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Influence of Ventilation Modes on Carbon Dioxide Fixation of *Chlorella vulgaris* in a Flat-Plate Photobioreactor

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Abstract: Microalgae have the ability to mitigate CO_2 emission with a high productivity, thereby having the potential for applications in reducing CO_2 with high concentration. To date the effect of different ventilation modes in photobioreactor on the high level CO_2 fixing capacity was still unclear. To explore an efficient, convenient and cheap aerating method for high concentration CO_2, an inner parallel flat-plate photobioreactor configuration (IPFP) was designed and the effect of inner-mixing ventilation and intermittent ventilation modes on the CO_2 fixation rate (CFR) in a laboratory-scale IPFP was determined. *Chlorella vulgaris*, a promising freshwater green algal strain with high CO_2 fixation rate under CO_2 concentration lower than 5% (v/v) but cannot survive under 15% CO_2, was used to perform the experiments. Results showed that both of the novel ventilation modes can effectively enhance microalgal performance on growth and carbon biofixation rates when 15% CO_2 was directly provided. The CFR of *Chlorella vulgaris* in this photobioreactor ranged 1.30 to 1.78 g $CO_2 \cdot L^{-1} \cdot d^{-1}$. The pH value of cultural medium was also determined. Results showed that the distribution of pH values was uniform in the IPFP cross section during the inner-mixing ventilation mode, which indicating a good mixing characteristic of the fluid in the IPFP. In the intermittent ventilation mode, the pH values demonstrated periodical variation with the maximum value of 8.2 and the minimum value of 6.5. Aerating 15% CO_2 for 12 minutes and air for 48 min in one hour (12 min 15% CO_2/48 min air) provided a longer period in pH<7.0 than that of aerating 15% CO_2 for 6 minutes and air for 54 min in one hour (6 min 15% CO_2/54 min air), and thus, was more beneficial to the CFR. IPFP with an inner-mixing ventilation mode effectively enhances the performance of *C. vulgaris* on microalgal growth and CO_2 biofixation, indicating that this PBR has the potential for use in the field of carbon reduction.

Keywords: Carbon Dioxide Biofixation, *Chlorella vulgaris*, Inner-mixing Ventilation, Intermittent Ventilation, Flat-plate Photobioreactor

1. Introduction

Bio-fixation using microalgae was suggested as a promising method for the post-combustion CO_2 capture and storage [1, 2], and the CO_2 fixation capability depends heavily on the biomass production [3, 4]. In the existing equipment, closed photobioreactors (PBRs) seem to be well suited to culture microalgae that are particularly dedicated to CO_2 fixation and rapid biomass accumulation in comparison to open ponds [5, 6]. Numerous types of PBRs, e.g. column, flat

plate, and spiral tube, have been designed for high-efficient CO_2 fixation of microalgae. Previous studies have proved that the flat-plate PBR had advantages of larger illumination surface area and smaller light path length, and thus, microalgae cultured in this PBR could achieve both relatively higher biomass yields accompanying with lower energy consumption [7-9].

Some physic-chemical parameters strongly affect the CO_2 fixation efficiency of microalgae, including geometry size, aeration rate, and ventilation model. Among these parameters, CO_2 ventilation (i.e., CO_2 concentration, feeding rate, and ventilation mode of CO_2) is considered as the most significant factor to influence the cell photosynthesis of microalgae [10, 11]. Even though each microalgal strain responds differently to the CO_2 concentrations, it has been widely reported that the maximum photosynthetic efficiency and productivity decreased dramatically for the vast majority of microalgae for CO_2 concentrations above 10% (v/v) [11, 12]. With regard to flue gases, high CO_2 concentrations are less likely to favor microalgae culture except for few species, e.g., *Botryococcus braunii* 765 [13], *Chlorella* sp. [14, 15], and *Nannochloris* sp. [16]. If either real or simulated flue gases were provided directly, drastic consequences would result for the microalgae lacking tolerance ability for high CO_2 concentration. Many efforts have been undertaken to dilute flue gases; however, the cost of associated equipment made it less likely to achieve a more cost-effective cultivation and biofixation [17-19]. The CO_2 feeding rate (SGV, vvm, volume gas per volume broth per minute) was a further factor influencing the hydro-dynamics in the reactor and the pH value of microalgal suspension combining with CO_2 concentration [20]. Exposure of microalgal cells to high gravitational and shear forces can damage cell structure. For the majority of closed cultivation, the commended ventilation rate was within a range of 0.1-1.0 vvm [21]. A further factor underlying the CO_2 fixation of microalgae is the CO_2 ventilation mode. Recent studies generally focus less on the comparison of the CO_2 fixation of different ventilation modes. Consistent with the previous argument, we are interested to learn how to obtain a smooth implementation via directly aerating a high concentration of CO_2, thus decreasing the dilution device cost and; furthermore, to determine whether the CO_2 mixture can be achieved inside the PBRs. In our previous publications, we proposed an innovative flat photobioreactor (IPFP) and the Chlorophyta *Chlorella vulgaris* could achieve a high carbon fixation rate under both [17, 22]. The objective of this study was to compare the CO_2 fixation rates (CFR) of these two modes, i.e. the inner-mixing ventilation and intermittent ventilation modes, and then investigate the possible mechanism in term of pH distribution in the suspension.

2. Materials and Methods

2.1. Organism and Culture Medium

C. vulgaris was obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology (FACHB-collection), the Chinese Academy of Sciences (Wuhan, China). Algal cells were pre-incubated in a 5 L Erlenmeyer flask at 25°C under cool-white fluorescent lighting at 80 μmol· m^{-2}·s^{-1} for two weeks. To avoid sticking, the flask was hand shaken five times daily during the pre-incubation experiment. Then, the microalgae cells were transported into a photobioreactor at a cell density of up to 1.0 × 10^9 cells/ml. During the experiments, microalga was cultivated in a modified BG11 medium with the composition of $NaNO_3$ (1.5 g·L^{-1}), K_2HPO_4 (0.04 g·L^{-1}), $MgSO_4$·$7H_2O$ (0.075 g·L^{-1}), $CaCl_2$·$2H_2O$ (0.036 g·L^{-1}), Citric acid (0.006 g·L^{-1}), Ferric ammonium citrate (0.006 g·L^{-1}), EDTA·Na_2 (0.001 g·L^{-1}), and trace mental solution (1 ml·L^{-1}). The trace mental solution consists of H_3BO_3 (2.86 g·L^{-1}), $MnCl_2$·$4H_2O$ (1.86 g·L^{-1}), $ZnSO_4$·$7H_2O$ (0.222 g·L^{-1}), $CuSO_4$·$5H_2O$ (0.079 g·L^{-1}), Na_2MoO_4·$2H_2O$ (0.390 g·L^{-1}), and Co $(NO_3)_2$·$6H_2O$ (0.049 g·L^{-1}) [23]. The pH value of the medium was adjusted to 7.1 via 1 M NaOH or 1 M HCl.

2.2. Bioreactor Setup and Semi-Continuous Operation

The main structure of IPFP is a 10 L acrylic plate vessel (24 cm long, 8 cm wide, and 50 cm high). Light was provided via three inner set flexible LED strips (LXHL-PW09, Philips Ltd., Holland) on the three transparent draft tubes of the IPFP (Figure 1). Two-side illuminations at 120 μmol·m^{-2}·s^{-1} were realized on each tube for the autotrophic growth of microalgae. Three identical draft tubes were vertically placed and equally distributed throughout the tank. The main design parameters of the IPFP were approximately: ratio of height to diameter = 2:1, working volume = 8.0 L, and ratio between the downcomer and riser cross sectional area = 3.0. Prior to each experiment, the bioreactor was soaked in a solution of 0.3% NaClO for 1 h and then, the remainder of disinfectant was removed via sodium thiosulfate under sterile condition. A high concentration of CO_2 (15%) was fed into the photobioreactor through CO_2 diffusers that settled at the bottom of the tank. 15% CO_2 was prepared using clean air and pure CO_2 gas (according to a ratio 17:3 v/v) in a gas mixer.

Before the stationary phase, 2 L microalgal culture was replaced daily with fresh medium on the fourth or the fifth day. All experiments were carried out in a two-step illumination strategy to maintain rapid and steady microalgal growth. During the early growth phase, the culture was operated in the first illumination phase at 120 μmol·m^{-2}·s^{-1}, while the irradiance increased to 240 μmol·m^{-2}·s^{-1} during the second illumination phase when the cell density reached 2.0 × 10^7 cells/ml. Both ventilation modes were realized by operating the three gas inlets displayed in Table 1.

2.3. pH Value Measurement

The pH values in experiments NO. 1, NO. 2, and NO. 5 were directly determined once per day using a PHS-3C pH meter with E-201-C-9 type combined electrode (Shanghai LIDA Instrument Factory, Shanghai, China). Results were illustrated via contour plots by using the software Surfer 13.0 (Golden Software, USA) together with the Kriging

interpolation method. The pH values in experiment NO. 3 and experiment NO. 4 were monitored and recorded every minute via calibrated pH meter.

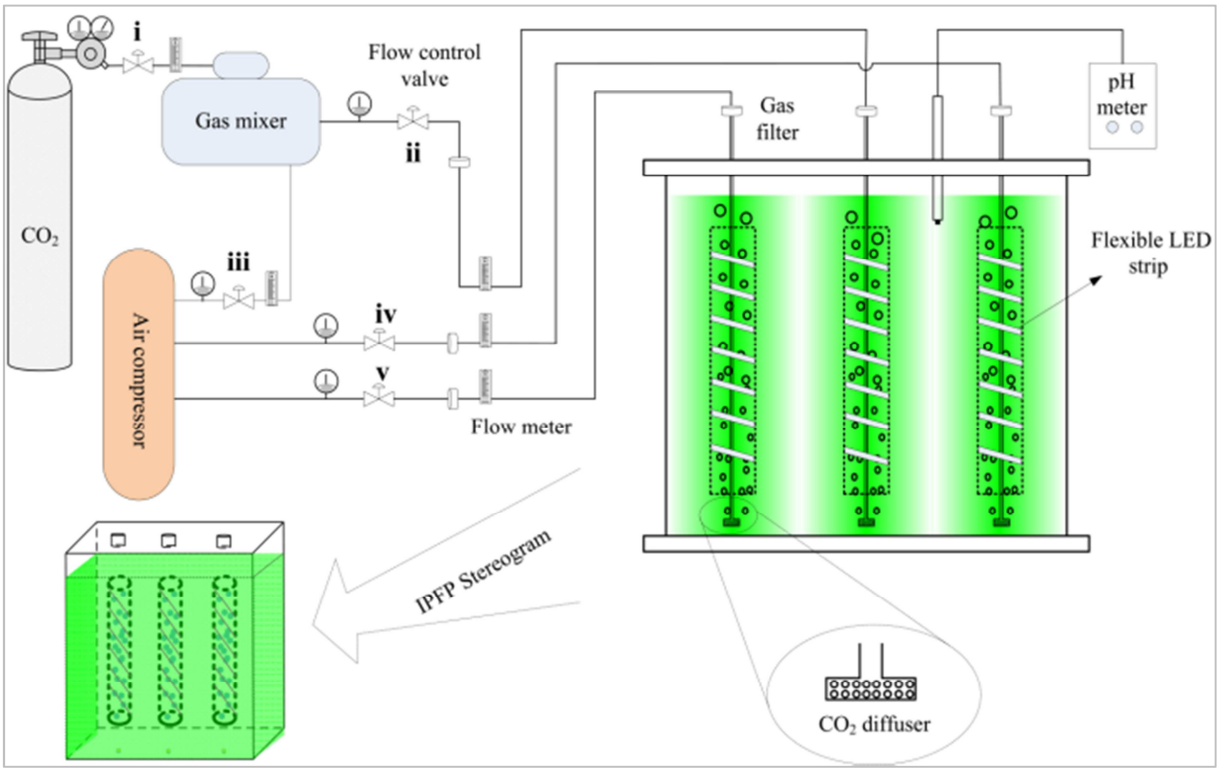

Figure 1. *Schematic diagram of the inner parallel flat-plate PBR.*

Table 1. *Ventilation modes and gas feeding conditions.*

	Ventilation modes	Air inlets and gas types A-B-C	SGV (vvm)[e]	Feeding methods
NO. 1 [a]	Inner-Mixing	Air-15% CO_2-Air	0.3	Continuously
NO. 2 [b]		N_2-15% CO_2-N_2		Continuously
NO. 3 [c]	Intermittent	15% CO_2 or Air	0.3	Alternately
NO. 4 [d]		15% CO_2 or Air		Alternately
NO. 5	Control	Air	0.3	Continuously

Note: a. Air was provided through inlets A and C and, 15% CO_2 was continuously provided through inlet B; b. N_2 was provided through inlets A and C, and 15% CO_2 was continuously provided through inlet B; c. in one hour, 15% CO_2 is fed through all inlets for 6 min, and then, air is fed through all inlets for 54 min alternately; d. in one hour, 15% CO_2 is fed through all inlets for 12 min and then, air is fed through all inlets for 48 min alternately; the air flow through inlet A, B, and C was equal in all experiments.

2.4. Determination of Growth Kinetic Parameters and CO_2 Fixation Rate

Microscopic counts were performed on the suspension samples using a hemacytometer and a 37XB inverted biological microscope. Algal growth was expressed via cell density (CD, cells·ml^{-1}) measured according to Chiu et al. [9].

Biomass was gravimetrically determined as follows: firstly, a known volume of *C. vulgaris* suspension was taken once per day and centrifuged at 1000 g for 15 min; then, the solid was washed twice using distilled water and was dried at 105°C for 16 h. The biomass productivity (P, g·L^{-1}·d^{-1}) was calculated according to Eq. (1):

$$P = (W_2 - W_1) / (t_2 - t_1) \quad (1)$$

where W_1 and W_2 represent the biomass (g·L^{-1}) at time t_1 and t_2, respectively.

The CFR (gCO_2·L^{-1}·d^{-1}) was calculated via the relationship between the carbon content and the volumetric growth rate of the microalgal cells, as indicated in Eq.(2) [17]:

$$CFR = P \times C_{carbon} \times M_{CO2} / M_C \quad (2)$$

where C_{carbon} represents the carbon content of the microalgae cell (%, w/w) determined via elemental analyzer. M_{CO2} and M_C represent the molar mass of CO_2 and carbon, respectively.

2.5. Statistical Analysis

SPSS 17.0 was employed for statistical analyses. The semi-continuous cultivation of each experiment had four operating cycles. The CFRs were expressed via mean values ± standard deviations (mean ± SD). The variations of CFRs were tested according to one-way analysis of variance (ANOVA). When significant differences were found, post-hoc comparison tests for means were conducted using Tukey test or Games-Howell test to identify which values were significantly different. Statistical significance was defined at $P < 0.05$.

3. Results and Discussion

3.1. pH Distribution in the IPFP Under the Inner-mixing Ventilation Mode

The pH monitoring location may potentially impact the comparison of the determination results between five experiments, especially between experiment NO. 1 and experiment NO. 2. Fifteen measuring points were uniformly dispersed on the cross section of the IPFP as shown in Figure 2. A low pH region (about 6.45) appeared in the IPFP cross section. On both sides of the photo bioreactor, pH showed a characteristic slight increment from the central axis to the sides with the value above 6.50. Generally, pH sharply decreased to pH 5.0 when the CO_2 concentration ≥ 10-20 % was directly aerated [24]. In experiment NO. 1 (without inoculation), 15% CO_2 was bubbled through the inlet B, and atmospheric air with extremely low CO_2 (about 0.04% v/v) was bubbled through both inlets A and B. The air flow through inlets A, B, and C was equal (0.1 vvm). Severe acidification was absent in the IPFP. This phenomenon indicates that the turbulent flow and the mixed gas-liquid-solid (CO_2-medium-microalgae) phases were appropriate for the inner dilution of 15% CO_2 in the IPFP. Compared to the growth of *C. vulgaris* at pH 6.2, its growth under pH 3.0-5.0 was limited to (29.7-60.5)% [25]. Using a special inner-mixing ventilation mode, strenuous acidification in the PBRs was avoided and, a direct utilization of a high concentration of CO_2 without any traditional external gas dilution operation could be realized.

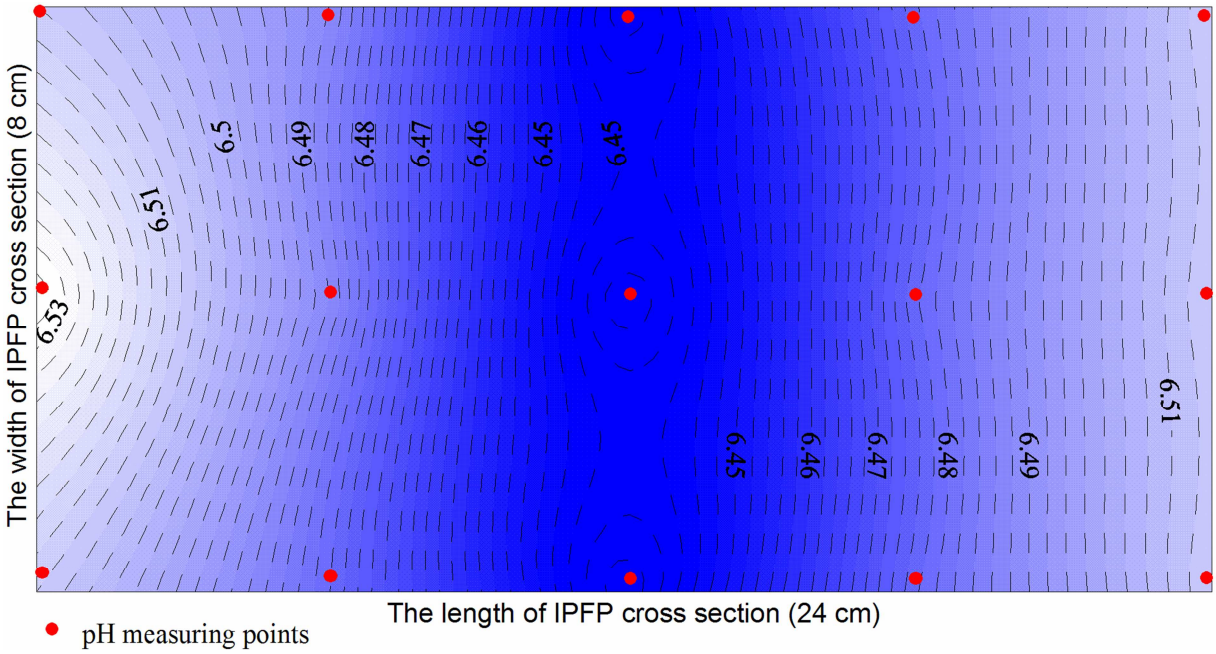

Figure 2. *Effect of monitoring position on the pH values of the microalgae suspension in the IPFP under inner-mixing aeration mode.*

3.2. pH Values Under the Intermittent Ventilation Mode

The relationship between the average pH value of the culture medium and the cultivation time of experiments NO. 3 and NO. 4 was illustrated in Figure 3. The pH value rapidly decreased from 6.98 to 6.62 (NO. 3, 6 min) or to 6.52 (NO. 4, 12 min) in response to 15% CO_2 ventilation, indicating that the chemical equilibrium moved toward accumulation of CO_2 and HCO_3^- for implementing photosynthesis and growth. When the gas type converted, the ventilation of air enabled CO_2 absorption, thus decreasd the concentration of H^+ in the medium and increasing the pH value. CO_2 concentrating mechanisms have been suggested to have a relation with the formation of carbon in medium. CO_2 firstly combines with H_2O to form H_2CO_3, which dissociates into HCO_3^- and H^+. HCO_3^- further dissociates into CO_2 or CO_3^{2-} depending on the pH value of the medium [26]. HCO_3^- is the main form of inorganic carbon, which could enter into microalgal cells through an active transport and then becomes organic carbon.

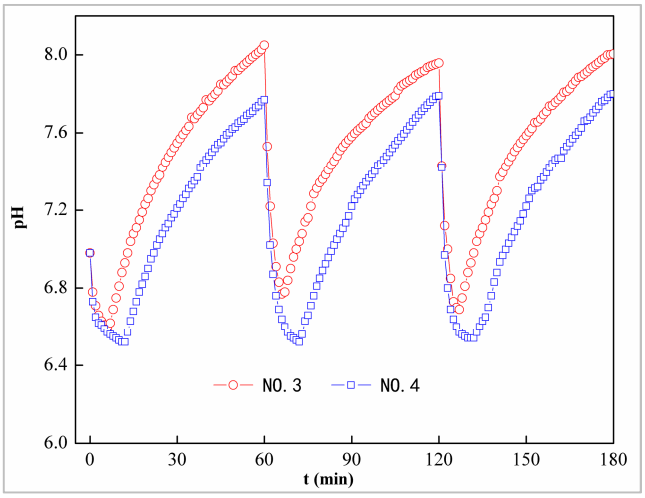

Figure 3. *pH values of C. vulgaris suspension under intermittent aeration mode.*

3.3. CO_2 Fixation Rate under Different Ventilation Modes

By daily withdrawing 25% culture and adding fresh medium, cells could be maintained at a non-limited growth. Moreover, during the second illumination phase at 240 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the light energy was sufficient for microalgal growth. The cell densities obtained from experiments NO. 1 to NO. 5 were (7.58 ± 0.24), (7.88 ± 0.21), (7.12 ± 0.25), (7.50 ± 0.36), and (6.38 ± 0.13) × 10^7 $cells \cdot ml^{-1}$, respectively (Figure 4). The sequence of the CFR was NO. 2 > NO. 4 ≥ NO. 1 ≥ NO. 3 > NO. 5 (Figure 5). In our previous study, *C. vulgaris* could not survive under 15% CO_2 aeration without any dilution. This result clearly indicated that both the inner-mixing ventilation mode and the intermittent mode benefit the growth of *C. vulgaris* under a supply of 15% CO_2. Lam and Lee reported that *C. vulgaris* grew more favorably at 5% CO_2 than at a combination of CO_2 bubbling and ambient air [27]. This study also indicated that *C. vulgaris* grew better in CO_2 supply than in air, which could mainly be attributed to the positive effect on the photosynthesis of plants for increasing CO_2 levels via CO_2 fertilization.

The CFR in the experiment NO. 2 was significantly higher than that in experiment NO. 1. Both experiments are different in the type of gases aerated from inlets A and C. The dissolved oxygen (DO) of algal suspension in NO. 2 was below 2.12 $mg \cdot L^{-1}$, which was far lower than that in NO. 1 (9.98-11.32 $mg \cdot L^{-1}$) (Figure 6). N_2 aerating from inlets A and C sharply lowered the DO. The improvement of CFR may be attributed to the growth enhancing under a low DO level. The bubbles formed by N_2 ventilation would benefit the elimination of the photo-inhibition effect of accumulative oversaturated DO on microalgal photosynthesis [28, 29].

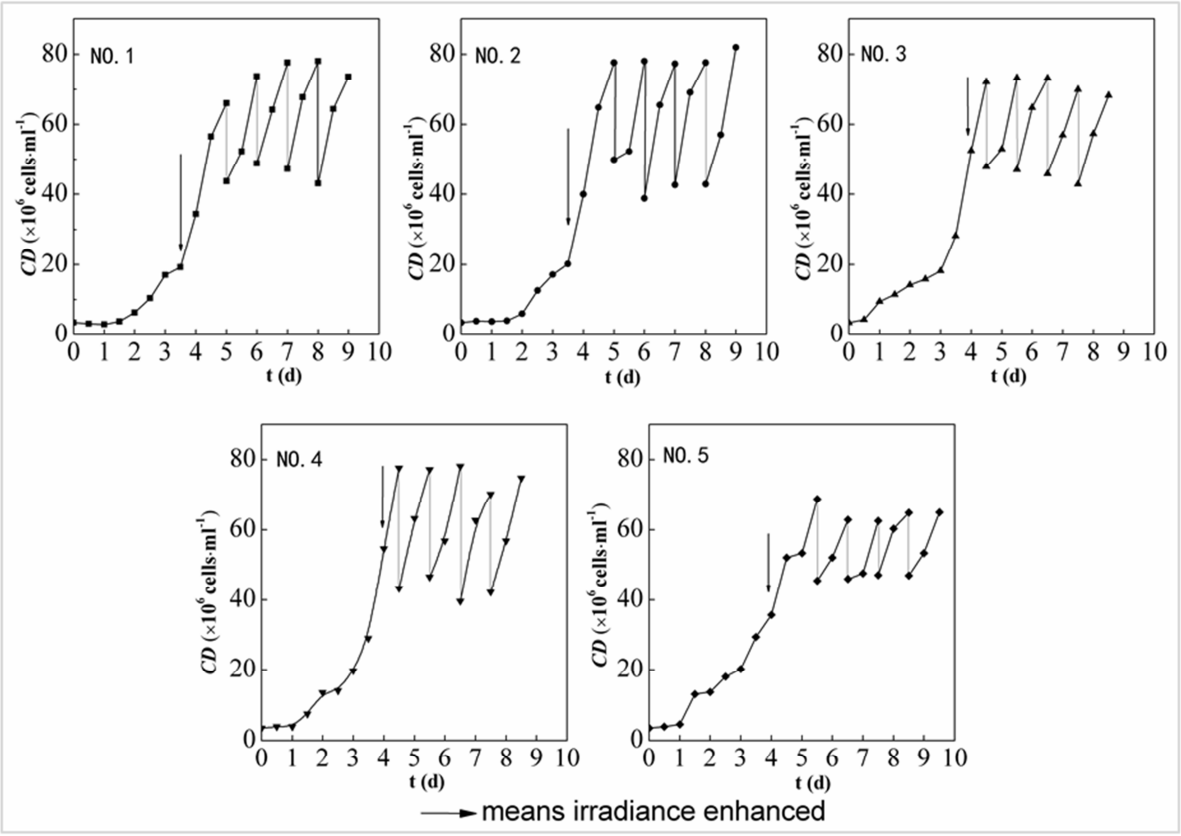

Figure 4. *Growth curves of C. vulgaris under different ventilation modes.*

Concerning the intermittent ventilation mode, the CFR in NO. 4 was (1.56 ± 0.08) $gCO_2 \cdot L^{-1} \cdot d^{-1}$, which was significantly higher than that of experiment NO. 3 ((1.30 ± 0.20) $gCO_2 \cdot L^{-1} \cdot d^{-1}$). Both results are higher than the highest CFR of previous studies that used a tubular PBR with *C. vulgaris* under similar condition (1.16 $gCO_2 \cdot L^{-1} \cdot d^{-1}$) [30]. Therefore, the new configuration of PBR favors the improvement of the cell growth rate and the CO_2 fixation ability of our microalga strain. Theoritically, longer ventilating time per hour should result in a more apparent inhibitory effect on microalgae growth. It can be concluded that there are other mechanisms that affect the microalgal carbon fixation in the IPFP in experiments NO. 4 and NO. 3.

Usually, CO_2 concentrating mechanisms (CCM) are believed to have a relation with formation of carbon in medium. CO_2 firstly combines H_2O to form H_2CO_3 which dissociates into HCO_3^- and H^+, HCO_3^- further dissociating into CO_2 or CO_3^{2-} depending on the pH of medium. CO_2 could enter into microalgal cells through active transport and then being biofixation. However, in the closed cultivation, aeration high level CO_2 increases the H^+ concentration in the medium, resulting in a pH value decrease. With the cell growth the

photosynthesis leads to the pH value gradually increasing. Also, it should be pointed out that, usually, low pH value can inhibit the growth of algae although there is different adaptivity to pH value in different microalgae, e.g., the microalga *Chlorella vulgaris* maintained the maximum growth rate in the wide range of pH between 6.0 and 9.0 but started to be inhibited from pH 5.0 while *Chlorella* strain KR-1 has a constant growth at pH 4.2 but was completely inhibited at pH 3.5 [31]. In most cases, fresh water eukaryotic algae prefer acidic environments (pH 5.0-7.0) [32, 33]. In the case of experiment NO. 4, the time per hour (21 min) under weak acidic conditions is longer than that of NO. 3 (13 min), which could be advantageous for microalgal growth.

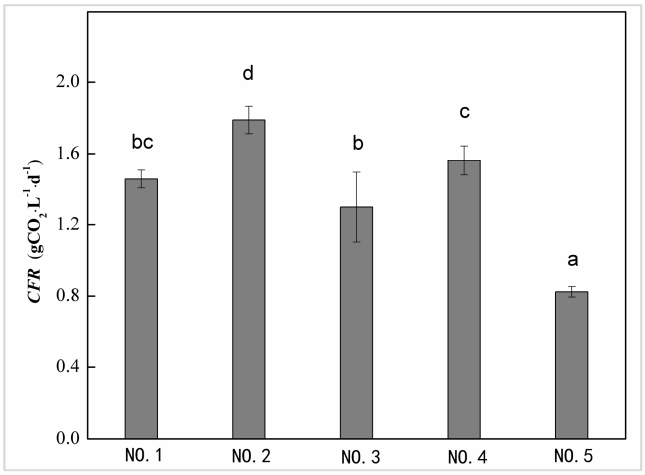

Figure 5. *CFR of C. vulgaris under different ventilation modes. Data with different letters (i.e. a, b, c, and d) are significantly different (P < 0.05).*

Table 1 summarized the microalgal CO_2 fixation rate using different photobioreactors for different microalgae species facing high CO_2 concentration. Results showed that under phototrophic cultivation, there is a large variation in the FD of microalgae, ranging from 0.26 to 6.56 g $CO_2 \cdot L^{-1} \cdot d^{-1}$, depending on the type of microalgae species and CO_2 concentration. Doucha et al. reported that FD of the microalga *Chlorella* sp. reached 6.56 g $CO_2 \cdot L^{-1} \cdot d^{-1}$ in an outdoor open thin-layer photobioreactor [39]. To date, this is the highest FD achieved, which can be mainly attributed to the much higher light absorption efficiency of thin liquid layer than that of general PBR. Moreover, the advantages of using *Chlorella* sp. to fix high concentration have been demonstrated in other publications [34, 37], although other species, e.g., *Scenedesmus* sp. and *Chlorococcum littorale*, also displayed excellent tolerance to high concentration of CO_2 (above 20%). However, most of these results were obtained in the PBR with a continuous gas feeding. No effort has been conducted to deal with the low pH value caused by high CO_2 aeration. This study proposed an inner-mixing ventilation mode, and achieved a high FD. This improvement could mainly attribute to the mitigation of low pH value in the medium which is usually recognized to have an adverse effect on growth of microalgae. Moreover, the aerated (closed) cultivation using N_2 could eliminate the photo-inhibition of accumulative oversaturated DO on microalgal photosynthesis. As a whole, the microalgal *C. vulgaris* was able to rapidly grow and effectively fix CO_2 when using the proper cultivation mode, indicating it has the potential for use in the field of carbon emission reduction and biomass energy.

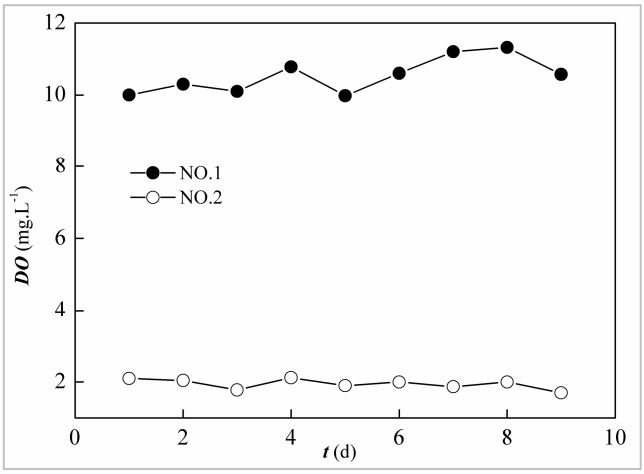

Figure 6. *CFR of C. vulgaris under different ventilation modes. Data with different letters (i.e. a, b, c, and d) are significantly different (P < 0.05).*

Table 2. *Comparation of the IPFP with other PBR based on FD when microalgae facing high concentration CO_2 (higher than 5%) directly.*

PBR	Species	CO_2 (%)	Cultural mode	*FD*	
IPFP	*C. vulgaris*	15	Semi-continuous	1.78	This study
L-F-PBR[a]	*Euglena gracilis*	11	Semi-continuous	0.27	[34]
V-PBR[b]	*Chlorococcum littorale*	20	Batch	0.90	[35]
BV-PBR[c]	*C. vulgaris*	10	Batch	0.51	[36]
BV-PBR	*Aphanothece microscopica Nägeli*	15	Batch	0.51	[37]
BV-PBR	*A. microscopica N.*	15	Batch	0.55	[38]
BV-PBR	*C. pyrenoidosa*	15	Batch	0.48	[39]
BV-PBR	*Microcystis aerusinosa*	15	Batch	0.49	[39]
BV-PBR	*M. ichthyoblabe*	15	Batch	0.52	[39]
BV-PBR	*Scenedesmus* sp.	15	Batch	0.61	[38]
BV-PBR	*S. quadricauda* SDEC-13	15	Batch	0.14	[40]
Airlift V-PBR	*A. microscopic N.*	15	Batch	1.43	[38]
Flask	*C. pyrenoidosa* SJTU-2	10	Batch	0.26	[41]
Flask	*C. pyrenoidosa* SJTU-2	20	Batch	0.22	[41]
Flask		30	Batch	0.15	[41]
Flask	*S. obliquus* SJTU-3	50	Batch	0.11	[41]
Flask		10	Batch	0.29	[41]

PBR	Species	CO_2 (%)	Cultural mode	*FD*	
Flask	*S. obliquus* SJTU-3	20	Batch	0.25	[41]
Flask		30	Batch	0.15	[41]
Flask		50	Batch	0.11	[41]
TL-PBR[d]	*Chlorella* sp.	6–8	Fed-batch	6.56	[42]

Note. a. L-F-PBR, L type flat photobioreactor; b. V-PBR, Vertical-column photobioreactor; c. BV-PBR, Bubbling Vertical-column photobioreactor; d, Thin layer open PBR.

4. Conclusion

Both the inner mixing ventilation mode and the intermittent ventilation mode can enhance *C. vulgaris* growth and carbon biofixation when 15% CO_2 was directly provided. The inner mixing ventilation mode is more effective. DO dilution and pH value both played important roles in microalgal fixation.

Acknowledgements

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