this project. Additionally, insufficient research exists regarding the influence mechanism of different flow velocity gradients on algal cells, which hinders effective ecological inhibition of algae growth using hydraulic principles at this stage. Therefore, this study focuses on investigating the impact law of flow velocity on cell growth for *Cyclotella meneghiniana*—a dominant diatom species found in the Henan section—and aims to explore how different flow velocity gradients affect algal cells' behavior. The findings will provide essential theoretical support for addressing algal damage through hydrodynamic principles within the middle route project of South-to-North Water Transfer.

Experimental inquiry

Experimental materials and devices

The diatom strain used in the experiment was isolated from *Cyclotella meneghiniana* (FACHB-2828) collected from the main canal of the middle route of the South-to-North Water Transfer Project. The corresponding medium (BG-11) was obtained from the Institute of Aquatic Biology, Chinese Academy of Sciences ($NaNO_3$: 0.075 g/L; NaH_2PO_4·H2O: 0.005 g/L; $Na_2SiO_3 \cdot 9H_2O$: 0.15 g/L).The algae strains were isolated, purified, and cultured in an incubator (*LRH-250F) according to specific requirements for subsequent experimental research. The portable intelligent flow rate instrument (LGY-II) is selected for the flow rate measurement.The self-made experimental device is a ring plexiglass tank. The experimental flow rate required by changing the turbine blade rotation speed is adjusted. A raised structure is set on the inner wall of the glass to disturb the flow to simulate the turbulent growth environment, as shown in Fig. 2.

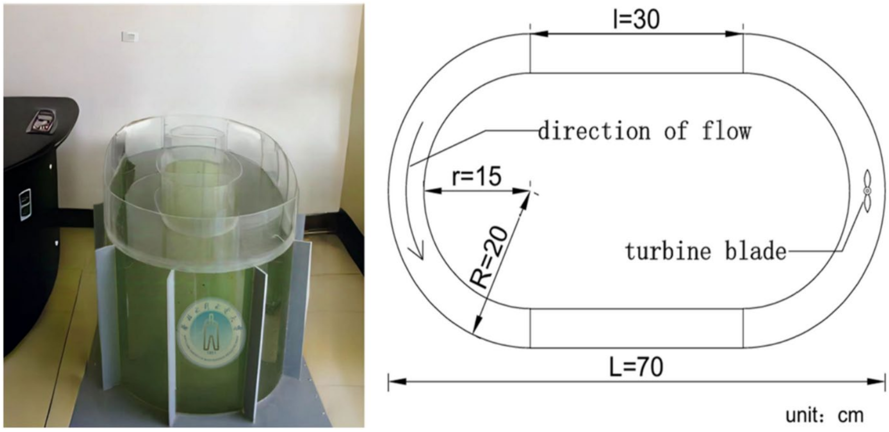

Fig. 2. Overhead diagram of the experimental device.

Experimental process

According to the operation of the middle route of the South-to-North Water Transfer project, six experimental flow velocity conditions were set at intervals of 0.2 m s^{-1} from 0 to 1.0 m s^{-1} (0 m s^{-1}, 0.2 m s^{-1}, 0.4 m s^{-1}, 0.6 m s^{-1}, 0.8 m s^{-1} and 1.0 m s^{-1}). The static water group was used as the control group for comparison purposes. Culture medium and micro-cyclic algae stock solution were added to each experimental group to ensure an initial algae density of 2.52×10^4 cells·$(mL)^{-1}$ throughout the experiment in order to meet the growth requirements of algae in terms of nutrient content and other factors. The water temperature was maintained at a constant level of 25℃, the photosynthetic photon flux density was 90 μmol photons·m^{-2} s^{-1}, and the ratio of light to darkness was set at 12 h:12 h, with the duration reaching 14 days. The experiment involved measuring algal density, specific growth rate, chlorophyll-a concentration (Chl-a concentration), and carotenoid concentration (car concentration). Growth indexes such as algal cell density and algal cell growth rate were recorded every48 hours. Three parallel samples were selected for each measurement to calculate their mean values ensuring accuracy in experimental data analysis.TP/TN concentrations were determined using a total nitrogen analyzer(MI200K) while Chl-a and carotenoid concentrations were measured using UV–visible spectrophotometry[19]. By using SR-algae counting frame to count directly under the microscope[20], algal cell density was recorded and specific growth rate was calculated. The formula for calculating specific growth rate was as follows:

$$\mu = \frac{\ln X_n - \ln X_{(n-1)}}{t_n - t_{(n-1)}} \quad (1)$$

where ln Xn – ln X(n – 1) is the pair value of the density difference between two adjacent algae; $t_n - t_{(n-1)}$ is the time difference between two adjacent samples (48 h).

Experimental result

Effect of flow velocity on cell growth of *Cyclotella meneghiniana*

The effect of flow velocity on the growth of small ring algae cells was characterized using two sets of data. Algal cell density (Fig. 3a) represents the total number of algal cells in the experiment, while the specific growth rate of algal cells (Fig. 3b) indicates their growth rate. Significant changes in algal cell density were observed after 4 days from the start of the experiment, whereas differences in specific growth rates decreased after 10 days. When experimental flow rates were below 0.4 m s^{-1}, small ring algal cells exhibited a faster increase in growth rate with increasing flow velocity; however, at a flow rate of 0.4 m s^{-1}, maximum cell growth was observed. Flow velocities exceeding 0.6 m s^{-1} inhibited algal cell growth, and this inhibitory effect became more pronounced with higher flow velocities. Therefore, based on our experimental measurements for *Cyclotella meneghiniana*, we propose that a critical flow velocity is reached at 0.4 m s^{-1}[21]. Simultaneously, based on the data of algal cell density in the experimental groups with flow velocities of 0.8 m s^{-1} and 1.0 m s^{-1}, it is evident that beyond a velocity threshold of 0.8 m s^{-1}, the influence of increased flow velocity on algal cell growth diminishes significantly compared to the static water group.

Changes of nutrient content in water under different flow velocity

The change rates of TP and TN in water at different flow rates were significantly different, as shown in Fig. 4. Algal cells need to absorb exogenous nitrogen and phosphorus as energy supply for maintaining cell metabolism and growth[22,23], so the change rate of TN and TP content can directly represent the intensity of cell metabolism in different experimental groups. For example, when the flow rate was 0.4 m s^{-1}, the change rates of TN and TP were 0.568 and 0.168, respectively, which were the highest in the experimental group, and the metabolic rate of algae cells was the highest in this flow rate environment. At the same time, when the flow velocity is greater than 0.6 m s^{-1}, the TN and TP change rates are negatively correlated with the flow velocity, and the TN and TP change rates of 0.8 m s^{-1} and 1.0 m s^{-1} are significantly lower than those of the static water group. It can be seen that the flow velocity environment is directly related to the metabolism of algal cells. When the flow velocity is lower

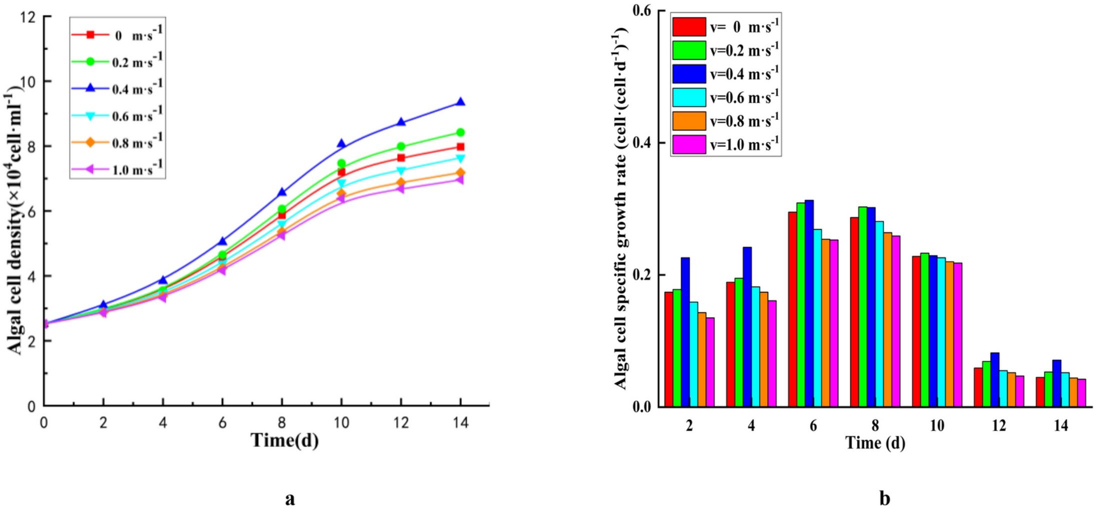

Fig. 3. (**a**) Changes in cell density of algae. (**b**) specific growth rate of algal cells.

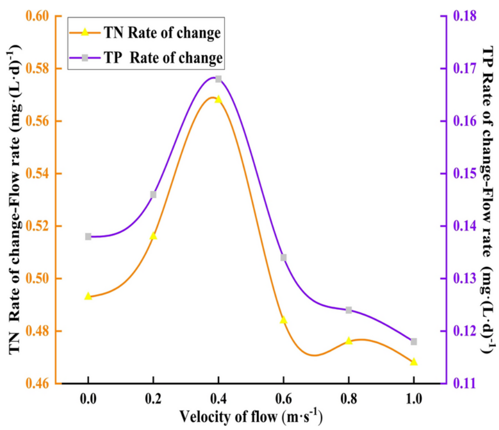

Fig. 4. Change rates of TP-TN under different flow rates.

than 0.4 m s^{-1}, the metabolic capacity of cells is enhanced with the increase of flow velocity, and the metabolic capacity of cells is the strongest at 0.4 m s^{-1}.

Effect of different flow velocity on photosynthetic pigment of alga

The effect of flow velocity on photosynthesis of algal cells was investigated by measuring photosynthetic pigment content. As shown in Fig. 5, the content of photosynthetic pigment (Chl-a, car) in all experimental groups was basically the same at the initial stage, and the difference of pigment content in all experimental groups became larger on the 6d of the experiment. At the same time, the photosynthetic pigment content of 0.4 m s^{-1} experimental group was significantly higher than that of other experimental groups, and the photosynthetic pigment content was positively correlated with the growth velocity lower than 0.4 m s^{-1}. When the growth velocity exceeded 0.6 m s^{-1}, a negative correlation was observed between flow velocity and carotenoid and chlorophyll a contents, indicating that higher flow velocities were associated with lower pigment concentrations. The experimental groups exposed to flow velocities of 0.8 m s^{-1} and 1.0 m s^{-1} exhibited significantly reduced pigment content compared to the static water group. These findings are consistent with the results reported by Istvanovic et al.[24] finding that there is a negative correlation between Chl-a and higher velocity based on the data analysis of three rivers in Eastern Europe for many years, and Ma et al.[25] based on Sri Lanka river water dynamics and river chlorophyll results mutually corroborate. At the same time, according to the comparison of pigment content changes in different experimental groups, it was found that the influence of flow velocity on chlorophyll a content was more significant than that of carotenoid content.

Analysis of experimental results

By comprehensively analyzing the changes of cell density, nutrient content and photosynthetic pigment content in the experiment, it can be concluded that under different flow velocity gradients, low flow velocity promotes cell growth and high flow velocity inhibits cell growth. This is consistent with the experimental conclusion of

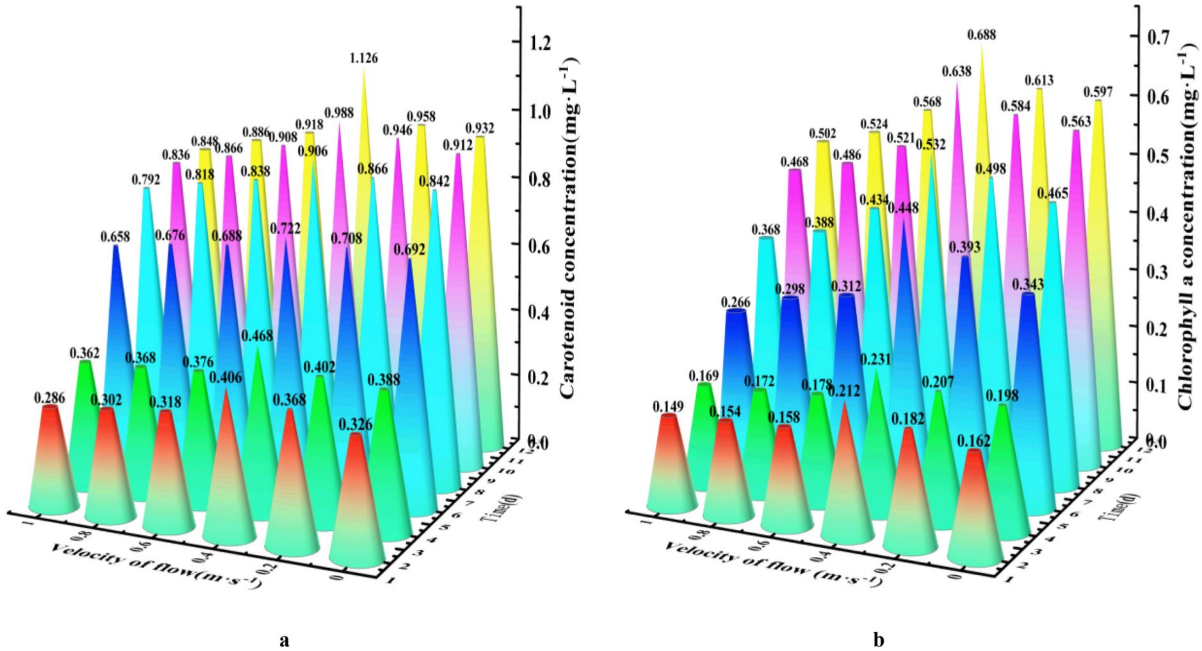

Fig. 5. (**a**) Changes of carotenoid content under different flow rates. (**b**) Changes of chlorophyll a content under different flow rates.

domestic and foreign scholars in related fields that the effect of flow velocity on algal cell growth presents "low promotion and high inhibition"[26–28]. At the same time, in order to further explore the influence mechanism of different flow velocity gradients on algal cell growth, the experimental flow velocity was divided into two gradient ranges, 0–0.4 m s^{-1} and 0.2–0.6 m s^{-1}, respectively, according to the above experimental results. It is convenient to explore the mechanism of velocity promoting algal cell growth and influencing the inflection point (0.4 m s^{-1}) of velocity under the condition of low velocity.

Exploring the mechanism of the effect of low velocity gradient on the cell growth of *Cyclotella meneghiniana*

Based on the experimental conclusion of domestic and foreign scholars in related fields, which found that algal cells grow better at lower flow velocity[29–31], combined with the above experimental results, the influence mechanism of low flow velocity gradient on the growth of *Cyclotella meneghiniana* cells was explored. By comparing the growth of algal cells in turbulent flow and laminar flow environment under the same conditions by changing the flow state, a turbulent control group was set up to explore the influence mechanism of promoting algal cell growth under low flow rate. The experimental results show that the growth state of algal cells in turbulent flow environment is better than that in laminar flow environment under the same flow velocity, and the greater the flow velocity, the more obvious the effect of promoting algal cell growth, as shown in Fig. 6. The experimental results are consistent with the conclusion of Musielak et al.[32,33] that turbulent environment is more conducive to algal cell growth through experimental exploration and analysis of algal cell growth in actual rivers by domestic and foreign scholars.

In addition, Clementina et al.[34]. found that appropriate disturbance would increase the contact frequency between nutrients and cell surface and increase the absorption efficiency of nutrients by algal cells, which explained the reason why low-velocity turbulent flow conditions were more conducive to algal cell growth than laminar flow. At the same time, the biggest difference between turbulent flow and laminar flow growth conditions lies in the contact frequency between algal cells and nutrients with mixed and increased fluid flow. Numerical simulation software was used to simulate fluid flow under turbulent flow and laminar flow conditions, as shown in Fig. 7. Although Chen et al.[35]. From a cytological perspective, it was observed that an appropriate increase in flow velocity could enhance the dispersion of nutrients around cells, facilitating their absorption and promoting cell growth by reducing the diffusion layer thickness of algal cells. In conjunction with experimental findings, Fig. 8 demonstrates that algae cells exhibited better growth under conditions of low flow velocity turbulence (0.2 m s^{-1}) compared to laminar flow at optimal velocity (0.4 m s^{-1}). This confirms that the frequency of contact between algal cells and nutrients is a decisive factor influencing algal cell behavior under low velocity gradients. In summary, the fundamental mechanism behind enhanced algal cell growth due to increased flow velocity under low gradient conditions lies in the augmented contact frequency between algal cells and nutrients, thereby facilitating nutrient uptake and promoting overall cellular development[36].

Exploring the influence mechanism of critical velocity on *Cyclotella meneghiniana*

In the experiment, the growth conditions of algal cells varied significantly under different flow gradient conditions. Among them, the critical flow rate, which refers to the optimal flow rate for promoting algal cell

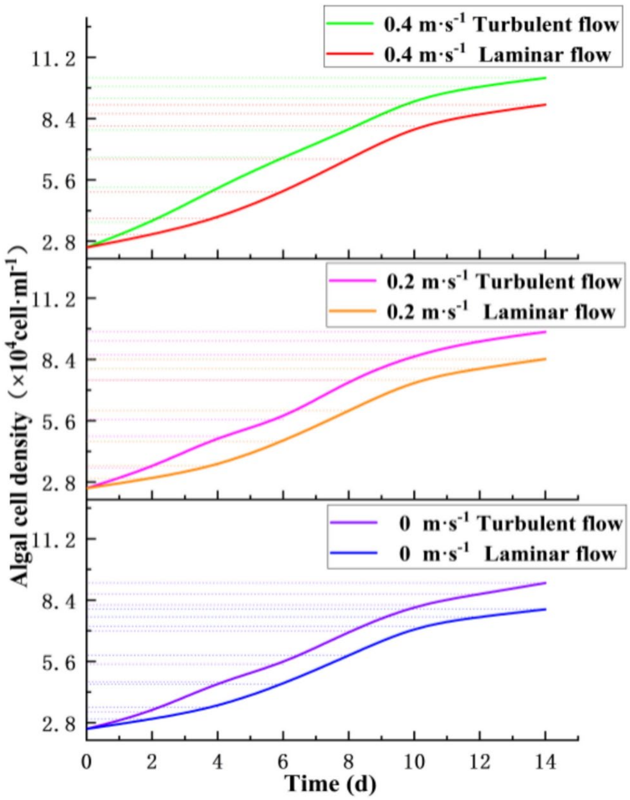

Fig. 6. Comparison of cell density changes under the same flow velocity (turbulent flow–laminar flow).

growth, exhibited variations across different algae species based on previous studies[37], in addition, the critical flow rate of the same algae under different trophic states is also different[21]. It can be seen that under the experimental conditions, the critical flow velocity of *Cyclotella meneghiniana* is 0.4 m s^{-1}.

In fact, the effects of different fluid environments on cell growth state have been studied in many fields. For example, in the field of microbiology, fluid shear stress can affect human endothelial cell structure and cell division[38]; Fluid shear stress has been found in the medical field to be the main cause of endothelial cell atherosclerosis[39]. Studies in the field of botany have found that stress also affects the morphology and structure of plant cells, and thus affects the function of plant cells[40]. Based on the above relevant studies, it can be seen that the cell growth state in fluid environment is closely related to fluid shear stress. Previous studies have shown that *Cyclotella meneghiniana* is a relatively robust alga with silicified frustules[41], and research has indicated that algae are highly sensitive to hydrodynamic stress and shear stress, with significant differences in their sensitivity[42]. By observing the TEM images of *Cyclotella meneghiniana* under different flow velocities, it is evident that the algal cell shell shows slight mechanical damage at a flow velocity of 0.4 m s^{-1}. Therefore, it can be inferred that the critical flow velocity may be caused by the destructive force of fluid shear stress on the algal cell structure, as shown in Fig. 9.

Due to variations in cell membrane structure across different trophic states, the critical flow rate for cell structure disruption differs based on fluid carrying capacity. Therefore, it is reasonable to propose that fluid shear stress can lead to this critical flow rate and explain the discrepancies observed in the same species of algal cells under different trophic states. While previous studies have primarily focused on investigating the direct influence of fluid shear stress on algal cell growth, little attention has been given to exploring its underlying mechanisms. In reality, changes in shear stress within a given fluid are caused by alterations in flow velocity, which directly impact cell structure integrity. This perspective aligns with findings from numerous scholars who have demonstrated that a certain level of fluid shear stress can indeed disrupt cellular structures[43–45]. In the case of small shear stress, the observed change in flow velocity coincides with variations in shear stress of the experimental variable fluid, aligning with the aforementioned mechanism highlighting the impact of low flow gradient on algal cells.

Conclusion and prospect

By simulating the growth environment of *Cyclotella meneghiniana* cells in the middle line project, we conducted an experiment to investigate the growth laws of these cells under different flow velocity gradients. Generally, our findings indicate that low flow velocities promote the growth of algal cells, while high flow velocities inhibit their growth. We determined that the critical flow velocity for promoting algal cell growth under experimental conditions is 0.4 m s^{-1}. Additionally, comparing turbulent and laminar flow patterns at the same velocity revealed that turbulent flow had a greater promotion effect on cell growth compared to laminar flow; specifically, algal cell

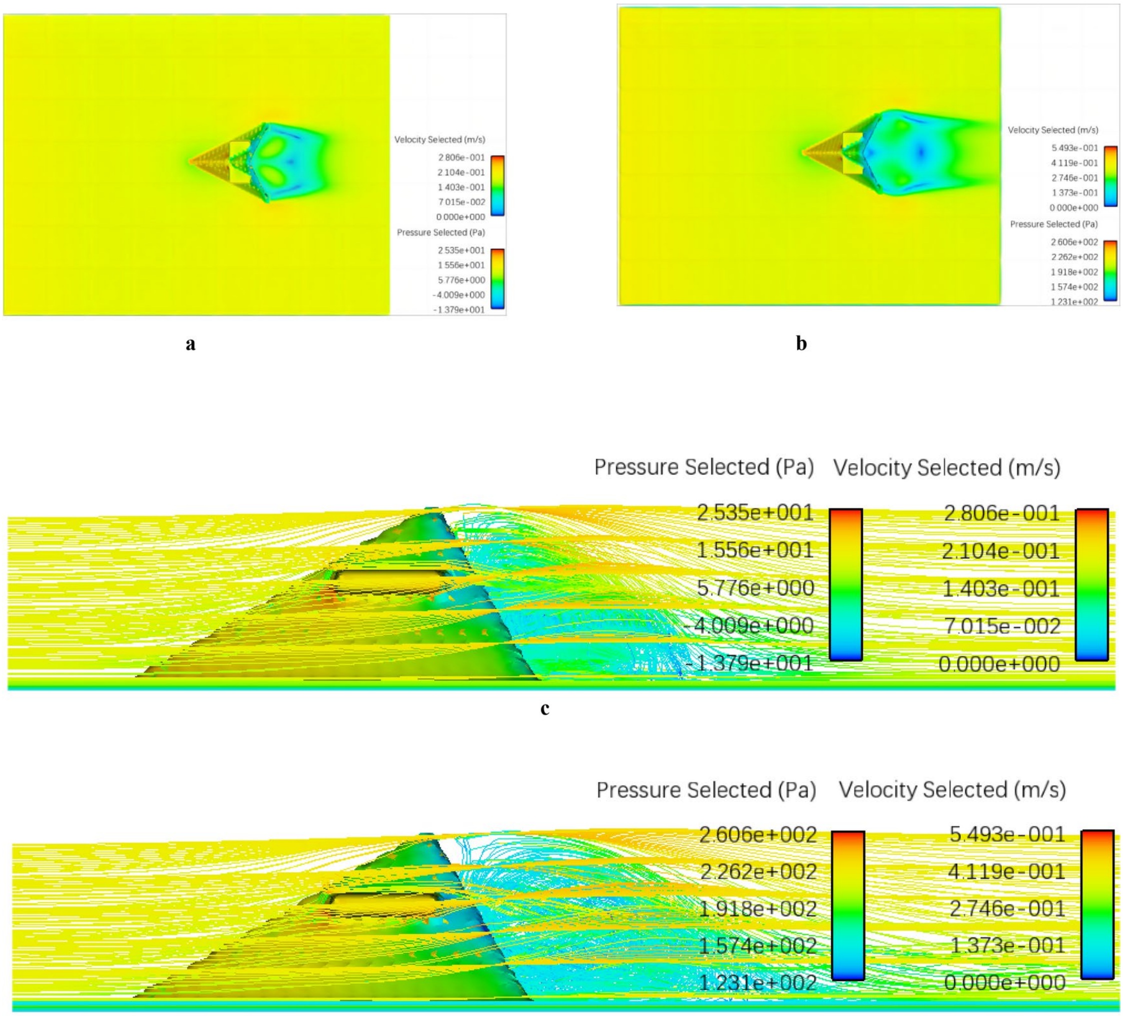

Fig. 7. (**a**) Top view of turbulent flow pattern in 0.2 m s^{-1} environment. (**b**) Top view of turbulent flow pattern in 0.4 m s^{-1} environment. (**c**) Side view of turbulent flow pattern in 0.2 m s^{-1} environment. (**d**) Side view of turbulent flow pattern in 0.4 m s^{-1} environment.

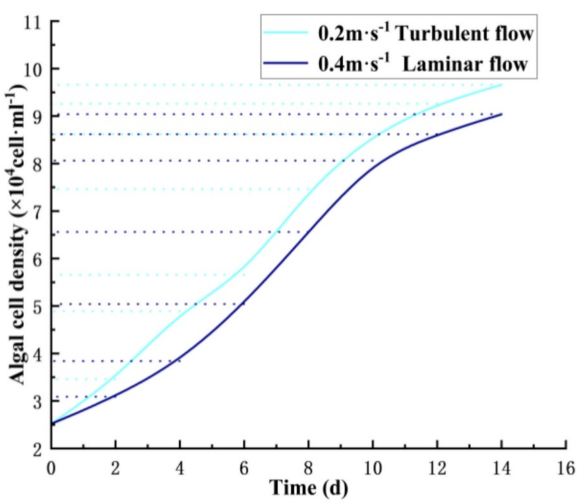

Fig. 8. Comparison of algal cell density between turbulent flow condition and laminar flow condition.

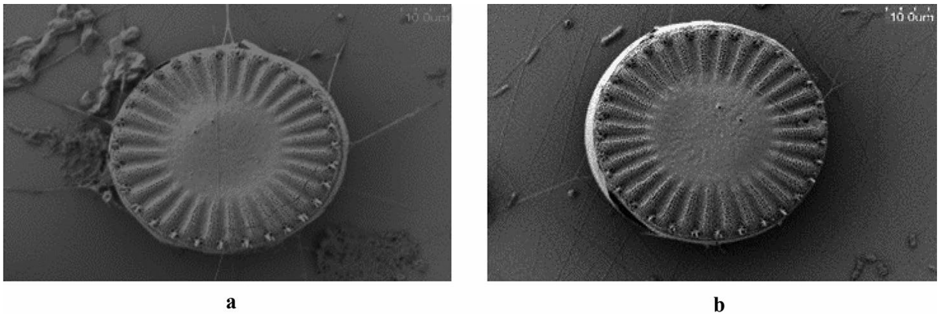

Fig. 9. (**a**) Electron microscopy of algal cells in the environment of 0 m s^{-1}. (**b**) Electron microscopy of algal cells in the environment of 0.4 m s^{-1}.

growth was better under a turbulent flow pattern with a velocity of 0.2 m s^{-1} than under a laminar flow pattern with a velocity of 0.4 m s^{-1}. We observed that as the flow rate increased, so did the growth rate of algal cells due to an increased contact frequency between cells and nutrients, thereby promoting their overall growth. The critical flow rate (0.4 m s^{-1}) resulted in mechanical damage to algal cells as observed through electron microscope analysis. It is worth noting that different algae species have varying critical flow velocities and even within one species, different trophic states can lead to variations in its critical flow velocity. Overall, our results support previous studies indicating differences in critical flows among various algae species and even within one species across different nutrient environments.

In recent years, there has been a gradual increase in studies on algal cell growth based on hydrodynamics both domestically and internationally, leading to certain research achievements. However, most existing studies focus on single influencing mechanisms, while the actual growth environment of algal cells is influenced by multiple factors. For instance, it has been demonstrated that temperature and pH have an impact on algal cell growth[46,47]. Future research should fully consider the practical engineering situation to explore the compound effects of multiple influencing factors on algal cell growth in real-world environments. Currently, domestic ecological hydraulic research has reached a mature stage. In the future, methods for algae growth inhibition should be integrated into ecological hydraulic design with ecological slope protection measures. Although indoor simulation experiments are used to investigate the effect of flow velocity on small ring algal cell growth, there exists a gap between these experiments and the actual engineering environment where algal cells grow. Therefore, it is necessary to combine data from numerous observation projects reflecting actual conditions of algal cell growth in order to enhance research outcomes. The investigation into the growth mechanism of algal cells based on hydrodynamics will play a crucial role in utilizing fluid characteristics for inhibiting algae growth in practical engineering applications and holds significant value for further research.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request. Corresponding authors: 2468083230@qq.com.

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Author contributions
J.Z.: Resources; investigation; data curation; funding acquisition; H.M.: Conceptualization; writing—original draft; writing—review & editing; G.S.: Investigation; data curation; visualization; writing—review & editing; Y.D.: Methodology; software; data curation; S.G.: Visualization; investigation.

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Declarations

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Correspondence and requests for materials should be addressed to G.S.