Title: Culture characteristics of the ARTP-mutated *Spirulina platensis* mutants in CO₂ aeration culture system for biomass production

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Abstract:

For biomass production of *Spirulina platensis* as feedstock of fermentation, the culture characteristics of three typical mutants of 3-A10, 3-B2 and 4-B3 generated by Atmospheric and

Room Temperature Plasmas(ARTP) mutagenesis were systematically studied by using CO₂ aeration culture system and compared with the wild strain. The specific growth rate of wild strain in the pure air aeration culture system exhibited a 76.2% increase compared with static culture, while the specific growth rates of the 3-A10, 3-B2 and 4-B3 in pure air aeration culture system were increased by 114.4%, 95.9% and 88.2% compared with their static cultures. Compared with static culture, the carbohydrate contents of wild strain, 3-A10, 3-B2 and 4-B3 in pure air aeration culture system dropped plainly by 51.0%, 79.3%, 85.5% and 26.1%. Increase of CO₂ concentration enhanced carbohydrate content and productivity. Based on the carbohydrate productivity, the optimal inlet of CO₂ concentration in aeration culture was determined to be 12%(v/v). Under this condition, 3-B2 exhibited the highest carbohydrate content (30.7%), CO₂ fixation rate (0.120gCO₂·g⁻¹·d⁻¹) and higher growth rate (0.093g·L⁻¹·d⁻¹), while 3-A10 showed the highest growth rate (0.118g·L⁻¹·d⁻¹) and higher CO₂ fixation rate (0.117gCO₂·g⁻¹·d⁻¹) but low carbohydrate content (24.5%), and 4-B3 showed the highest chlorophyll(Chl) content (3.82mg·g⁻¹). The most outstanding mutant by static culture in terms of growth rate and carbohydrate productivity (3-B2), was also demonstrated by CO₂ aeration culture system. This study revealed that the ARTP mutagenesis could generate the *S. platensis* mutants suitable for CO₂ aeration culture aiming at biomass production.

Keywords ARTP mutagenesis, biomass production, *Spirulina platensis*, CO₂ aeration culture,

carbohydrate content, CO₂ fixation rate

Introduction

Microalgae has the superior capacity in photosynthetic efficiency, carbohydrate and protein synthesis, hydrogen production and lipid accumulation (1-3). Additionally, the CO₂ capturing capacity of microalgae also makes the cultivation of microalgae an attractive option for the mitigation of the greenhouse effect.

Spirulina platensis as a kind of carbohydrate-containing microalgae, is a filamentous cyanobacteria characterized by cylindrical and multicellular trichomes, which possesses high contents of carbohydrate, protein, and multivitamins (4). Typical contents of carbohydrate and protein in *S. platensis* reach 8~17% and 46~63%, respectively (5). The sugar composition of *S. platensis* reportedly consists of glucose (54.4%) along with rhamnose (22.3%), mannose (9.3%), xylose (7.0%) and others (6). Moreover, *S. platensis* grows under the conditions of high pH and high salinity could avoid the bacterial contamination. These unique characteristics made *S. platensis* biomass a potential feedstock for the bio-production of chemicals and fuels such as ethanol fermentation (7, 8). According to the recent studies on the bioethanol fermentation by using *S. platensis* cells, the biomass of the microalgae with the carbohydrate content between 48~71% could be effectively fermented(8-10). Sasano *et al.* found that the carbohydrate content of *S. platensis* could accumulate up to 55~65% under nitrogen starvation(11), Markou *et al.* indicated

that under the phosphorus starvation, the carbohydrate content reached 63%(10), and Aikawa *et al.* showed that under the light intensity and nitrate supply optimization, carbohydrate content reached 50%(5). However, the accumulation of carbohydrate content under the starvation strategy would cause the low biomass productivity, low growth rate and difficulty in operation.

Mutagenesis is another approach to solving those bioprocess problems. Several conventional methods were used in the previous studies to obtain *S. platensis* mutants with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), nitrosoguanidine (NTG) and UV mutagenesis(12-14). While the physical and chemical mutagenesis approaches used in *S. platensis* mutation have their own shortages due to the limited efficiency and safety. Recently, a new mutagenesis tool – Atmospheric and Room Temperature Plasmas (ARTP) developed by our group (15-18) has been successfully applied to create the mutant library of *S. platensis*(16). Moreover, ARTP mutagenesis system has been successfully applied in various microorganisms to improve diverse traits. Wang LY *et al.* obtained a *Streptomyces avermitilis* mutant which avermectin B1a productivity was increased by 40 % compared with the wild strain after 3 min ARTP treatment (19), Li HP *et al.* reported that MMO activity of *Methylosinus trichosporium* OB3b mutants was increased to 2-fold of the wild strain (20), Jin LH *et al.* obtained a oleaginous *Rhodospiridium toruloides* ACCC 20341 mutant with high lipid yield which was improved to 2.2-fold of wild strain (17), and Qi F *et al.* mutated same strain with ARTP and the mutants capable of tolerating to all the cellulose hydrolysis inhibitory compounds were obtained (21).

With the establishment of ARTP mutation and high throughput screening using the micro plate and static flask culture, we eventually obtained 3 representative mutants, named as 3-A10, 3-B2 and 4-B3. By the static flask culture, the growth rate ($0.058 \text{ g}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$) and Chl content ($1.07 \text{ mg}\cdot\text{g}^{-1}$) of 3-A10 were lower than wild strain ($0.084 \text{ g}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$, $3.53 \text{ mg}\cdot\text{g}^{-1}$), but its carbohydrate content (26.1%) was higher than the wild strain (18.6%), 3-B2 had the similar growth rate ($0.079 \text{ g}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$) and Chl content ($3.51 \text{ mg}\cdot\text{g}^{-1}$) as the wild strain, but this mutant showed the highest carbohydrate content (33.1%, w/w) and productivity among the four strains; 4-B3 had the highest growth rate ($0.093 \text{ g}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$) and Chl content ($4.52 \text{ mg}\cdot\text{g}^{-1}$), but its carbohydrate content (13.1%, w/w) was lower than that of the wild strain(16). These mutants showed high genetic stability by the subculture (16).

The stability of the improved phenotypes of *S. platensis* mutants in large-scale culture is important in industrialization. For the biomass production of *S. platensis* as the feedstock of fermentation to fix CO_2 , CO_2 aeration culture system should be used to evaluate the large-scale culture characteristics of the selected mutants, as the static flask culture for the screening might be different from the aeration culture conditions including the mixing and light irradiation (22, 23). As widely observed, the growth rate of microalgae is related to the intensity of illumination and CO_2 fixation rate (24, 25). The energy supplied in microalgae cultures for mixing and CO_2 supply could force the cells to move between dark and light zones to enhance the light regime in the aeration culture system, and provide adequate CO_2 for the photosynthesis of microalgae, which

would improve the CO₂ fixation efficiency and enhance the biomass productivity compared with the static culture (26, 27). On the other hand, while sodium carbonate is used as the carbon source in the static flask culture, concentration of sodium ion will be accumulated with the cell growth, which would adversely affect the growth condition of *S. platensis* (28, 29). Since CO₂ aeration culture system uses CO₂ and air to supply the mixed gases, it can avoid the sodium ion accumulation. To demonstrate the feasibility of the ARTP-mutated mutants screened out by our previous procedure for biomass production, the culture characteristics of the different strains using CO₂ aeration culture system need to be investigated.

In this paper, we aimed to systematically evaluate the culture characteristics of the typical mutants (3-A10, 3-B2 and 4-B3) generated by ARTP and the wild strain (control) in aeration culture system. First, we established an aeration culture system feeding CO₂ and air continuously, and *S. platensis* wild strain was used to evaluate the performance in the aeration culture system. Then, we optimized the inlet CO₂ concentration and studied the culture characteristics of *S. platensis* wild and mutant strains in the aeration culture system with three inlet CO₂ concentrations.

Materials and Methods

Organism and culture conditions

Spirulina platensis wild type of FACHB-904 was obtained from Freshwater Algae Culture

Collection of the Institute of Hydrobiology, Wuhan, China. Typical *S. platensis* mutants of 3-A10, 3-B2 and 4-B3 strains were generated by the ARTP mutagenesis system combined with high throughput screening and maintained by the static flask culture reported previously by Fang MY *et al.* (16). These *S. platensis* strains were cultivated in 250 ml flask with 120 ml fresh Zarrouk's medium (30) and 30 ml inoculum. *S. platensis* strains were routinely grown in Zarrouk's medium and cultivated in an artificial climate incubator at 26°C with white fluorescent lamp. The light intensity was set at $53 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, with 12 h light and 12 h dark period cycle. In CO₂ aeration culture system, *S. platensis* strains were grown in Zarrouk's medium without NaHCO₃ and Na₂CO₃. We used NaOH to adjust pH value of the medium to 9.5 prior to inoculation and every day during the culture.

CO₂ aeration culture

The CO₂ aeration system consisted an inner-loop, upward flow and outside surrounded illumination. CO₂ was the only carbon source in this culture system and was continuously supplied during the cultivation period. The configuration of the aeration culture system consisted flow meter, membrane filter, air compressor, distributor, aerator, pure CO₂ and tank bioreactor (12 cm (D) × 16 cm (H)) with an effective volume of 1000 ml (Fig. S1). The flow rates of CO₂ and air were controlled by the respective flow meters with the fixed total gas rate of 1.0 L·min⁻¹. In the CO₂ aeration system, 200 ml inoculum of the *S. platensis* pre-culture was inoculated into 800 ml fresh Zarrouk's medium.

Specific growth rate assay

The optical density of *S. platensis* was determined at 560 nm (16, 31) with UV spectrophotometer (UV757CRT, Shanghai, China), and converted to the dry cell weight (DCW) by using Eq.(1), where x and y mean DCW and OD_{560} , respectively(16).

$$y = 1.251x - 0.01925 \quad (1)$$

The specific growth rate was calculated by Eq. (2).

$$\mu = (\ln x_2 - \ln x_1) / (t_2 - t_1) \quad (2)$$

Where x_2 and x_1 represent the DCW at the culture time t_2 and t_1 , respectively.

Carbohydrate content assay

The carbohydrate content was measured according to the method reported previously by Fang MY *et al* (16). In each sampling, culture broth of 5 ml was collected and washed. Later, it was hydrolyzed by adding 6 N HCl and heated at 95°C for 10 min, and then neutralized by 6 N NaOH. The reduced sugar was the calculated according to DNS method (32).

Chlorophyll content assay

The chlorophyll (Chl) content was measured according to the method reported previously by Fang MY *et al.* (16). The 5 ml *S. platensis* culture was collected and washed, followed by the addition of 90% methanol solution, and then sonication. UV-spectrophotometer was used to measure the absorbance of the extracted solution at 665 nm and 650 nm. Then, Chl content ($\text{mg} \cdot \text{L}^{-1}$) was evaluated according to following Eq. (3)

$$Chl\ content = 16.5 \times Abs_{665} - 8.3 \times Abs_{650} \quad (3)$$

151 **CO₂ fixation rate assay**

152 The inlet CO₂ was converted into bicarbonate and carbonate thereby transferred into the cells.
 153 The CO₂ fixation rate of *S. platensis* was measured by removing the bicarbonate in the medium
 154 from the cells and detected the final carbon ratio in the dried biomass (33). The sampled *S.*
 155 *platensis* cells were collected and washed, and then dried up at 105°C for 24 h. Subsequently, the
 156 dried cells were grinded to powder for measurement by elemental analyzer (vario EL III,
 157 Germany), and then CO₂ fixation rate was evaluated according to Eqs. 4 and 5.

$$n_{CO_2} = (x_2 - x_1) \times C\% / 12 \quad (4)$$

$$q_{CO_2} = (n_{CO_2} \times 44) / ((t_2 - t_1) \times \bar{x}) \quad (5)$$

158 Where *C%* indicates the carbon ratio of the dried biomass, and $\bar{x}$ means the average DCW of
 159 *x₂* and *x₁*.

160

161 **Results and discussion**

162 We first evaluated the culture difference of *S. platensis* between the static flask culture and
 163 CO₂ aeration culture using the wild strain. As shown in Figure S1, only air or the mixed gas of air
 164 and CO₂ was supplied from the bottom of the reactor. Culture characteristics including
 165 morphology, growth rate, carbohydrate content and Chl content, were compared for the two
 166 culture systems.

The morphology of the wild strain between the static culture and the aeration culture with only air was shown in Figure S2. Similar morphology in the aeration and the static cultures was observed. Table S1 summarized the characteristics of the wild strain in the static culture and aeration culture with only air at the flow rate of $1.0 \text{ L}\cdot\text{min}^{-1}$ ($n=3$), and the growth rate and Chl content of the wild strain in aeration culture system was $0.148 \text{ g}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ and $3.67 \text{ mg}\cdot\text{g}^{-1}$, which were increased by 76.2% and 26.1%, respectively, compared with those of the static culture. The main reason for the increment of growth rate and Chl content by the aeration culture was probably due to the better mass transfer (34) and light radiation than the static culture. On the other hand, the carbohydrate content of the wild strain in the aeration culture system with only air was decreased by 51.0% compared with that of the static culture. The formation of photosynthetic product of *S. platensis* is always accompanied by the produce of O_2 and the uptake of CO_2 . In the aeration culture system supplied with only air, the respiration might be enhanced (35-37), thereby resulting in the low carbohydrate content.

Characteristics of *S. platensis* mutants cultivated in the aeration culture system

The aeration culture system supplied with only air was then used to evaluate the culture characteristics of the *S. platensis* mutants for the comparison with the subsequent CO_2 aeration culture. As shown in Table 1, the specific growth rates of the mutants of 3-A10, 3-B2 and 4-B3 were $0.125 \text{ g}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$, $0.154 \text{ g}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ and $0.175 \text{ g}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$, which were increased by 114.4%, 95.9% and 88.2% compared with those of the static culture (16), respectively. The order of growth rate

for the 4 strains in the aeration culture were the same as that observed in the static flask culture (16), i. e., 3-A10 < wild strain 3-B2 < 4-B3. In the aeration culture system, 4-B3 showed the highest growth rate. Furthermore, 4-B3 also showed the highest CO₂ fixation rate and Chl content, which were 0.120 gCO₂·g⁻¹·d⁻¹ and 3.83 mg·g⁻¹, respectively.

On the other hand, the carbohydrates content of the 3 mutants in the aeration culture with only air were at the similar level (within the range of 4.8% to 5.4%), which were much lower than that of the static culture (within the range of 13.1% to 33.1% (16)), and also lower than the previously reported carbohydrate content of 8~14% for *S. platensis* (5). The lower carbohydrates contents in the aeration culture with only air supply than the static flask culture for all the strains might be caused by the respiration that enhanced by the high oxygen supply (35-38). Increase of the inlet CO₂ concentration in the gas supply for microalgae culture can expectedly enhance the photosynthesis (23, 39-41). However, overmuch CO₂ concentration would adversely inhibit the growth of microalgae (42). In order to accumulate higher carbohydrate content while keeping the higher growth rate for the *S. platensis* mutants, inlet CO₂ concentration supplied to the aeration culture system needs to be optimized.

Optimization of inlet CO₂ concentration for aeration culture system

Pure CO₂ gas was mixed with air to increase the inlet CO₂ ratio for the aeration culture system. Three different inlet CO₂ concentrations (6%, 12% and 15% (v/v)) in the mixed gas were examined. During the culture period, pH was manually controlled at 9.5 every day and the light intensity was

53 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, with 12 h light and 12 h dark period cycle.

Figure 1 showed the growth curves of the wild strain and 3 mutants. For all the strains, the growth rate and maximum cell density increased with the increase of CO₂ ratio, but were decreased when the CO₂ ratio reached 15% (Table S2). Thus, mixed gas with 12% CO₂ was considerably suitable for *S. platensis* strains in the CO₂ aeration culture. Several studies also pointed out that CO₂ ratio higher than 15% in the inlet gas would inhibit the growth of microalgae (41, 43-45). As pointed out by Miyachi *et al* (40), dissolved inorganic carbon is transported into the cells through HCO₃⁻ transporter. When CO₂ concentration is increased to certain level, the pH value in the cytoplasm will decrease and lower down the carbonic anhydrase activity, which will hinder the growth of *S. platensis*.

The increase of inlet CO₂ concentration in the mixed gas increased the final carbohydrate content significantly (Fig. 2). At 6% inlet CO₂, each mutant strain reached its highest carbohydrate content, and among all the mutants, 3-B2 showed the highest carbohydrate content. The respective carbohydrate content of the wild strain, 3-A10, 3-B2 and 4-B3 were 23.2%, 28.9%, 34.0% and 31.6% (w/w), which was 3.64-fold, 4.35-fold, 6.08-fold and 5.32-fold compared with those of the aeration culture with the only air, respectively. It showed that increase of inlet CO₂ concentration could effectively improve the carbohydrate accumulation in the cells. However, the carbohydrate content for the all strains showed a slow descending trend while CO₂ ratio was increased from 6% to 15%. The reasons for this result need further study. Besides, the highest carbohydrate

concentration was obtained at 12% inlet CO₂ (Table S2). The final carbohydrate concentrations reached after the cultivation at 12% inlet CO₂ for the wild strains, 3-A10, 3-B2 and 4-B3 were 0.220 g·L⁻¹, 0.281 g·L⁻¹, 0.320 g·L⁻¹ and 0.295 g·L⁻¹, respectively (Table S2). The 3-B2 showed the highest carbohydrate content and concentration at 12% inlet CO₂ concentration. Furthermore, the highest average carbohydrate productivity of 3-B2 mutant was also achieved at 12% inlet CO₂ concentration, which was 28.5 mg·L⁻¹·d⁻¹ (Table 1).

Chlorophyll is a kind of pigment that is important in photosynthesis. Chlorophyll content of a microalgae strain is related to the ability of photosynthesis, affecting the metabolism. As reported previously (16), ARTP mutagenesis could generate the mutants of *S. platensis* with altered chlorophyll content in addition to changes of growth rate and carbohydrate content. Figure 3 showed the change in Chl contents of the 4 strains during the aeration cultivation with three different inlet CO₂ concentrations. The Chl content for all the strains rose at first and then declined later during the 21 d culture. In this study, the medium was not added except the supply of CO₂ during the culture period, which would presumably lead to a slight drop in the final Chl content as the decrease of nitrogen source would influence the protein synthesis and chlorophyll synthesis (46, 47). While the inlet CO₂ was increased from 6% to 15%, the final Chl content of the wild strain decreased. However, the final Chl contents of 3-A10, 3-B2 and 4-B3 at 12% CO₂ were the highest among three inlet CO₂ concentrations, reaching 3.41 mg·g⁻¹, 3.24 mg·g⁻¹ and 3.82 mg·g⁻¹, respectively (Fig. 3).

Since CO₂ was the sole carbon source continuously supplied in the CO₂ aeration culture system, high CO₂ fixation rate would represent the high productivities of *S. platensis* biomass. The CO₂ fixation rates for the wild strain, 3-A10, 3-B2 and 4-B3 were shown in Figure 4. At 12% inlet CO₂ concentration, 3-B2 showed the highest CO₂ fixation rate (0.120 gCO₂·g⁻¹·d⁻¹) after 21 d culture, followed by 3-A10 (0.117 gCO₂·g⁻¹·d⁻¹), 4-B3 (0.077 gCO₂·g⁻¹·d⁻¹) and the wild strain (0.067 gCO₂·g⁻¹·d⁻¹). The highest CO₂ fixation rate, carbohydrate content and higher growth rate for 3-B2 in CO₂ aeration culture suggested that the fixed CO₂ might eventually be used in cell synthesis and glycogen storage (24, 25). Besides, 4-B3 had the highest CO₂ fixation rate and specific growth rate (Table S2) at 6% inlet CO₂ concentration, at the same time its carbohydrate content stayed at a higher level.

From the characteristics of the 3 mutants in the aeration culture system with the different inlet CO₂ concentrations, 12% was the optimal CO₂ concentration for *S. platensis* culture. Table 2 listed the characteristics of the 3 mutants and the wild strain in 12% inlet CO₂ concentration and compared with the respective characteristics of the static flask culture. 3-B2 showed the best culture performance at 12% inlet CO₂ concentration. At this condition, 3-B2 exhibited the growth rate of 0.093 g·L⁻¹·d⁻¹, carbohydrate content of 30.7% (w/w) and CO₂ fixation rate of 0.120 gCO₂·g⁻¹·d⁻¹. Moreover, the carbohydrate productivity at 12% inlet CO₂ concentration was increased by 25.6% compared to that at 6% inlet CO₂ concentration. At 12% inlet CO₂ concentration, the specific growth rate of 3-B2 was increased by 18.1% compared with that of the

static flask culture (16) (Table 2). The CO₂ aeration during the culture could overcome the problem of the sodium ion accumulation in the medium as seen in the static culture (28, 29), thereby effectively enhancing the growth rate of *S. platensis* mutants and the wild strain. Although the volume for the aeration culture system was 19-fold larger than that of the static flask culture, the carbohydrate content and carbohydrate productivity of wild strain and 4-B3 showed a significantly improved, which was increased by 29.0%, 116.8% and 34.3%, 132.8%, respectively. The chlorophyll content of 3-A10 also showed a significantly improvement, increased by 2-fold compared with that of the static flask culture. Moreover, the carbohydrate content and carbohydrate productivity as well as Chl content for the 3-B2 in both cultures remained the same as those obtained in the static flask culture (Table 2), indicating the large-scale culture feasibility of the *S. platensis* mutants generated by ARTP mutagenesis and the screening procedure established previously.

In conclusion, we demonstrated that CO₂ aeration culture with the appropriate inlet CO₂ concentration was suitable for effectively growing the ARTP-generated *S. platensis* mutants screened out by the static culture in terms of cell growth and carbohydrate productivity, and ARTP mutagenesis established previously could generate the ideal mutants of *S. platensis* for mass production of microalgae biomass with high content and productivity of carbohydrate by fixation of CO₂, which could be used as the feedstock for fermentation (7, 8). Moreover, ARTP

mutagenesis can also generate salt-tolerant mutants of *S. platensis* capable of growing well with high carbohydrate content in seawater by CO₂ aeration culture, which is undergoing now.

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Figure 1. Growth curve of wild strain and mutants cultivated in aeration system with 3 different inlet CO₂ concentration (6%, 12% and 15%).

a) wild strain , b) 3-A10, c) 3-B2 and d) 4-B3

Figure 2. Changes of carbohydrate contents of wild strain and mutants cultivated in aeration system with 3 different inlet CO₂ concentration of CO₂ (6%, 12% and 15%).

a) wild strain , b) 3-A10, c) 3-B2 and d) 4-B3

Figure 3. Changes of chl contents of wild strain and mutants cultivated in aeration system with 3

different inlet CO₂ concentration of CO₂ (6%, 12% and 15%).

a) wild strain , b) 3-A10, c) 3-B2 and d) 4-B3

Figure 4. CO₂ fixation rate of wild strain and mutants cultivated in aeration system with 3 different inlet CO₂ concentration of CO₂ (6%, 12% and 15%).

Table 1. Characteristics of the mutants and the wild strain (control) in aeration culture for 21 d with only air at the flow rate of $1.0 \text{ L} \cdot \text{min}^{-1}$ (n=3)

Strains	Growth rate	Carbohydrates content	Chl content	CO ₂ fixation rate
	$/\text{g} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$	$/\text{g} \cdot \text{g}^{-1}$	$/\text{mg} \cdot \text{g}^{-1}$	$/\text{gCO}_2 \cdot \text{g}^{-1} \cdot \text{d}^{-1}$
Control	0.148 ± 0.001	0.050 ± 0.003	3.67 ± 0.11	0.114 ± 0.002
3-A10	0.125 ± 0.002	0.054 ± 0.005	2.49 ± 0.20	0.107 ± 0.001
3-B2	0.154 ± 0.003	0.048 ± 0.002	3.77 ± 0.01	0.117 ± 0.001
4-B3	0.175 ± 0.001	0.050 ± 0.002	3.83 ± 0.01	0.120 ± 0.001

Table 2. Characteristics of the mutants and the wild strain (control) in aeration culture for 21 d with inlet 12% CO₂ concentration at 1.0 L·min⁻¹ and static flask culture(n=3)

Strain	Culture system	Growth rate	Carbohydrate	Carbohydrates	Chl	Chl	CO ₂ fixation	Reference
		/g·L ⁻¹ ·d ⁻¹	content	productivity	content	productivity	rate	
			/g·g ⁻¹	/mg·L ⁻¹ ·d ⁻¹	/mg·g ⁻¹	/mg·L ⁻¹ ·d ⁻¹	/gCO ₂ ·g ⁻¹ ·d ⁻¹	
Control	Aeration culture system	0.088±0.001	0.240±0.012	21.1±2.1	3.48±0.07	0.306±0.010	0.067±0.001	This study
	Static culture	0.084±0.018	0.186±0.045	15.7±3.8	3.53±0.07	-	-	[16]
3-A10	Aeration culture system	0.108±0.001	0.245±0.001	26.5±1.3	3.21±0.02	0.347±0.003	0.117±0.001	This study
	Static culture	0.0583±0.016	0.261±0.051	15.2±3.0	1.07±0.08	-	-	[16]
3-B2	Aeration culture system	0.093±0.001	0.307±0.018	28.5±2.5	3.24±0.09	0.380±0.004	0.120±0.001	This study
	Static culture	0.079±0.017	0.331±0.049	26.0±3.9	3.51±0.04	-	-	[16]
4-B3	Aeration culture system	0.101±0.001	0.284±0.005	28.4±3.2	3.82±0.05	0.386±0.006	0.077±0.004	This study

Static culture

0.0930 ± 0.025

0.131 ± 0.046

12.2 ± 4.3

4.52 ± 0.45

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[16]