



Turbulent mixing mediates the photosynthetic activities and biochemical composition of *Anabaena*: implications for bioengineering

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Abstract

Cyanobacteria exhibit a variety of adaptive strategies that allow them to thrive in ever-changing aquatic environments. Here, successive steady states of continuous cultures were used to investigate the effects of quantified turbulence on the biochemical compounds and physiological processes of *Anabaena flos-aquae* in a photobioreactor under different dilution rates. A rapid increase in cell density was clearly observed following an increase in the turbulent dissipation rate at all growth rates of *A. flos-aquae*. The photosynthetic response to irradiance curves showed that the turbulence-treated strains exhibited lower photosynthetic oxygen evolution and saturating irradiance as well as higher respiration in rapidly growing young cells, indicating that they might not be very adaptable to high turbulent dissipation rates. Additionally, there was an increase in the protein levels of *A. flos-aquae* with increasing turbulence at all growth rates, whereas carbohydrate formation and lipid accumulation demonstrated the opposite trends. At a high growth rate, the level of carbohydrates decreased whereas that of lipids increased, which was interpreted as reflecting an adaptation to the turbulent environment. These findings suggest that turbulence sensitivity is shear regimen- and growth rate-dependent in *A. flos-aquae*. The high respiration capacity, low saturating irradiance, and conversion of carbohydrates to lipids represent effective measures for revealing the adaptive strategies of rapidly growing young cells under hydrodynamic regimes. The results regarding optimum lipid accumulation with specific growth traits have important implications for the design of cultivation methods of cyanobacteria resource utilization with respect to regulating turbulence.

Keywords *Anabaena* · Biochemical compounds · Cultivation strategy · Photosynthetic · Turbulence

Introduction

Cyanobacteria receive attention and study worldwide due to their ability to proliferate extensively and form massive blooms in the upper water layer. Frequent outbreaks of cyanobacterial blooms have adverse effects on aquatic ecosystems and human health

(Merel et al. 2013). Cyanobacteria have been recognized as a unique group of microorganisms because they exhibit characteristics of both algae and bacteria. Similar to eukaryotic microalgae, cyanobacteria perform oxygenic photosynthesis and show rapid growth and high biomass productivity. To develop cyanobacteria as a resource some researchers have attempted to develop cultivation technologies to apply cyanobacteria as precursors for fuels and other products (Grondahl 2009; Steinhoff et al. 2014).

The synthesis of valuable compounds in high amounts in microalgae can be amplified under stress or stimulation conditions (Suali and Sarbatly 2012; Han et al. 2015). However, stress conditions can also have negative effects on microalgal growth (Markou and Nerantzis 2013). Cyanobacteria exhibit a variety of adaptive strategies that allow them to perform well under environmental stress conditions, including low or high light intensity, high ultraviolet light intensity, high temperature, turbulent mixing, grazing pressure, and scarce or overabundant nutrients (Oliver et al. 2012; Paerl and Otten 2013). Several studies have

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proposed various hypotheses regarding how cyanobacterial species achieve this versatility by adjusting their buoyancy, using atmospheric nitrogen, utilizing phosphorus and inorganic carbon efficiently, optimizing cellular nutrient storage, and transforming their phenotype (Litchman et al. 2010; Carey et al. 2012). Cyanobacteria can accumulate intracellular compounds in response to harsh environmental conditions; therefore, they have the potential to be transformed into valuable fuels (Quintana et al. 2011; Kumar et al. 2017). However, cyanobacteria as a potent candidate for efficient production have not been given much attention.

Studies have shown that changes in component synthesis, including lipids, carbohydrates, proteins, and chlorophyll, may be induced by extreme temperature and light fluctuations, physical disturbances, pH, nutrient depletion, and predators (Tedesco and Duerr 1989; Tang et al. 2004; Han et al. 2015; Çelekli et al. 2016). Among several environmental parameters, turbulent mixing has been widely accepted as a source of external energy in the planktonic environment. Turbulence has a variety of effects on phytoplankton, such as affecting the light/dark frequencies in a well-documented phenomenon known as the “flashing light effect” (Grobelaar 1994; Rubio et al. 2003), changing the diffusive sublayers and regulating the nutrient fluxes of cells (Guasto et al. 2012), causing mechanical damage (Chisti 2010; Michels et al. 2016) and altering behavior (Prairie et al. 2012; Sengupta et al. 2017). The impacts of turbulence on phytoplankton are complex and can influence a wide range of physiological aspects of organisms (Hondzo and Wüest 2009). In-depth researches of photosynthesis and nutrient strategies of phytoplankton in response to turbulence have been obtained from a few key genera, including *Phaeodactylum*, *Porphyridium*, *Cyclotella*, *Fragilaria*, *Chlorella*, *Chlamydomonas*, *Alexandrium*, *Lingulodinium*, *Nodularia*, and *Aphanizomenon*. (Moisander et al. 2002; Sullivan et al. 2003; Sobczuk et al. 2006; Wang et al. 2012; Leupold et al. 2013). Recently, the importance of turbulent mixing in shaping algal nutrient stoichiometry and its consequences for biochemical compounds has received attention. Hansen et al. (2013) suggested that mixing had an effect on the swimming speed of *Chlamydomonas reinhardtii* by affecting the biochemical composition, for example, through fatty acid accumulation. Chengala et al. (2013) reported that certain levels of moving fluid flow in a bioreactor enhanced the growth, protein, and fatty acid accumulation of *Dunaliella primolecta*. Our previous study found that cyanobacteria can adjust their growth rates in response to turbulence levels via the asynchronous cellular stoichiometry of C, N, and P (Xiao et al. 2016). Although the effects of turbulence on the cellular responses of phytoplankton have been extensively studied, such studies are lacking for the great majority of cyanobacteria. Furthermore, studies have demonstrated that carbon partitioning between carbohydrates and lipids in several species can be improved by applying stress, and

carbon storage can differ greatly among the different adaptive strategies of algae (Michel et al. 2010; Pandit et al. 2017). However, few studies have focused on the turbulence conditions and growth traits that are associated with the accumulation of compounds that could serve as substrates for biofuel production. The partitioning of carbon into different cyanobacterial storage products in response to turbulence has not yet been fully evaluated.

In this work, we studied the filamentous cyanobacterium *Anabaena flos-aquae* which can be easily observed in freshwater systems and has specific responses for adapting to environmental changes (Singh et al. 2017). Few studies have investigated the potentially beneficial aspects of turbulence as a stress cultivation condition in promoting *Anabaena* growth. Compared to batch systems continuous cultivation by dilution with fresh media has many benefits that enable the culture environment to remain at the steady state (Tang et al. 2012). In this context, continuous cultivation under different turbulence levels and dilution rates was adopted in the current study to investigate the photosynthetic performance of *A. flos-aquae* and its partitioning of carbon into different storage products. The results contribute to the understanding of the specific property depending on species/strains growth under turbulence regimes and can be used to increase the production of valuable metabolites in *Anabaena*.

Materials and methods

Strain and culture conditions

The axenic freshwater filamentous cyanobacterium *Anabaena flos-aquae* FACHB-245 was kindly provided by the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences (FACHB-Collection; Wuhan, China). The strain was maintained in sterile BG11 medium (Rippka et al. 1979) under a cool-white fluorescent light intensity of $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ with a 12-h:12-h light:dark cycle at a temperature of $25 \pm 1 \text{ }^\circ\text{C}$. Cells in the logarithmic growth phase in batch mode were incubated in a turbulence-controlled (0, 150, 200, and 50 rpm) continuous photobioreactor under three different dilution rates and then transitioned to continuous mode.

Following the methodology described in Xiao et al. (2016), a two-phase flow Computational Fluid Dynamics (CFD) model of the continuous flow reactor was applied to simulate the hydrodynamic conditions within the reactor. The computational domain of the model was the whole continuous flow reactor, which had slight differences from the batch reactor described in Xiao et al. (2016) (Fig. 1). The domain was discretized using unstructured grids, with finer grids applied in the impeller and shaft regions (maximum size = 1 mm). Approximately 1,200,000 total computational grids

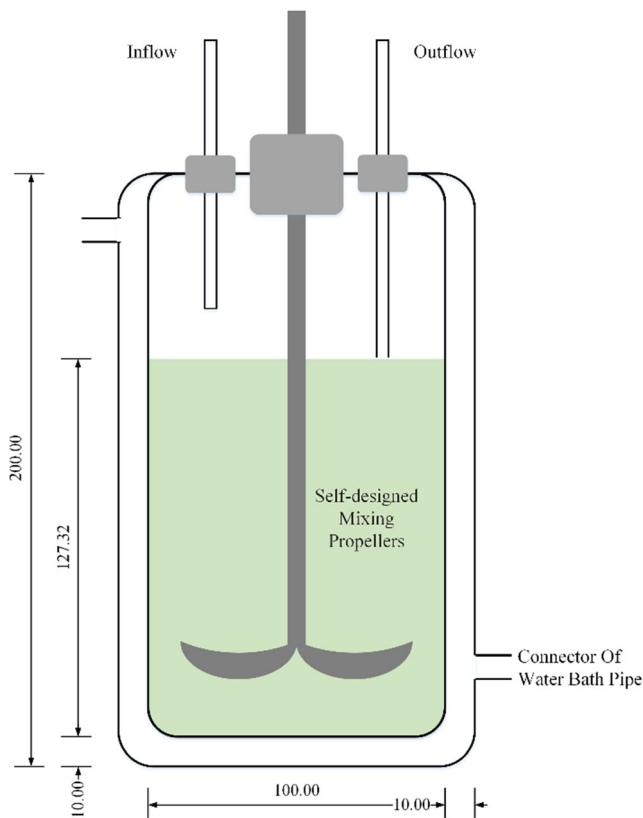


Fig. 1 The continuous photobioreactor system used to culture *Anabaena flos-aquae* (unit: mm)

(maximum size = 2 mm) were created using the tetra mesh option of ANSYS ICEM CFD (ANSYS Inc.) to obtain the grid-independent solution for the flow. The simulation of the process was considered as a steady state and was numerically solved by using the commercial software package CFX 12.0 (ANSYS Inc.). The calibration and validation approach of the model was as discussed in Xiao et al. (2016). The turbulent mixing rate and Kolmogorov microscale at different levels of rotation speed were estimated at steady state. The integrated results are shown in Table 1.

Photobioreactor setup and determination of dilution rate

Continuous cultivation of *A. flos-aquae* was conducted in a modified photobioreactor system (Fig. 1), which was made of a double glass vessel with a width, height, and thickness of 120, 200, and 10 mm, respectively. The working volume (V_0) was 1 L with an environmentally controlled growth chamber under an 80- $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ light intensity at 25 °C with a water jacket connected to a water bath. Sufficient mixing was achieved through axial flow impellers; thus, the system was assumed to be a continuous stirred-tank reactor with different turbulent mixing rates (defined above). Axenic fresh culture medium was pumped

Table 1 Turbulent dissipation rate and Kolmogorov microscale at different levels of rotation speed as estimated by the CFD hydrodynamic model

	Turbulence		
Rotation speed (rpm)	150	200	250
Turbulent dissipation rate ($\text{m}^2 \text{s}^{-3}$)	0.007	0.015	0.030
Kolmogorov microscale (mm)	0.123	0.099	0.083

into the photobioreactor through silicone rubber tubing with flow rates (F) of 0.1, 0.3, and 0.6 L day⁻¹, corresponding to dilution rates $D = F/V_0$ of 0.1, 0.3, and 0.6 day⁻¹, respectively. The culture medium was withdrawn at the same flow rate from the photobioreactor. In continuous cultivation at steady state, all culture parameters remain constant (culture volume, dissolved oxygen concentration, nutrient and product concentrations, pH, cell density, etc.). Growth of microorganisms occurs at a constant specific growth rate (μ), which is numerically equals to the dilution rate (D) (Tang et al. 2012). The test was performed in triplicate under the conditions described above.

Measurement of cell density and chlorophyll *a*

The cell density was determined using a hemocytometer under an Olympus CX43 microscope (Olympus, Japan). Prior to cell counting, *A. flos-aquae* filaments were ultrasonicated for 30 or 60 s at 20 kHz and 100 W (JY92-2D, SCIENTZ, China) to obtain single cells. Chlorophyll *a* was extracted with 90% acetone and measured spectrophotometrically according to Nusch (1980).

Photosynthetic oxygen evolution analysis

The photosynthetic oxygen evolution was measured by a Clark-type oxygen electrode (Chlorolab 2, Hansatech, UK). The temperature of the chamber was controlled by a thermostatic circulator at 25 °C. Illumination was set from 25 to 1000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, with oxygen evolution measured for at least 10 min at each irradiance. The parameters for the photosynthetic response to irradiance curves (P–E curves) were obtained according to Walsby (1997): $P = P_m[1 - \exp(-\alpha E/P_m)] + R_d + \beta E$, where E is irradiance, P is the photosynthetic rate at irradiance E , P_m is the maximum photosynthesis rate, α is the initial slope of P–E curve, R_d is the dark respiration rate, β is the slope at high irradiance, and E_k is the saturating irradiance for photosynthesis ($E_k = P_m/\alpha$). The photosynthetic rates were normalized to chlorophyll *a*, and non-linear curve fitting of the data was performed with Origin 9.0 (OriginLab, USA).

Carbon contents and carbonic anhydrase activity

Samples were harvested by centrifugation (6300×g, 15 min, 25 °C), washed twice, and homogenized in liquid nitrogen with cold Veronal Buffer (12 mM, pH 8.3). The carbonic anhydrase (CA) activity was measured by the method of Wilbur and Anderson (1948). The time required for the pH to change from 8.3 to 7.3 was measured at 0–2 °C after the addition of 3 mL of CO₂-saturated water to 3 mL of buffer samples. CA activity was calculated as an enzyme unit, $EU = (T_0/T) - 1$, where T_0 is the time for the uncatalyzed reaction and T is the time for the enzyme-catalyzed reaction. CA activity was normalized to Chl *a*.

To measure the cellular carbon content of *A. flos-aquae*, 30 mL culture suspensions were collected from each sample, filtered onto precombusted GF/F filters (Whatman, UK) and dried at 65 °C for 24 h. The dried suspensions were then removed and wrapped in weighted tinfoil and then weighed again to obtain the mass of samples. The carbon concentrations were analyzed by using a Vario El cube elemental analyzer (Elementar, Germany).

Biochemical composition determination

Anabaena flos-aquae culture suspensions were collected from each sample and centrifuged at 6300×g for 15 min (4 °C). Then the pellets were washed twice with distilled water and used to determine biochemical composition, including total carbohydrates, proteins, and lipids. The carbohydrate content was quantified spectrophotometrically by the phenol-sulfuric acid method using glucose as the standard (Dubois et al. 1956). The protein content was determined with Coomassie Blue G-250 reagent using bovine serum albumin as the standard (Bradford 1976). All contents were measured under steady-state conditions and normalized to cell counts. Lipid extraction was based on Bligh and Dyer (1959). Pellets were frozen at –80 °C for 24 h and then dried in a freeze dryer for up to 4 days. Dried cells were ground into powder and treated with chloroform/methanol (2:1, v/v). The extracted lipid was centrifuged, and the chloroform layer, including lipids, was collected. The residues were subjected to repeated extraction five times. All of the extracts obtained were then combined and the lipid content was evaluated by its weight relative to the weight of dry organism biomass.

Statistical analysis

The data are presented as the mean ± standard deviation (SD). Significant differences between controls and treated samples were determined by ANOVA. All statistical analyses were carried out with Origin 9.0 (OriginLab, USA). Differences were considered significant at $P < 0.05$.

Results

Cell density of *A. flos-aquae* at steady-state conditions

Anabaena flos-aquae continuous cultivation in a 1 L photobioreactor was performed under different turbulent mixing levels. Each continuous culture in the steady-state condition was conducted for 20 days. Figure 2 shows the steady-state condition of cell density at various dilution rates (from 0.1 to 0.6 day⁻¹). Changes in the dilution rate of the continuous culture had significant impacts on the steady-state cell density. As expected, reducing the dilution rate increased the steady-state cell density, which ranged from 2.18×10^6 to 3.92×10^7 cells mL⁻¹. Under the same dilution rate, the steady-state cell density of *A. flos-aquae* increased with increasing turbulence intensity. For instance, the value of the steady-state cell density under a turbulent dissipation rate of 0.03 m² s⁻³ was approximately 1.4×, 1.7× and 2.2× higher than that under the stagnation condition at a dilution rate of 0.1, 0.3, and 0.6 day⁻¹, respectively (ANOVA, $P < 0.05$).

Photosynthetic characteristics of *A. flos-aquae*

The photosynthesis–irradiance (P–E) curves and parameters of cultured *A. flos-aquae* displayed marked differences among dilution rates and turbulent mixing levels (Fig. 3 and Table 2). The maximum photosynthesis rate (P_m) increased gradually with increasing turbulence intensity at a dilution rate of 0.1 day⁻¹ but decreased gradually at a dilution rate of 0.6 day⁻¹. At a dilution rate of 0.3 day⁻¹, P_m and photosynthetic efficiency (α) were increased under the turbulence treatments, with maximum values observed at the dissipation rate of 0.007 m² s⁻³. Moreover, α increased with turbulence intensity at the dilution rate of 0.6 day⁻¹. Concomitantly, the value of the dark respiration rate

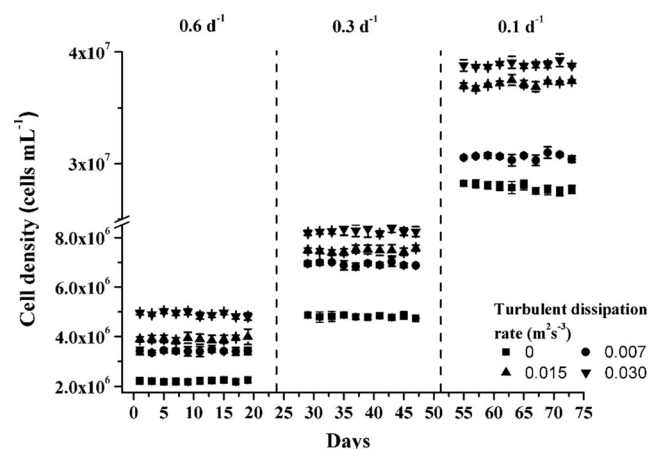


Fig. 2 Cell density of *Anabaena flos-aquae* under steady-state conditions at different dilution rates and turbulent mixing levels. Data are the means ± SD (n = 3)

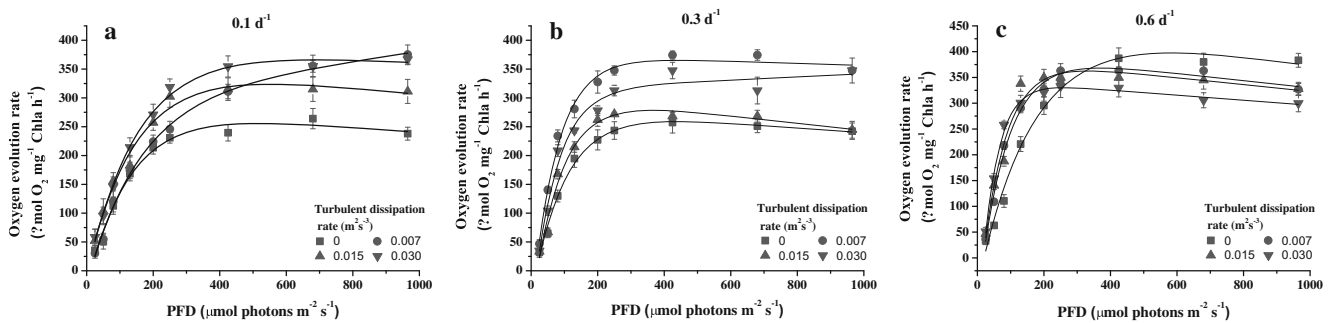


Fig. 3 Photosynthetic oxygen evolution as a function of the incident photon flux density (PFD) in *Anabaena flos-aquae* grown at different dilution rates and turbulent mixing levels. (a) Dilution rate of 0.1 day⁻¹. (b) Dilution rate of 0.3 day⁻¹. (c) Dilution rate of 0.6 day⁻¹. Data are the means ± SD (n = 3)

(R_d) and saturating irradiance for photosynthesis (E_k) increased significantly (ANOVA, $P < 0.05$) under the turbulence treatments of *A. flos-aquae* at a dilution rate of 0.1 day⁻¹. In contrast, the R_d value dramatically decreased with increasing turbulent dissipation rate at dilution rates of 0.3 and 0.6 day⁻¹ (ANOVA, $P < 0.05$), as indicated by the higher oxygen consumption rates per cell. The lowest R_d value was $-147.98 \pm 6.46 \mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$, which occurred at a turbulent dissipation rate of $0.03 \text{ m}^2 \text{ s}^{-3}$ and dilution rate of 0.6 day⁻¹. Relative to E_k under the stagnation condition, E_k was lower in the 0.007, 0.015, and $0.03 \text{ m}^2 \text{ s}^{-3}$ treatments by 0.5, 0.4, and 0.3 times, respectively.

Changes in cellular carbon with the chlorophyll a ratios of *A. flos-aquae*

Carbon is used for maintenance, growth, and respiration related to growth. The C/Chl *a* ratio is a key physiological variable

that can be used to evaluate the normalizing photosynthetic rate and carbon fixation efficiency. The results (Fig. 4) demonstrated that under a higher dilution rate, *A. flos-aquae* had a higher ratio of C/Chl *a*, while the C/Chl *a* ratio was decreased in the turbulent treatments relative to the stagnation condition except at low dilution rates. At a dilution rate of 0.1 day⁻¹, the C/Chl *a* ratio slightly increased with increasing turbulent dissipation rate, although not significantly (ANOVA, $P > 0.05$). However, the ratios were significantly lower at a higher turbulence intensity ($0.03 \text{ m}^2 \text{ s}^{-3}$) than they were under the control treatments at dilution rates of 0.3 and 0.6 day⁻¹ (ANOVA, $P < 0.05$).

Carbonic anhydrase activity of *A. flos-aquae*

To further investigate the effects of turbulence intensity and dilution rate on carbon fixation efficiency, the CA

Table 2 Parameters of the photosynthesis–irradiance curves for *Anabaena flos-aquae* grown at different dilution rates and turbulent mixing levels. P_m , maximum photosynthesis rate, $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$; α , the initial slope of the P–E curve, $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$

($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)⁻¹; R_d , dark respiration rate, $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$; β , the slope at high irradiance, $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$ ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)⁻¹; E_k , saturating irradiance for photosynthesis, $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$

Dilution rate (day ⁻¹)	Turbulent dissipation rate (m ² s ⁻³)	P_m	α	R_d	β	E_k
0.1	0	313.49 ± 7.55	2.43 ± 0.42	-29.81 ± 7.87	-0.04 ± 0.01	128.87 ± 11.25
	0.007	320.78 ± 7.24	2.03 ± 0.32	-22.85 ± 6.05	0.08 ± 0.03	157.64 ± 9.46
	0.015	366.29 ± 3.31	2.49 ± 0.35	-2.24 ± 0.16*	-0.06 ± 0.01	147.17 ± 8.34
	0.030	398.82 ± 5.67	2.60 ± 0.33	-8.55 ± 0.14*	-0.03 ± 0.01	153.60 ± 6.35
0.3	0	328.43 ± 7.83	3.28 ± 0.75	-47.44 ± 2.43	-0.04 ± 0.01	100.24 ± 5.29
	0.007	446.95 ± 8.85	5.83 ± 0.11	-72.32 ± 2.41*	-0.02 ± 0.01	76.69 ± 4.71
	0.015	378.70 ± 7.53	4.44 ± 0.94	-72.11 ± 2.75*	-0.06 ± 0.02	85.38 ± 4.85
	0.030	396.74 ± 9.89	5.46 ± 0.20	-82.23 ± 3.86*	0.03 ± 0.01	72.66 ± 3.86*
0.6	0	525.10 ± 8.41	3.17 ± 0.43	-58.23 ± 2.65	-0.06 ± 0.02	165.49 ± 10.58
	0.007	494.16 ± 1.75	5.90 ± 0.11	-94.08 ± 4.63*	-0.07 ± 0.01	83.79 ± 2.69*
	0.015	493.21 ± 4.60	6.87 ± 0.41	-103.85 ± 5.25*	-0.07 ± 0.01	71.77 ± 3.85*
	0.030	493.03 ± 1.24	9.05 ± 0.64	-147.98 ± 6.46*	-0.05 ± 0.01	54.48 ± 2.84*

Data are means ± SD (n = 3)

*Significant difference ($P < 0.05$)

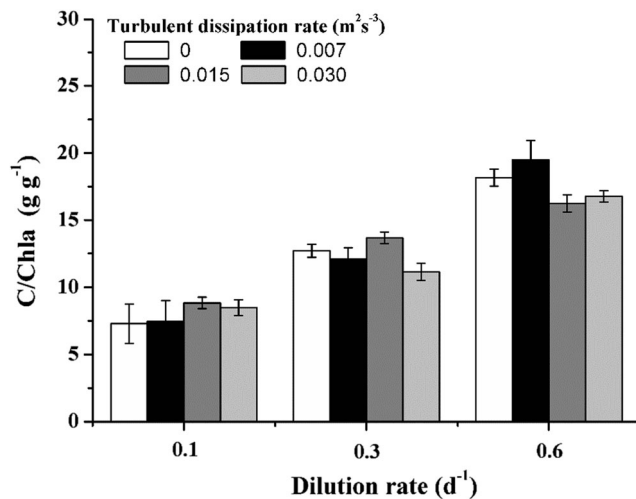


Fig. 4 Changes in the cellular carbon to chlorophyll *a* ratio of *Anabaena flos-aquae* grown at different dilution rates and turbulent mixing levels. Data are the means \pm SD ($n=3$)

activity of cultured *A. flos-aquae* was measured under different turbulence intensities and at various dilution rates. The results showed that increasing the turbulence intensity had no significant effect on CA activity at the dilution rates of 0.1 and 0.6 day⁻¹ (ANOVA, $P>0.05$). At a dilution rate of 0.1 day⁻¹, CA activity remained approximately stable, and at a dilution rate of 0.6 day⁻¹, it showed a slight increase. However, there was a significant decrease at higher turbulence intensity at the dilution rate of 0.3 day⁻¹ (ANOVA, $P<0.05$). In addition, higher CA activity was observed at the higher dilution rate. The CA activity of *A. flos-aquae* cultured at a dilution rate of 0.6 day⁻¹ was almost two times higher than that observed at a dilution rate of 0.3 day⁻¹ in each case (Fig. 5).

Variation in the biochemical composition of *A. flos-aquae*

The composition varied among the different turbulence intensity and dilution rate treatments (Fig 6). Slight increases in the carbohydrate content per cell were generally observed in the turbulent conditions relative to the stagnation condition at the lowest dilution rate (0.1 day⁻¹). However, a significant decline in carbohydrate synthesis was observed with increasing turbulent dissipation rate after exposure to the higher dilution rates (ANOVA, $P<0.05$). The carbohydrate content per cell in the 0.03 m² s⁻³ group was 40.3 and 72.2% lower than that in the control group at dilution rates of 0.3 and 0.6 day⁻¹, respectively. The protein content per cell increased over all of the culture treatments relative to the control treatments. The turbulent samples exhibited a higher protein content per cell than did the control samples at all dilution rates (ANOVA, $P<0.05$). The maximum protein content per cell was 2.86, 7.89, and 17.37 pg cell⁻¹ at dilution rates of 0.1, 0.3, and 0.6 day⁻¹,

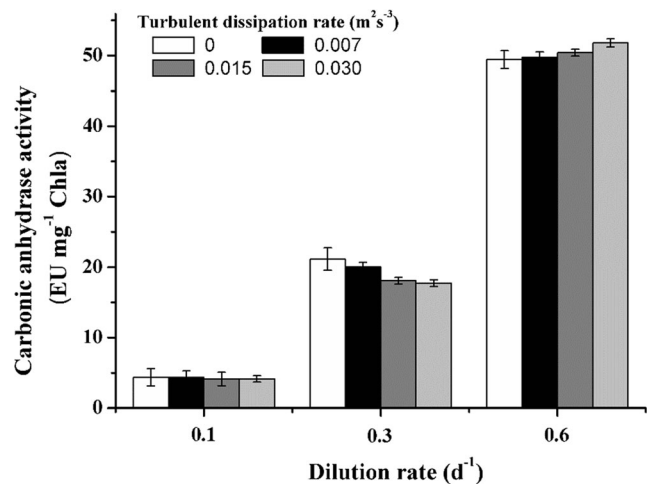


Fig. 5 Carbonic anhydrase activity of *Anabaena flos-aquae* grown at different dilution rates and turbulent mixing levels. Data are the means \pm SD ($n=3$)

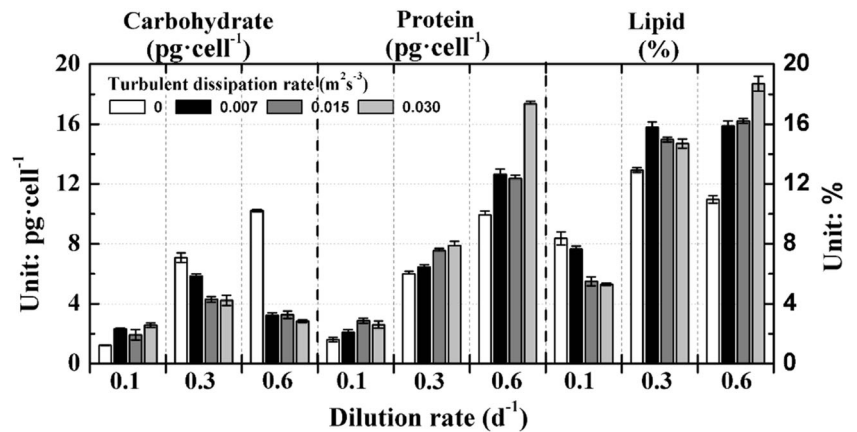
respectively. However, lipid content demonstrated the opposite trend. Total lipid accumulation decreased gradually with increasing turbulence intensity at a dilution rate of 0.1 day⁻¹, while compared to the control samples, the turbulent samples exhibited markedly increased lipid proportions at dilution rates of 0.3 and 0.6 day⁻¹ (ANOVA, $P<0.05$). The lipid content peaked (15.8 and 18.7%) under turbulent dissipation rates of 0.007 and 0.03 m² s⁻³ and dilution rates of 0.3 and 0.6 day⁻¹, respectively.

Discussion

Cyanobacteria have different degrees of sensitivity to turbulence, which may vary among mixing intensities and species (Peters and Marrasé 2000). This work used continuous cultures to study photosynthetic performance and carbon partitioning in relation to growth traits and turbulence intensity in *A. flos-aquae*. The turbulent dissipation levels in the photobioreactor covered the range of intensities experienced by cyanobacteria in the field, and the dilution rate, which refers to the growth rate at the steady state in continuous cultivation, was below the maximum specific growth rate of *A. flos-aquae* (0.78 day⁻¹) reported in the literature (Reynolds 2006).

Continuous cultivation at the laboratory scale has been recognized as useful for holding other relevant parameters constant, thus enabling the optimal levels of factors of interest to be determined (Czitrom 1999; Guedes et al. 2014). In the present study, the impact of turbulence on cells was first evaluated by direct measurement of cell density. A rapid increase in cell density was clearly observed following an increase in the turbulent dissipation rate at all dilution rates of *A. flos-aquae* under the steady-state conditions (Fig. 2). The dilution

Fig. 6 Comparison of the biochemical composition of *Anabaena flos-aquae* grown at different dilution rates and turbulent mixing levels. Data are the means \pm SD ($n = 3$)



rate determines the mean residence time of the population and, thus, the average age of the cells in the reactor (Mirón et al. 2003). At high dilution rates, rapidly growing young cells are more sensitive to an increase in turbulence mixing than are cells cultured at lower dilution rates, exhibiting greater increases in cell density compared to control cells. However, previous studies have proposed that turbulent forces that do not kill a cell may still produce stress responses, such as decreased photosynthetic efficiency (Thomas et al. 1995).

The carbon and energy requirements for phytoplankton metabolism are all derived from the photosynthetic process (Wilhelm and Jakob 2011). The photosynthetic rate, carbohydrate accumulation, and utilization of stored photosynthates may vary among cells with different activities (Olesen and Ganf 1986). Defining the functional relationships between turbulence and the photosynthetic performance of *A. flos-aquae* in the laboratory is perceived as a first step towards determining the partitioning of carbon into storage under turbulence effects. In this study, the responses of *A. flos-aquae* to turbulence intensities at varied growth rates varied widely based on the P-E curves and parameter measurements. The maximum oxygen production rates in the turbulence cultures were higher than those of the control culture at low and medium growth rates, whereas the opposite trend was observed at the high growth rate of 0.6 day^{-1} . It appears that the rapidly growing, younger cells were less adaptable to the high dissipation rate than were the older cells. Regarding photosynthesis, the slope of the P-E curve, α , increased with turbulence level at all growth rates of *A. flos-aquae* (Table 2). This result is consistent with the findings of Chengala et al. (2013) who concluded that turbulent conditions facilitated algal physiology, particularly in photosynthesis, as indicated by the higher O_2 levels in the bioreactor. Wang et al. (2012) reported that turbulence imparts a positive effect on the structure of PS II in diatom species. However, in a study of the dinoflagellate *Gonyaulax polyedra*, photosynthesis was not as sensitive to turbulence as was population growth (Thomas et al. 1995). We speculate that these discrepancies might be due to study variation in the turbulent conditions employed and differences

among species in response pattern. The present study showed that respiration was increased with the turbulent dissipation rate at a growth rate of 0.6 day^{-1} relative to the stagnant condition. Such negative effects could cause reductions in the oxygen production rate. Previous studies of the impacts of turbulence on photosynthesis in phytoplankton have primarily attributed the impacts to the “flashing light effect”, which refers to the changes in the light received by organisms due to the changes in the frequency of light-dark cycling (Grobbelaar 1994; Rubio et al. 2003). Nevertheless, in the case of *A. flos-aquae*, turbulence significantly decreased the saturating irradiance for photosynthesis at growth rates of 0.3 and 0.6 day^{-1} . The E_k value under a high dissipation rate at a growth rate of 0.6 day^{-1} was even lower than the experimental light intensity supplied. When planktonic organisms are cultured under light conditions that exceed their light saturation threshold, mixing can improve the light exposure to individual cells (Sobczuk et al. 2006). Therefore, it is likely that the rapidly growing, younger cells of *A. flos-aquae* can optimize their saturating irradiance to adapt to turbulence. The range of E_k values in our experiment reflects the plasticity of *A. flos-aquae*, exhibiting different growth traits in response to differences in turbulence. Such plasticity might be a significant contributor to this cyanobacterium’s environmental adaptability.

Turbulence consistently affected the carbon to chlorophyll *a* ratio in *A. flos-aquae*, but there was considerable variability in the response among the different growth rates. This variability may arise from at least three sources: (1) Photosynthesis control pathways from light requirement to the capacity for carbon dioxide fixation. Dark respiration, which can promote the consumption of cellular carbon, increased with increasing turbulent dissipation rate in the rapidly growing, younger cells of *A. flos-aquae*. (2) Turbulence weakened the Calvin Cycle in *A. flos-aquae* at the growth rate of 0.3 day^{-1} , as shown by the changes in CA activity, and reduced cellular carbon, as the maximum rate of carbon dioxide fixation is related to the amounts of CA or RuBisCo (MacIntyre et al. 2000). (3) At a given growth rate, cells under

continuous cultivation were in different physiological states (with younger cells at the high dilution rate and older cells at the low dilution rate). The shear sensitivity of filamentous cyanobacteria may vary depending on the growth stage, as demonstrated in *Nodularia* (Moisander et al. 2002).

It is believed that microorganisms alter their biosynthetic pathways in an attempt to maintain their growth rates or increase survival under unfavorable environmental conditions (Li et al. 2011). In general, the biochemical contents measured in the present study were all higher at the high growth rates than at the low growth rates. However, the partitioning of photosynthetic carbon into biochemical components in response to turbulence varied among the different growth rates in our experiment. At all growth rates, *A. flos-aquae* contained increasing amounts of protein as the turbulent dissipation rate increased, and the rapidly growing young cells contained more protein than older cells. Proteins facilitate the growth of cells and participate in the metabolic and structural functions of the cell. The results of our study are in agreement with Chengala et al. (2013), who reported that turbulence favored the accumulation of protein in *Dunaliella primolecta*. The proportion of protein in the cells increased partly due to the synthesis of new proteins in the rapidly growing young cells and partly due to the consumption of some storage components, mainly carbohydrates (Mirón et al. 2003). It has been reported that carbohydrates share the same precursors with triacylglycerols, which are largely produced under stimulating growth conditions (Klok et al. 2014). However, to date, the relationships between carbohydrate and lipid synthesis remain unclear (Li et al. 2010). The present study showed that the processes of carbohydrate formation and lipid accumulation are asynchronous, and they demonstrated opposing trends at a given growth rate. As shown in Fig. 6, at the low growth rate, the carbohydrate content increased with increasing turbulent dissipation rate, whereas the lipid content decreased. At the high growth rate, the level of carbohydrates decreased with increasing turbulent dissipation rate, whereas that of lipids increased. These trend differences might be related to the differences in the specific shear sensitivity under different growth rates in *A. flos-aquae*, which indicated that the rapidly growing cells were more sensitive than were the older cells to turbulence. In the rapidly growing young cells, the respiration level increased and saturating irradiance decreased in response to a high turbulent dissipation rate, which can partially explain the negative effects on carbohydrate level. Under stress, some algal species can inhibit the carbohydrate biosynthetic pathway and redirect photosynthetic carbon flux towards lipid biosynthesis (Li et al. 2011). Lipid biosynthesis and accumulation occur in different growth phases (Yu et al. 2017). At high dilution rates, young cells contain lower amounts of storage lipids than do cells cultured at lower dilution rates (Reis et al. 1996). Our results demonstrated that the optimum level of lipid accumulation in response to turbulence was exhibited

by the younger cells of *A. flos-aquae*, indicating that carbon partitioning by turbulence, which underlies the improvements in lipid accumulation, is sensitive to growth rate. Turbulent mixing has been shown to regulate the nutrient fluxes of cells (Karp-Boss et al. 1996). Increments of nitrogen and phosphorus fluxes in the rapidly growing young cells were also observed under the high turbulent dissipation rate (data not shown), resulting in the acceleration of lipid synthesis. Moreover, cyanobacterial lipids play important roles in thylakoid membrane structural components (Chen et al. 2013); therefore, a high lipid content is vital for photosynthetic processes. Taken together, the results suggest that altering the allocation of biochemical compounds might be a strategy for adapting to unfavorable conditions at high growth rates in *A. flos-aquae*. The results may serve as a theoretical guide for the enhancement of lipid productivity by regulating the turbulence supply. For future research, we suggest that fatty acid composition analysis be conducted and that multiple cyanobacterial species be studied to better understand their implications for the design of cultivation strategies for cyanobacteria resource utilization.

Conclusion

This study revealed shear regimen- and growth rate-dependent sensitivity to turbulence in a strain of *Anabaena*. Under the continuous culture conditions, a high respiration capacity, low saturating irradiance, and the conversion of carbohydrates to lipids were observed in the rapidly growing young stage of *A. flos-aquae*. These responses might reflect adaptive strategies employed to promote survival under high turbulence intensity. From a biotechnological perspective, such strategies represent safe, natural approaches for promoting lipid accumulation in rapidly growing young cells.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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