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Forced light/dark circulation operation of open pond for microalgae cultivation

Yu Chen, Junfeng Wang, Wei Zhang, Lin Chen, Lili Gao, Tianzhong Liu*

Key Laboratory of Biofuels, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao 266101, China

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ABSTRACT

Open pond has been widely used for microalgae culture. However its lower biomass productivity damaged its economic viability used as cultivation systems for feedstock production. In this study we introduced a forced L/D circulation operation to conventional open pond, in which the culture medium was pumped to circulate between an illuminated shallow pond and a fully darkened tank. The growth of microalgae was dominated by the photic retention time and dark/light ratio of the forced L/D circulation. The optimal values were determined to be 3.98 for dark/light ratio and 5.80 min for photic retention time by response surface methodology, at which, a biomass productivity of 36.5 g m^{-2} d^{-1} for *Scenedesmus dimorphus* was approached in laboratory. Outdoor cultivation practice with the forced light/dark circulation of pond was also carried out and averaged 28.5 g m^{-2} d^{-1} biomass productivity was achieved, which is double of that by conventional open pond cultivations in 250 mm or 50 mm water depth without forced circulation.

1. Introduction

Microalgae are considered as the most promising feedstock for biofuel production due to the high productivity potential, less competition with food production and less negative impact on the environment when compared with other biomass feedstock options [1–3]. Although intensive efforts have been made on microalgae biofuel research, no commercial production systems have achieved economic viability [3–7] mainly due to the low cultivation efficiency and high cost. Of the prevailing microalgae culturing devices, open pond has the widest applications [8–10] because of its easy construction, mature scale-up and power input effective [11]. However, the poor biomass productivity at field level is its fatal drawback considering the competition of land for traditional crops when commercial developments of microalgae biofuel are promoted.

In order to improve the cultivation efficiency of open pond, many methods to enhance the mixing and CO_2 transfer have been developed. However, solar illumination is the most dominative factor to influence the growth of microalgae at field level of pond [12]. Open pond is generally operated at the water level higher than 200 mm. Because of the attenuation of solar light, there are several irradiate zones with different light intensities alone the light path. The illumination intensity of the pond is below the photo-compensation point for algae growth or totally blacked except the upmost thin layer [13]. As a result, photosynthesis of microalgae is limited for its growth. Though mixing in light path direction may improve the cultivation efficiency, the paddle-wheel is ineffective to produce such kind of mixing at economic power input.

The whole photosynthesis process includes two stages, viz. 'light' stage in which the light energy is absorbed to oxidize the water and produce NADPH and ATP, 'dark' stage in which

* *Corresponding author*. Tel./fax: +86 532 80662735.
E-mail address: liutz@qibebt.ac.cn (T. Liu).
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the NADPH and ATP are fixed into carbon hydrate. This character of photosynthesis provides a way of alternate illumination by light/dark (L/D) cycles to improve the microalgae growth [13–17] without extra illumination. The frequency of the intermittent irradiation is thought to be an important factor to influence solar utilization and photosynthetic efficiency [18]. Grobbelaar [16] has grouped the fluctuating L/D cycles into three scales: (i) High frequency fluctuation of less than 100 ms (10 Hz); (ii) Medium frequency fluctuation from seconds to minutes; (iii) Low frequency cycles from hours to natural day–night alternation. The first scale gives rise to the "flashing effect", which has been reported to lead to higher growth rates [19–22]. However such high frequency fluctuation is unfeasible in engineering practice and power input for mass cultivation. Low frequency cycle from hours to natural day–night alternation has no meaning for improvement of microalgae cultivation because all current open pond cultivations are natural day/night alternation. What's more interesting is the medium frequency fluctuations of seconds-to-minutes level because of their economic feasibility in practice. Merchuk [23], Bosca [24] and Wang [25] have showed that algae exposed to certain L/D cycles irradiation has high photosynthetic activity and specific growth rates. However, what is the appropriate frequency is conflicting of most references. For example, Janssen [26] found L/D cycles in the range 6–87 s leads to similar or lower growth rates. Grobbelaar [16] found no influence of L/D cycles in the range 1–263 s on the volumetric productivity. Vejrazka [27] showed that using a duty cycle of 0.5, L/D cycles of 1 and 10 Hz resulted about 10% lower biomass yield, but of 100 Hz resulted about 35% higher biomass yield than the yield obtained in continuous light. Xue et al. [28] found that larger photosynthetic enhancement could be expected with the increase of L/D frequency at higher light intensity, while light integration effect was totally absent under low light fractions.

Here we introduced the forced L/D circulation to open pond, by which the conventional open pond with deep water level (ca. 200 mm depth for example) was replaced by a shallow lighted open pond (ca. 50 mm depth for example) connected with a fully dark tank (containing all other culture medium). The volume ratio of the shallow pond to the dark tank and the flow rate could be adjusted to produce different frequencies of the forced L/D circulation. The influence of the photic retention time and dark/light volumetric ratio on biomass productivity was investigated, and the optimal values of the two factors were determined by response surface methodology. Outdoor experiments were also conducted to validate the enhancement on algal growth.

2. Materials and methods

2.1. Algal strain and growth medium

Microalgal species of *Scenedesmus dimorphus* was isolated from local wastewaters (Qingdao, China) and maintained in the CAS Key Laboratory of Biofuel. The strain was maintained in 250 cm^3 Erlenmeyer flasks containing 100 cm^3 of modified BG-11 medium [29] in an incubator at a temperature of 25 °C and under continuous illumination of 100 μmol m^{-2} s. The composition of modified BG-11 was (g m^{-3}): $NaNO_3$, 1500; $MgSO_4 \cdot 7H_2O$, 75; $CaCl_2 \cdot 2H_2O$, 36; citric acid, 6.0; Na_2EDTA, 1.0; ferric ammonium citrate, 6.0; Na_2CO_3, 20.0; $KH_2PO_4 \cdot H_2O$, 40.0; $ZnSO_4 \cdot 7H_2O$, 0.222; $CuSO_4 \cdot 7H_2O$, 0.079; $MnCl_2 \cdot 4H_2O$, 1.81; $NaMoO_4 \cdot 2H_2O$, 0.39; $Co(NO_3)_2 \cdot 6H_2O$, 0.0494; H_3BO_3, 2.86. The initial culture was inoculated with biomass concentration about 800 g m^{-3} and 300 g m^{-3} for indoor and outdoor experiment, respectively.

2.2. Experimental methods

2.2.1. Indoor experiments

A laboratory device was constructed as shown in Fig. 1. The setup consists of a glass bubbling column and a glass tank. The column with the diameter of 50 mm, containing 700 cm^3 of culture medium, was bubbled with CO_2 in air (volume ratio = 3%) at a gas to liquid mixing rate of 0.15 m^3 m^{-3} min^{-1} to support carbon source and maintain the pH within the range of 7.5–8.5. A glass tank containing different volume of BG11 culture medium was fully wrapped by aluminium foil and connected with the glass bubbling column. A peristaltic pump was used to control the flow rate of the circulation of culture medium. The glass tank was also aerated with CO_2 in air (volume ratio = 3%) at a gas to liquid mixing rate of 0.15 m^3 m^{-3} min^{-1}. Continuous illumination of 400 μmol m^{-2} s^{-1} from one side of the column was provided by cold fluorescence lamps. The illuminated surface area of the glass column was calculated as 280 cm^2 (area for the cross-section of the column). The temperature of the culture broth was kept at 25 ± 3 °C during the experiments. All the experiments had three duplicates.

In this research, two parameters were defined to describe the frequency of the forced circulation.

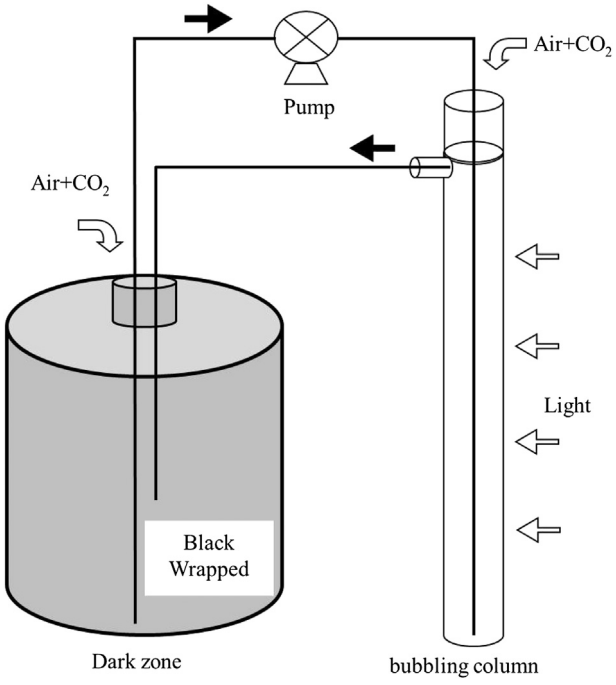

Fig. 1 – Schematic figure of the forced L/D circulation culture device in laboratory.

1) t_b: photic retention time. It presented how long the algal cells will be illuminated in photic zone in each L/D cycle. It was calculated as the result of the volume of culture medium in photic zone (V_b, in cm^3) divided by the flow rate of the culture broth controlled by the peristaltic pump (U, in $cm^3\ min^{-1}$).

$$t_b = \frac{v_b}{U} \quad (1)$$

2) K: dark/light ratio. It presented how many algal cells are in dark and how many cells are lighted in a circulation cycle. It could be calculated as the ratio of the volume in dark zone to the volume in photic zone, as shown in following:

$$K = \frac{V_d}{V_b} \quad (2)$$

where, V_d presented the volume of the culture medium in dark zone.As the results, the period, T of each forced L/D circulation cycle could be calculated by equation (3):

$$T = \frac{V_d + V_b}{U} = (1 + K)t_b \quad (3)$$

The influence of t_b and K on the biomass productivity was investigated and then central composite rotatable design (CCRD) was used to optimize the above two factors. The CCRD experiment comprised 13 experimental runs with 4 factorial points, 4 axial points, and 5 central points for replication (Table 1).

A second-order polynomial regression (equation (4)) was used to setup the correlation between the areal biomass productivity with parameters K and t_b.

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ij} x_i x_j + \sum \beta_{ii} x_i^2 \quad (4)$$

where Y presented areal biomass productivity, x_i and x_j are the independent variables (K and t_b); β_0 was the regression coefficient at centre point; β_i was the linear coefficient; β_{ij} is the interaction coefficient and β_{ii} was the quadratic coefficient.

Analysis of variance (ANOVA) was performed using Design Expert statistical package (version 7.1.3, Stat-Ease Inc., USA) to evaluate the significance of the model and coefficients.

2.2.2. *Outdoor validation practice*

Three kinds of ponds at bench scale were constructed, as shown in Fig. 2. Ponds A and B were the same size but had different water depth of 50 mm and 250 mm (loading of 0.035 m^3 and 0.175 m^3 of culture broth) respectively. The hybrid system C consisted a pond (the same as B but containing 35 l of culture broth) serving as the photic zone and a dark tank loading 0.14 m^3 of culture broth serving as the dark zone. The illuminated surface area of all the three culture devices was the same of 0.7 m^2. The medium was pumped from a dark tank by a diving pump (Atman AT-101, China) and enters the photic pond through an aperture pipe (the length of the aperture pipe is about 400 mm with 40 holes and the diameter of the hole is 3 mm). The photic retention time (t_b) was fixed as 6 min by the diving pump and the dark/light ratio (K) was fixed as 4 (0.14/0.035). CO_2 enriched compressed air (volume ratio = 3%) was injected into the three ponds with a gas to liquid mixing rate of 0.15 $m^3\ m^{-3}\ min^{-1}$ to supply carbon. All the experiments were done in May of 2011, at Qingdao (latitude: 36°57′N; longitude: 120°22′E), China, and the data shown later (Fig. 6A and B) was the average of two batch cultivations.

Table 1 – Central composite design (CCD) and experimental values.

Runs	Photic ratio, K (–)	Photic retention time, t_b (min)	Areal biomass productivity, (g m^{-2} d^{-1})	
			Observed values	Predicted values
1	1.7	2.2	27.8	26.1
2	5.3	2.2	30.3	29.8
3	1.7	7.8	29.5	29.0
4	5.3	7.8	33.8	32.8
5	1.0	5.0	24.8	25.9
6	6.0	5.0	30.8	31.2
7	3.5	1.0	27.4	28.3
8	3.5	9.0	31.9	32.6
9	3.5	5.0	34.4	36.1
10	3.5	5.0	37.4	36.1
11	3.5	5.0	36.6	36.1
12	3.5	5.0	37.0	36.1
13	3.5	5.0	35.0	36.1

2.3. *Analytical methods*

The volumetric biomass density (kg m^{-3}) at 24 h intervals was measured according to the method described by Chiu [30]. The areal biomass density (g m^{-2}) (all the algal biomass per square meter of illumination) and areal biomass productivity (g m^{-2} d^{-1}) (all the net increase of algal biomass per square meter of illumination per day) were calculated to compare the cultivation efficiency of different forced L/D circulation operation.

3. Results and discussion

3.1. *Effect of dark/light ratio (K) and photic retention time (t_b) on biomass productivity*

The effects of the dark/light ratio on the cultivation were elucidated in Fig. 3. The volumetric density of algal biomass

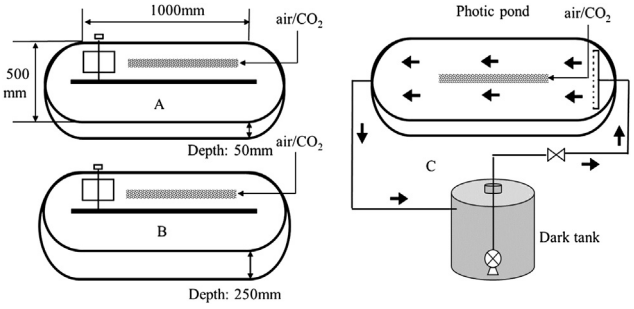

Fig. 2 – Schematic figures three kinds of open pond cultivation systems, A: open pond with 250 mm water depth, B: open pond with 50 mm water depth, C: open pond with forced L/D circulation.

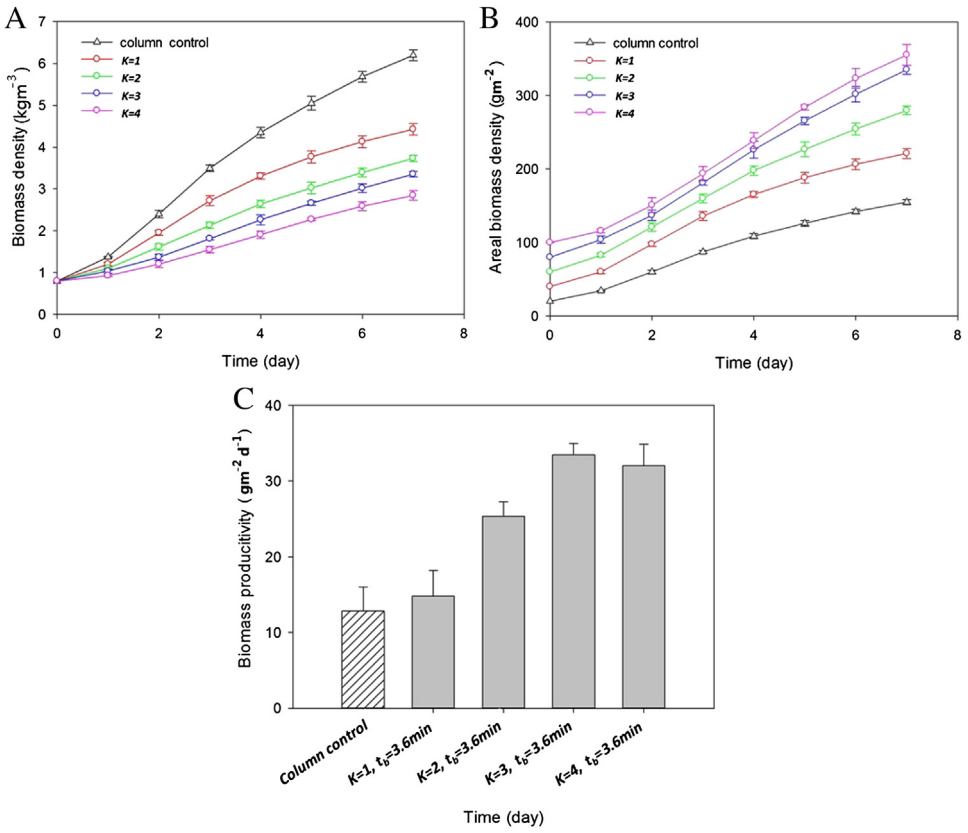

Fig. 3 – Effects of dark/light ratio on microalgae growth, A: volumetric density of microalgae, B: areal biomass density of microalgae, C: areal biomass productivity.

decreased with the increase of dark/light ratio and the control of single bubbling column had the highest volumetric density (Fig. 3A). Higher dark/light ratio led to a higher areal biomass density and the control column had the lowest areal biomass density. The averaged areal biomass productivities of 7 days culture at different volumetric ratio were shown in Fig. 3C. The increase of the total volume of culture medium by increase of dark/light ratio with the same retention time has led to bigger biomass productivity. The highest productivity of 33.5 g m^{-2} d^{-1}, more than doubling to that in column control was obtained at the dark/light ratio of 4. Further increase of the dark/light ratio did not increase the biomass productivity further. In facts, the net increase of algal biomass at any cultivation device is the sum of the biomass production by photosynthesis and the consumption by algal cells respiration. At the same circulation flow rate, more volume of culture in dark zone means more biomass loss by algal cells respiration. Only when the increase of the algal growth is higher than the consumption by algal cells respiration, the forced circulation performed the enhancement effect for biomass productivity.

The effects of the photic retention time on algal volumetric density and areal biomass density at the fixed dark/light ratio of 4 were shown in Fig. 4A and B. For the forced L/D circulations, the areal biomass density was different with different photic retention time. It should be noted that for the forced L/D circulations, the total volume of the culture medium with different photic retention time was the same, so the difference of the biomass density was only produced by the photic retention time. From Fig. 4B, even at very slow forced circulation rate (t_b = 350 min, equalling about one cycle circulation per day, for example), the biomass output had a slight increase compared with the control column without forced circulation. The highest biomass productivity of averaged 33.5 g m^{-2} d^{-1} of 7 days cultivation was reached when the photic retention time was 3.6 min with the dark/light ratio of 4 (Fig. 4C).

3.2. *Optimization of forced L/D circulation by response surface methodology*

The above result has exposed the important effects of both the dark/light ratio and photic retention time on the biomass productivity of microalgae in the forced L/D circulation. Central composite design (CCD) was used (Table 1) to identify the interaction and to determine the optimal values of the two factors. The response surface plot of biomass production as a function of dark/light ratio and photic retention time was shown in Fig. 5. An increase of K with t_b up to the optimal point increased the biomass productivity to a maximum level, and a further increase in k with t_b the trend was reversed. The maximum response of areal biomass productivity of 36.5 g m^{-2} d^{-1} was occurred at K = 3.98 and t_b = 5.80 min. At this point, the period time of the forced

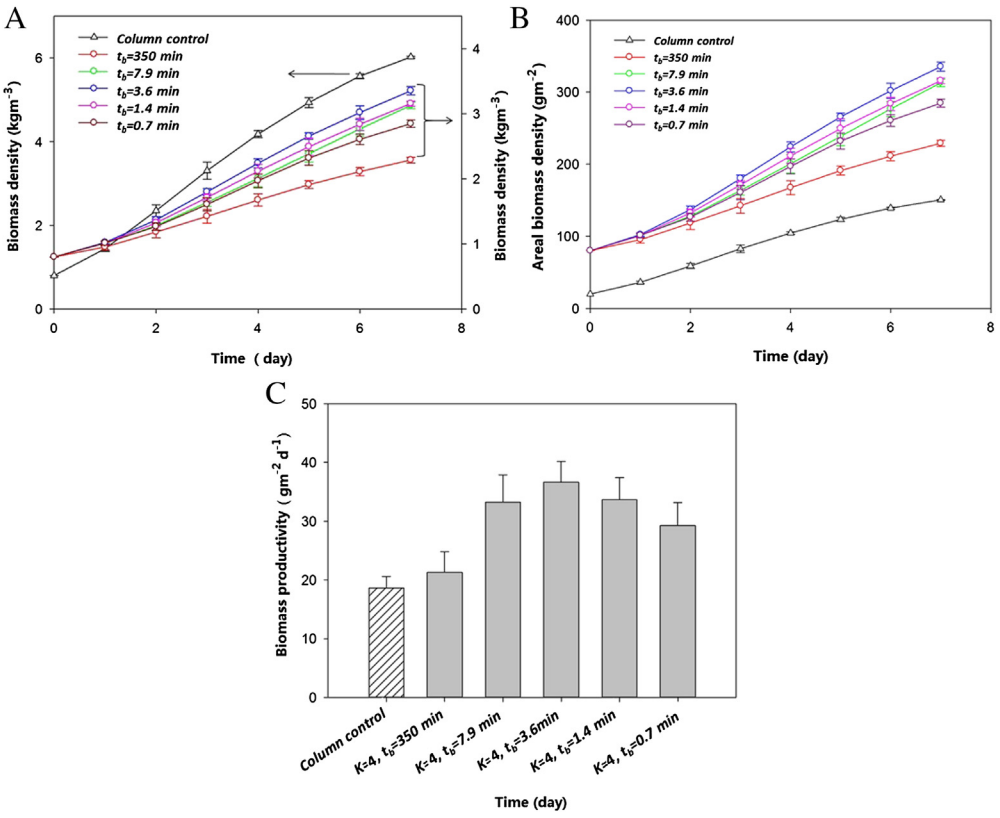

Fig. 4 – Effects of photic retention time on microalgae growth, A: volumetric density of microalgae, B: areal biomass density of microalgae, C: areal biomass productivity.

circulation was $T = (1 + 3.98) \times 5.8 = 28.9$ min. It should be noted that such circulation period of 28.9 min and the photic retention time of 5.80 min were located in the range of the 'middle level' as defined by Grobbelaar [16]. Such frequency in 'middle level' was engineering practicable.

The significance of the dark/light ratio, photic retention time and their interaction on the biomass productivity was analysed by Fisher's *F*-test (Table 2). It was found that all the linear and quadratic terms coefficients for both *K* and t_b were significant. What is interest is that there were no interactions on biomass output between *K* and t_b, which meant that the circulation period time *T* (see equation (4)) had no significant influence on the biomass productivity. A second-order polynomial function to correlate the areal biomass productivity and with both *K* and t_b, was obtained in equation (5). The analysis of variance (ANOVA) showed that this regression model was statistically significant at a 95% confidence level ($p < 0.05$).

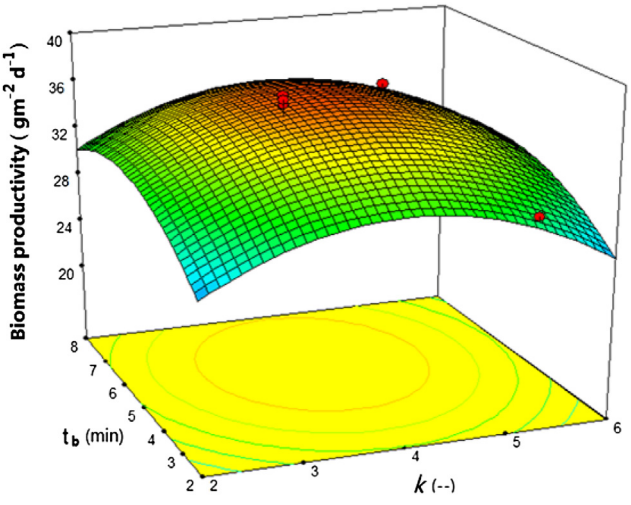

Fig. 5 – Response surface plot of areal biomass productivity as a function of dark/light ratio and photic retention time.

$$Y = 6.29 + 9.45K + 4.03t_b - 1.20K^2 - 0.35t_b^2 \quad (5)$$

According to the equation (5), the predicted response along with the experimental data of different *K* and t_b were presented in Table 1. It revealed a close correspondence between the values.

3.3. *Outdoor cultivation with forced L/D circulation*

To validate the statistical model, outdoor cultivation experiments in three kinds of ponds (Fig. 6) were carried out. The dark/light ratio and photic retention time were set as $K = 4$ and $t_b = 6$ min, which were very close to the above optimal point ($K = 3.98$, $t_b = 5.8$ min). Though the cell concentration in shallow pond (50 mm culture depth) cultivation reached about 2 kg m^{-3} after 7 days cultivation (Fig. 6A), the averaged areal biomass productivity was only 12.1 g m^{-2} d^{-1}. It almost equalled to that of 11.3 g m^{-2} d^{-1} for conventional pond with

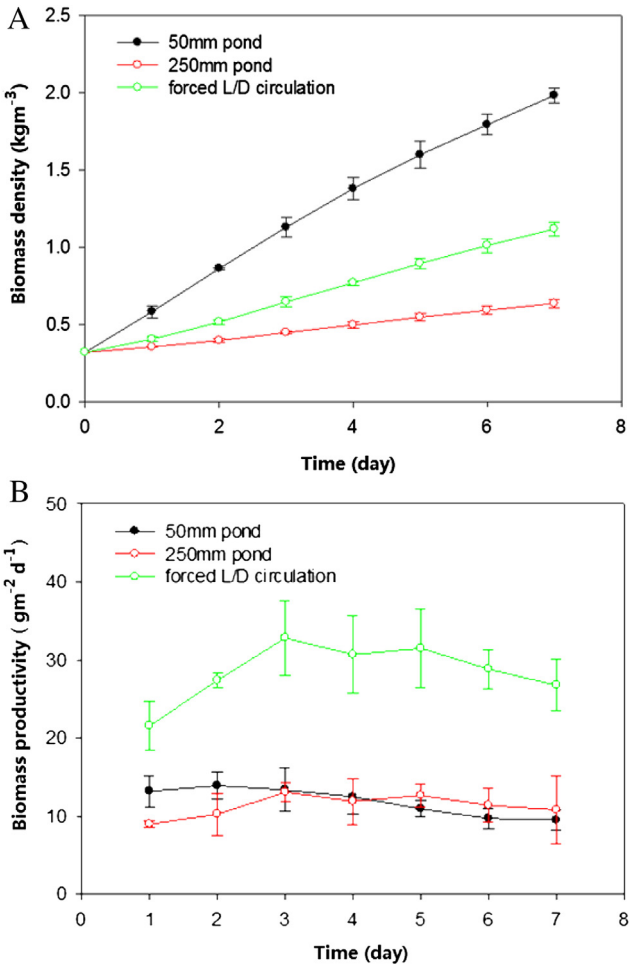

Fig. 6 – Biomass density and productivity of three open pond, A: volumetric biomass density.

250 mm culture depth (Fig. 6B). When *S. dimorphus* was grown in the forced L/D circulation pond, biomass productivity ca. 22–33 g m^{-2} d^{-1} (averaged value was 28.5 g m^{-2} d^{-1}) had been achieved in the 7 days culture. This value was more than two times of that in 50 mm shallow pond and 250 mm depth pond. It should be noted that all these three cultivation systems occupied the same land area because in the engineering aspects, the dark tank could be constructed underneath of the photic pond to save the illuminated land for the forced L/D circulation cultivation. A noticeable point is that the outdoor productivity of 28.5 g m^{-2} d^{-1} for forced L/D circulation is much lower than the expected value of 36.4 g m^{-2} d^{-1} according to equation (2). The difference in illumination time might be the reason of this restrained biomass productivity. The open ponds were only illuminated 12 h (from 6 am to 6 pm), while indoor culture was illuminated for 24 h a day. Nevertheless the result obviously validated the benefit of forced L/D circulation on microalgae biomass production, and such an operation was feasible in application without engineering obstacle.

Table 2 – The significance of the dark/light ratio, photic retention time and their interaction by Fisher's *F*-test.

Source	Sum of squares	*df*	Mean square	*F*-value	*p*-Value	Significance
Model	181.54	4	45.39	26.82	0.0001	Significant
X_1–K	17.71	1	17.71	10.47	0.0120	Significant
X_2–t_b	17.27	1	17.27	10.21	0.0127	Significant
X_1^2–K^2	97.39	1	97.39	57.55	<0.0001	Significant
X_2^2–t_b^2	54.97	1	54.97	32.48	0.0005	Significant

4. Conclusion

It was demonstrated that the forced light/dark circulation benefited the growth of microalgae in open pond. The dark/light ratio and photic retention time are the two key factors of the forced L/D circulation to influence the biomass productivity of microalgae. The determined optimal frequency by response surface methodology was the dark/light ratio of 4 and photic retention time of 6 min. Biomass productivity of 28.5 g m^{-2} d^{-1} in outdoor experiments validated the enhancement for algal growth by the forced circulation.

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