

## Lipid accumulation and CO<sub>2</sub> utilization of two marine oil-rich microalgal strains in response to CO<sub>2</sub> aeration

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### Abstract

Biological CO<sub>2</sub> sequestration by microalgae is a promising and environmentally friendly technology applied to sequester CO<sub>2</sub>. The characteristics of neutral lipid accumulation by two marine oil-rich microalgal strains, namely, *Isochrysis galbana* and *Nannochloropsis* sp., through CO<sub>2</sub> enrichment cultivation were investigated in this study. The optimum culture conditions of the two microalgal strains are 10% CO<sub>2</sub> and *f* medium. The maximum biomass productivity, total lipid content, maximum lipid productivity, carbon content, and CO<sub>2</sub> fixation ability of the two microalgal strains were obtained. The corresponding parameters of the two strains were as follows: ((142.42±4.58) g/(m<sup>2</sup>·d), (149.92±1.80) g/(m<sup>2</sup>·d)), ((39.95±0.77)%, (37.91±0.58)%), ((84.47±1.56) g/(m<sup>2</sup>·d), (89.90±1.98) g/(m<sup>2</sup>·d)), ((45.98±1.75)%, (46.88±2.01)%), and ((33.74±1.65) g/(m<sup>2</sup>·d), (34.08±1.32) g/(m<sup>2</sup>·d)). Results indicated that the two marine microalgal strains with high CO<sub>2</sub> fixation ability are potential strains for marine biodiesel development coupled with CO<sub>2</sub> emission reduction.

**Key words:** *Isochrysis galbana*, *Nannochloropsis* sp., CO<sub>2</sub> enrichment cultivation, neutral lipid, biodiesel, open raceway pond

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### 1 Introduction

The CO<sub>2</sub> concentration in the atmosphere continuously increases; as a consequence, this increase significantly affects the global environment. Extensive CO<sub>2</sub> emissions from anthropogenic activities cause global warming (Ramanan et al., 2010). These emissions can be reduced through biological CO<sub>2</sub> sequestration by using photosynthetic microalgae because these organisms unlikely compete with food crops for arable land and fresh water resources (Kumar et al., 2010). Cultivated microalgae not only exhibit high CO<sub>2</sub> fixation ability but also produce significant amounts of renewable biomass for biofuels. These organisms also yield value-added products from biomass, such as proteins, fatty acids, and dietary supplements for humans, animals and fish (Pulz and Gross, 2004).

Studies on carbon sequestration based on microalgae, specifically the CO<sub>2</sub> tolerance of microalgae, have achieved certain progress. However, research objects mostly include freshwater algae, such as *Scenedesmus* sp., *Spirulina platensis*, and *Chlorella* sp., and the CO<sub>2</sub> tolerance of these algae ranges from 5% to 40% (Yang et al., 2011). Present research on carbon sequestration of

marine microalgae is mainly concentrated on several species, such as *Nannochloropsis* sp., *Dunaliella salina*, *Chlorella* sp., and *Phaeodactylum tricornutum* (Salih, 2011). Results show that the growth rate and biomass accumulation are inhibited to a certain degree when the CO<sub>2</sub> concentration exceeds 5% (Lee and Tay, 1991), and the CO<sub>2</sub> emission concentration of power plants is approximately 10% (Bai et al., 2006). Therefore, carbon emission reduction based on marine microalgae must withstand 10% of CO<sub>2</sub>. Meanwhile, research on lipid accumulation of these microalgae under carbon-rich conditions, especially the accumulation of neutral lipid, is rarely undertaken.

Biodiesel is formed by the fatty acid methyl esters derived from a transesterification reaction between triglycerides and methanol (Chisti, 2008). Neutral lipids or triglycerides, which are mainly found as storage lipids in microalgae, are essential for biodiesel production (Wang et al., 2010). The chemical and physical qualities of biodiesel are closely related to the properties of its parent oil. Therefore, the feasibility of microalgal species as a biodiesel feedstock depends on the optimization of its biomass and neutral lipid content. To enhance the novel feedstock, re-

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searchers should select suitable strains and CO<sub>2</sub> concentration for mass scale cultivation. Adverse environmental effects of high CO<sub>2</sub> emissions can be reduced by using biofuels. Biodiesel can be produced from microalgae in a small scale. However, large-scale biodiesel production by microalgae remains economically unviable (Liu et al., 2006; Xu et al., 2011). However, the characteristics of neutral lipid accumulation under CO<sub>2</sub>-enriched cultivation in covered raceway ponds have yet to be reported. Therefore, microalgal cultivation under CO<sub>2</sub>-enriched conditions in covered raceway ponds must be improved.

Two marine oil-rich microalgal strains, namely, *Isochrysis galbana* CCMM5001 and *Nannochloropsis* sp. CCMM7001, exhibit high environmental adaptability (Wei et al., 2015; Liu and Wang, 2014). The outdoor-cultured biomass of these microalgae can reach 26.4 g/(m<sup>2</sup>·d) (Liu et al., 2013) and 9.9 g/(m<sup>2</sup>·d) (Bondioli et al., 2012), respectively. The lipid content of these marine microalgae is also high. Their lipid content is 7.0%–40.0% (Mata et al., 2010) and 22.7%–52.0% (Bondioli et al., 2012; Moazami et al., 2012). The two microalgal strains can strongly tolerate CO<sub>2</sub> (>15%, v/v) (Chiu et al., 2009). The CO<sub>2</sub> concentrations of the two microalgal strains in industrial flue gases from power plants usually range from 10% to 15%, and these concentrations may provide a carbon source for large-scale microalgal cultivation. *Isochrysis galbana* and *Nannochloropsis* sp. were selected from marine microalgal strains with high biomass and high lipid contents (neutral lipid and total lipid) in our preliminary experiment. Mass cultivation coupled with CO<sub>2</sub> emission reduction was conducted in a covered raceway pond. This study provided a basis for CO<sub>2</sub> sequestration and biodiesel technology.

## 2 Materials and methods

### 2.1 Microalgal strains

*Isochrysis galbana* CCMM5001 and *Nannochloropsis* sp. CCMM7001 were provided by Han Xiaotian from the Institute of Oceanology, Chinese Academy of Science. These strains were maintained in *f/2* medium at (20±1)°C with continuous illumination at 100 μmol/(m<sup>2</sup>·s).

### 2.2 The open raceway pond

The open raceway pond was 200 cm×30 cm×40 cm in dimension, the water surface height is 30 cm. The speed is 30 r/min (Moheimani and Borowitzka, 2007).

### 2.3 Culture conditions

The two marine microalgal strains were grown in a 100 L photobioreactor, and inoculated in the exponential phase with an initial cell concentration of 2.12×10<sup>6</sup> cells/mL in an open raceway pond in autumn of 2014 in Qingdao (35°35′–37°09′N; 119°30′–121°E). The culture temperature ranged from 10°C to 30°C, and illumination ranged from 447 μmol/(m<sup>2</sup>·s) to 1 081 μmol/(m<sup>2</sup>·s). Light intensity was measured at 12 o'clock. Seawater continuously bubbled with filtered air at the aeration rate of 0.1 vvm by flow counter for 12 h (6:00–18:00). The range of CO<sub>2</sub> concentrations (0%, 0.04%, 10%, and 15%), nutrient concentrations (*f/4* [0.5-fold *f/2* medium], *f/2*, *f* [two-fold *f/2* medium], and *2f* [four-fold *f/2* medium]) were set up and compared. This experiment was performed in triplicate. Sampling was conducted by transfer liquid gun and graduated flask at a fixed time every other day. The biomass was harvested at the plateau phase.

### 2.4 Dry weight and neutral lipid analysis

The tube was dried at 105°C for 24 h and weighed (*DW*<sub>0</sub>).

After growth, the biomass was separated from the medium by centrifugation at 10 000 g for 10 min, then freeze dried (*DW*<sub>1</sub>)

Dry weight was calculated by the equation:

$$M = (DW_1 - DW_0) / 0.01, \quad (1)$$

where *M* is the dry algae biomass (g/L).

Neutral lipid was determined with Nile red method (Chen et al., 2009, 2011). Unit volume of fluorescence value (excitation wavelength 480 nm, emission wavelength 580 nm) was measured to characterize the change of neutral lipid accumulation.

Fluorescence value in single algal cell was calculated by the equation:

$$FI = (F_{NL} - F) / Y, \quad (2)$$

where *FI* is fluorescence value in single algal cell, *F*<sub>NL</sub> is the total of fluorescence value, *F* is fluorescence value in the medium, and *Y* is cell concentration (Wang et al., 2010).

### 2.5 Biomass productivity, total lipid and lipid productivity analysis

The biomass productivity was calculated by the equation:

$$W = (m_2 - m_1) / (t_2 - t_1) \times 1000 \times 0.3, \quad (3)$$

where *W* is biomass productivity (g/(m<sup>2</sup>·d)), *m* is biomass dry weight (g/L) when the time is *t*, *t* is culture time (d).

Lipid productivity was calculated by the equation:

$$L = WP, \quad (4)$$

where *L* is lipid productivity (g/(m<sup>2</sup>·d)), *W* is biomass productivity (g/(m<sup>2</sup>·d)), *P* is the content of lipid (%) (Huerlimann et al., 2010; Tang et al., 2011).

### 2.6 Total lipid and fatty acids analysis

Microalgae were harvested at the late exponential growth phase by centrifugation at 10 000 g for 5 min. Total lipid was extracted and determined by the modified method (Huerlimann et al., 2010; Tang et al., 2011). Fatty acids were extracted and determined by the method (Yang et al., 2013).

### 2.7 Carbon content and CO<sub>2</sub> fixation rate analysis

CO<sub>2</sub> fixation rate was calculated by the equation:

$$F_{CO_2} = C_c \times W \times (M_{CO_2} / M_c) / 100, \quad (5)$$

where *F*<sub>CO<sub>2</sub></sub> is CO<sub>2</sub> fixation rate (g/(m<sup>2</sup>·d)), *C*<sub>c</sub> is carbon content (g/g), *W* is the average of biomass productivity (g/(m<sup>2</sup>·d)), *F*<sub>CO<sub>2</sub></sub> is molecular weight of CO<sub>2</sub> (44 g/mol), *M*<sub>c</sub> is molecular weight of carbon (12 g/mol) (Zhao et al., 2011).

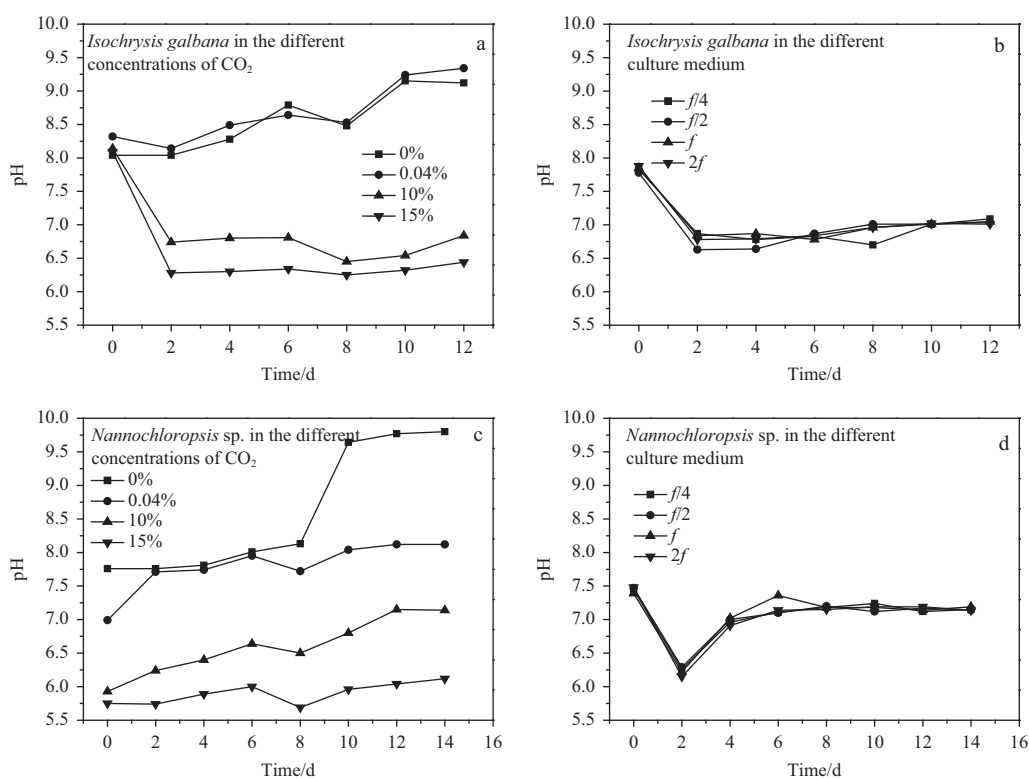
### 2.8 Statistical analysis

All data were obtained by using at least three replicated biological samples. Experimental results were expressed as mean value±SD. Statistical analysis was performed using the SPSS11.5 statistical package. The statistical significance was achieved when *p*<0.05.

### 3 Results

#### 3.1 The pH changes and growth curve of the two marine microalgae

The pH value is shown in Fig. 1. The pH value of the culture medium with CO<sub>2</sub> remains generally stable (acid condition pH 6.5). Under different concentrations of CO<sub>2</sub>, the pH values of the culture medium differ significantly. The concentration of CO<sub>2</sub> is high, and the pH value is low. Under conditions without CO<sub>2</sub> access, the pH value of the culture medium is higher than 7.0, and baseline drift occurs. The pH value of the culture medium will increase with culture time. Under the non-inflation condition, the pH values of the two strains of microalgae are kept at 9.5. Under conditions with or without CO<sub>2</sub>, the pH values of the two strains of microalgae retain stability in the plateau phase.



**Fig. 1.** The pH changes of two marine microalgae strains in different culture conditions. a. pH of *I. galbana* cultured in different concentrations of CO<sub>2</sub>, b. pH of *I. galbana* cultured in different culture medium, c. pH of *Nannochloropsis* sp. cultured in different concentrations of CO<sub>2</sub>, and d. pH of *Nannochloropsis* sp. cultured in different culture medium.

#### 3.2 The dynamics changes of the neutral lipid accumulation of the two marine microalgae

The dynamic changes of the neutral lipids in the two marine microalgae are shown in Fig. 3. With increased culture time, the fluorescence intensity of the neutral lipid of a single algal cell initially decreased then increased. Neutral lipid accumulates in the plateau phase. No significant difference in the content of neutral lipid of the two marine microalgae was observed under the different concentrations of CO<sub>2</sub> in the plateau phase. However, under different nutrient concentrations, the fluorescence value of a single algal cell in *I. galbana* is  $f/4 > f/2 > f > 2f$ . In particular, the fluorescence intensity of the neutral lipid of *I. galbana* can be as high as  $490.00 \pm 4.60$  in the case of  $f/4$ . With increased nutrient concentrations, the content of neutral lipid in *Nannochloropsis* sp. gradually decreases. After being accumulated in the plateau

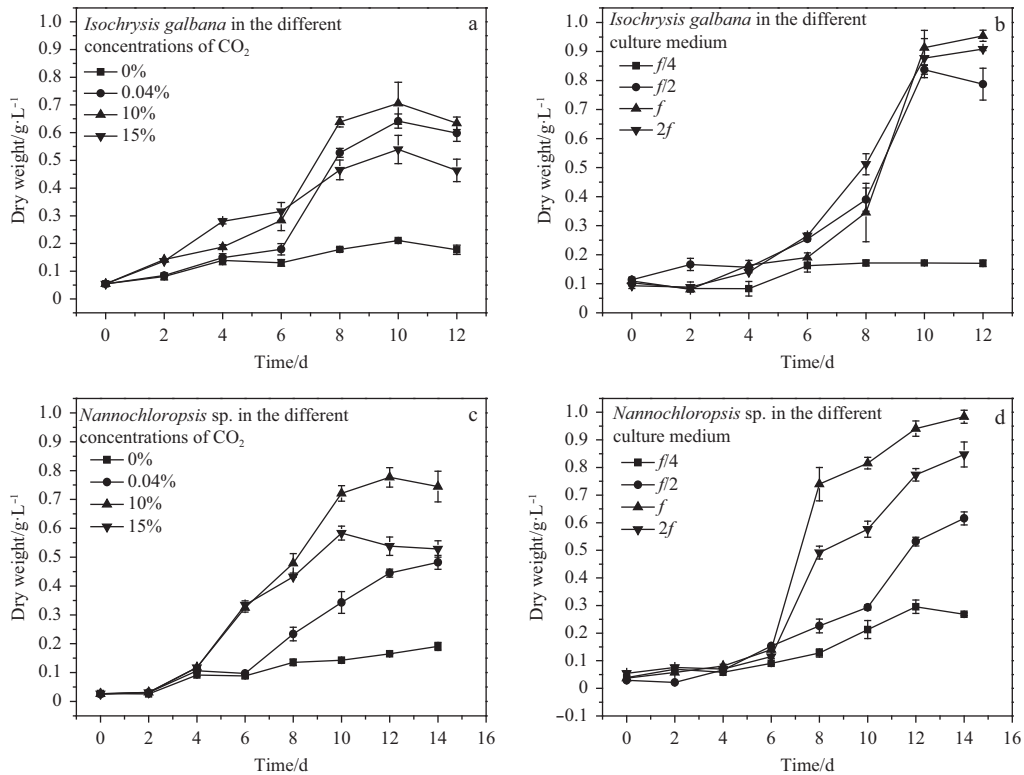
phase, the fluorescence intensity of the neutral lipid is  $f/4 > f/2 > f > 2f$ , the highest value of which is  $322.00 \pm 20.60$ .

The growth curves of the two strains of microalgae under different culture conditions are shown in Fig. 2. *Isochrus galbana* enters the exponential growth phase under different CO<sub>2</sub> and nutrient concentrations on the 4th day and enters the plateau phase on the 10th day. Except for the low-nutrient concentration ( $f/4$ ), the biomass of *I. galbana* exhibits no significant difference with the increase of the nutrient concentration. *Nannochloropsis* sp. enters the exponential growth phase under different concentrations of CO<sub>2</sub> and nutrients on the 4th day and enters the plateau phase in the 12th day. The cell density of *Nannochloropsis* sp. increased with nutrient concentration. The highest biomass is  $(0.98 \pm 0.02)$  g/L. With CO<sub>2</sub> access, a significant increase in biomass is observed. Optimal growth of the two marine microalgae were detected under the 10% CO<sub>2</sub> concentration ( $(0.71 \pm 0.08)$  g/L and  $(0.78 \pm 0.03)$  g/L).

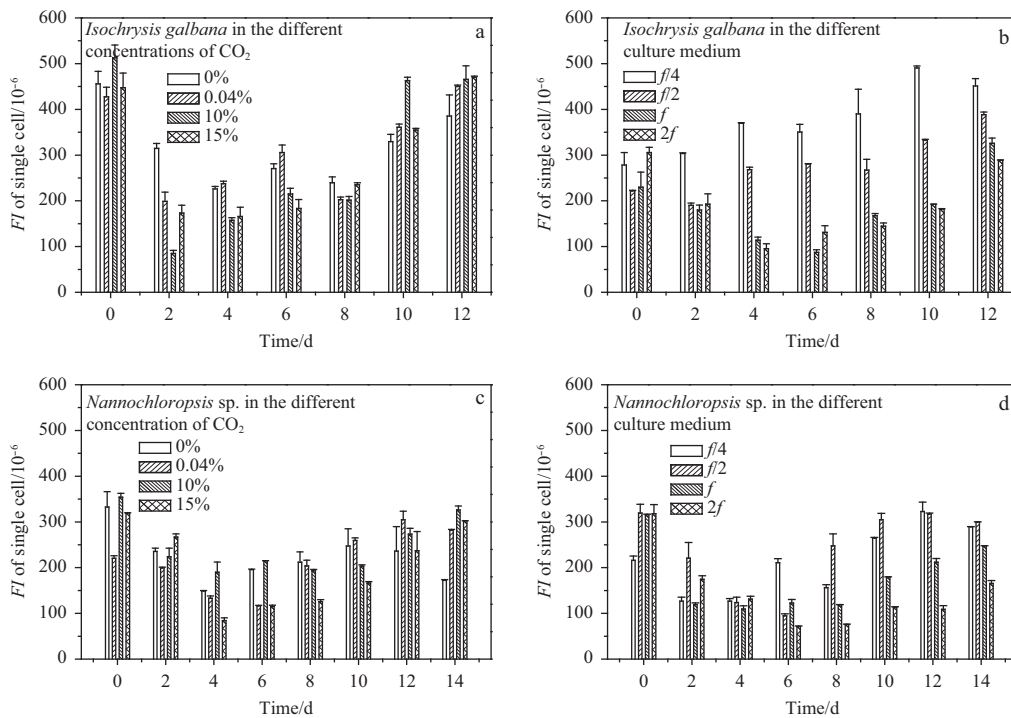
phase, the fluorescence intensity of the neutral lipid is  $f/4 > f/2 > f > 2f$ , the highest value of which is  $322.00 \pm 20.60$ .

#### 3.3 The dry weight and total lipid content of the two marine microalgae

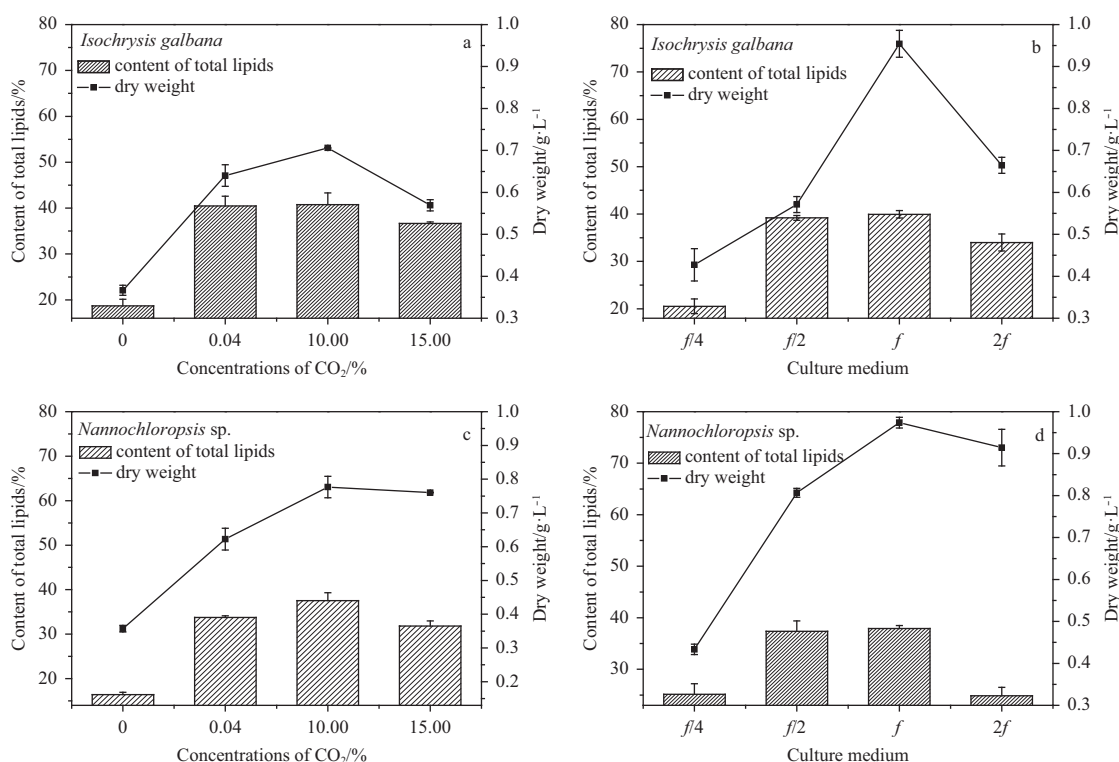
The dry weight and total lipid content of the two marine microalgae are presented in Fig. 4. The biomass and total lipid content of the two marine microalgae significantly increased and reached the highest values ( $(0.71 \pm 0.08)$  g/L,  $(40.78 \pm 2.54)\%$ ) and ( $(0.78 \pm 0.03)$  g/L,  $(37.54 \pm 1.78)\%$ ) under the 10% CO<sub>2</sub> concentration. Under different nutrient concentrations, the biomass and total lipid content of *I. galbana* in the  $f/4$  treatment group were the lowest, and those of the  $f$  treatment group were the highest ( $(0.95 \pm 0.03)$  g/L and  $(39.95 \pm 0.77)\%$ ). The biomass and total lipid content of *Nannochloropsis* sp. were the highest ( $(0.98 \pm 0.01)$  g/L



**Fig. 2.** The growth curve of two marine microalgae strains in different  $\text{CO}_2$  concentrations and culture medium (mean $\pm$ SD). a. The growth of *I. galbana* cultured in different concentrations of  $\text{CO}_2$  and *f/2* culture medium, b. the growth of *I. galbana* cultured in different culture medium and 10%  $\text{CO}_2$ , c. the growth of *Nannochloropsis* sp. cultured in different concentrations of  $\text{CO}_2$  and *f/2* culture medium, and d. the growth of *Nannochloropsis* sp. cultured in different culture medium and 10%  $\text{CO}_2$ .



**Fig. 3.** Dynamics of neutral lipid accumulation of two marine microalgae strains in different conditions (mean $\pm$ SD). a. Dynamics accumulation of neutral lipid of *I. galbana* cultured in different concentrations of  $\text{CO}_2$  and *f/2* culture medium, b. dynamics accumulation of neutral lipid of *I. galbana* cultured in different culture medium and 10%  $\text{CO}_2$ , c. dynamics accumulation of neutral lipid of *Nannochloropsis* sp. cultured in different concentrations of  $\text{CO}_2$  and *f/2* culture medium, and d. dynamics accumulation of neutral lipid of *Nannochloropsis* sp. cultured in different culture medium and 10%  $\text{CO}_2$ .



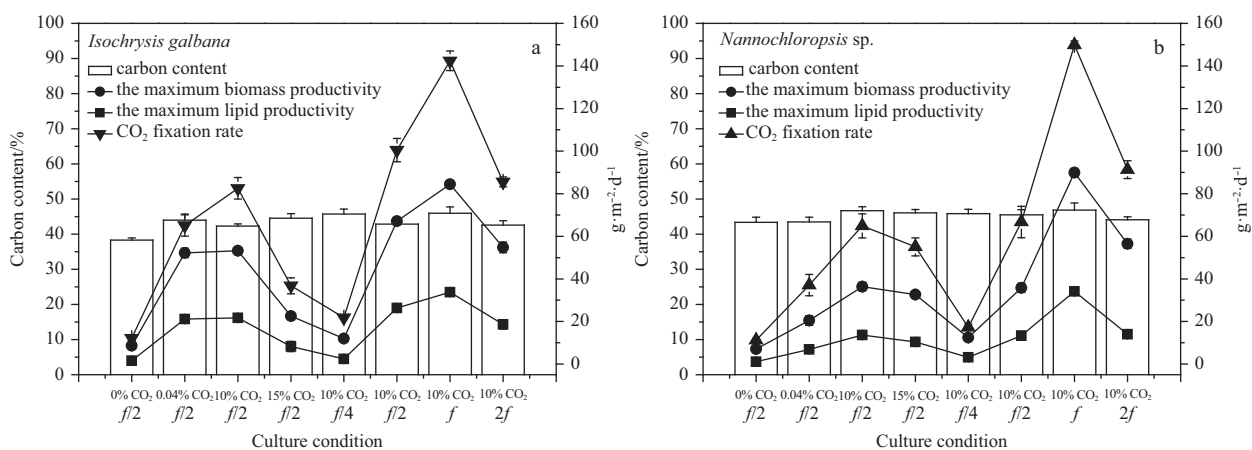
**Fig. 4.** Dry weight and total lipids of two marine microalgae strains in different culture conditions (mean±SD). a. The CO<sub>2</sub> concentration effect on dry weight and lipid content of *I. galbana*, b. the culture medium effect on dry weight and lipid content of *I. galbana*, c. the CO<sub>2</sub> concentration effect on dry weight and lipid content of *Nannochloropsis* sp., and d. the culture medium effect on dry weight and lipid content of *Nannochloropsis* sp..

and (37.91±0.58%) in the *f* treatment group. However, the total lipid content is the lowest ((24.85±1.63)%) in the 2*f* treatment group.

### 3.4 The carbon content, the maximum biomass productivity, the maximum lipid productivity and CO<sub>2</sub> fixation rate of the two marine microalgae

Figure 5 shows the carbon contents, maximum biomass productivity, maximum lipid productivity, and CO<sub>2</sub> fixation rate of

the two marine microalgae. The optimum culture conditions of the two microalgal strains are 10% CO<sub>2</sub> and *f* medium. The carbon content, maximum biomass productivity, maximum lipid productivity and CO<sub>2</sub> fixation ability of the two microalgal strains were as follows: ((45.98±1.75)%, (46.88±2.01)%), ((142.42±4.58) g/(m<sup>2</sup>·d), (149.92±1.80) g/(m<sup>2</sup>·d)), ((84.47±1.56) g/(m<sup>2</sup>·d), (89.90±1.98) g/(m<sup>2</sup>·d)), and ((33.74±1.65) g/(m<sup>2</sup>·d), (34.08±1.32) g/(m<sup>2</sup>·d)).



**Fig. 5.** The carbon content, the maximum biomass productivity, the maximum lipid productivity and CO<sub>2</sub> fixation rate of two marine microalgae strains in different culture conditions (mean±SD). a. The CO<sub>2</sub> concentration and culture medium effects on the carbon content, the maximum biomass productivity, the maximum lipid productivity and CO<sub>2</sub> fixation rate of *I. galbana*, and b. the CO<sub>2</sub> concentration and culture medium effects on the carbon content, the maximum biomass productivity, the maximum lipid productivity and CO<sub>2</sub> fixation rate of *Nannochloropsis* sp..

### 3.5 The fatty acid compositions of the two marine microalgae

The fatty acid compositions of the two marine microalgae are shown in Tables 1 and 2. The fatty acid compositions of *I. galbana* are mainly C14:0, C16:0, C18:1, and C20:6. Under different concentrations of CO<sub>2</sub>, no significant changes in C20:6 and C14–C18 are observed. Their concentrations are maintained at 14% and 83%, respectively. The content of PUFAs will increase with the concentrations of CO<sub>2</sub>. With increased nutrient concentration, C14–C18 and SFAs of *I. galbana* gradually decreased and C20:6 and PUFAs significantly increase. The fatty acid compositions of *Nannochloropsis* sp. are mainly C16:0, C16:1, C18:1, and C20:5. The most important difference is that access to CO<sub>2</sub> facilitates the accumulation of C20:5 and PUFAs and prevents the accumulation of C14–C18 (the main components of biodiesel). Under the low nutrient concentrations (*f/4*), *Nannochloropsis* sp. exhibits low content of C20:5 and PUFAs, and high contents of C14–C18, SFAs, and MUFAs.

### 4 Discussion

The main carbon source of marine microalgae through the open culture is CO<sub>2</sub>. Different microalgae exhibit different resistances to CO<sub>2</sub> (Wang et al., 2014; Salih, 2011). CO<sub>2</sub> access will cause the pH of the algal solution to decrease. The pH will directly influence the solubility of CO<sub>2</sub> and the dissolved oxygen concentration and consequently affect the carbon fixation of microalgae (Kumar et al., 2010). The results show that the increase

of CO<sub>2</sub> concentration facilitates the growth and lipid accumulation of *Nannochloropsis* sp. and *I. galbana*. However, if the concentration of CO<sub>2</sub> is more than 10%, the growth indices of the two microalgae gradually decrease. The concentration of CO<sub>2</sub> significantly affects the fatty acid composition of the two marine microalgae. However, compared with that of control group without CO<sub>2</sub>, the contents of EPA and PUFAs in *I. galbana* increased and that of C14–C18 (the main components of biodiesel) decreased. The results of Hu and Gao (2003, 2006) and Hoshida et al. (2005) are consistent with that of this work. The changes in EPA and PUFA contents may be attributed to the synthesis of polyunsaturated fatty acids in the microalgae, which starts from C16:0. Increasing the concentration of CO<sub>2</sub> is equivalent to reducing the concentration of oxygen in the air, which will influence the activity of related desaturases. For the preparation of biodiesel by microalgae lipid, the increase in the contents of unsaturated fatty acids influences the combustion of biodiesel. Thus, CO<sub>2</sub> emission reduction and the preparation of microalgae biodiesel using *I. galbana* can guarantee the quality of biodiesel.

Nutrients are one of the reasons why large-scale cultivation of marine microalgae is expensive. The dosage of nutrients influences the production of biodiesel. This factor also has a certain influence on the ecological environment resources (Fixen, 2007). Therefore, determining the appropriate dosage of nutrient is important. The sufficiency of the nutrients was associated with optimal algal growth and lipid accumulation. This is confirmed by

**Table 1.** The fatty acid composition (%) of *Isochrysis galbana* in different culture conditions

Factor FAMES	Concentrations of CO <sub>2</sub>				Different culture medium			
	0%	0.04%	10%	15%	<i>f/4</i>	<i>f/2</i>	<i>f</i>	<i>2f</i>
C14:0	18.14	18.50	17.69	18.83	16.38	19.09	17.08	14.25
C16:1	5.64	4.59	5.60	7.03	3.36	8.72	6.13	8.79
C16:0	18.61	18.48	18.66	17.23	20.89	15.01	14.60	10.54
C18:3	0.1	0.25	0.41	0.33	0.12	1.51	0.14	1.23
C18:2	2.74	4.03	4.03	4.12	5.59	6.97	7.21	13.56
C18:1	37.51	37.52	35.81	34.47	37.09	28.39	33.67	22.16
C18:0	0.76	0.95	1.33	1.01	0.84	0.47	0.58	0.48
C20:5	2.10	1.92	1.52	2.70	3.03	3.94	2.96	7.74
C20:6	14.45	13.76	14.47	14.28	12.69	15.89	17.63	21.26
C14–C18	83.45	84.32	83.53	83.02	84.28	80.17	76.41	71.00
SFAs	37.51	37.93	37.68	37.07	38.11	34.57	32.26	25.27
MUFAs	43.15	42.11	41.41	41.49	40.46	37.12	39.80	30.95
PUFAs	19.34	19.96	20.91	21.43	21.42	28.31	27.94	43.79

**Table 2.** The fatty acid composition (%) of *Nannochloropsis* sp. in different of culture conditions

Factor FAMES	Concentrations of CO <sub>2</sub>				Different culture medium			
	0%	0.04%	10%	15%	<i>f/4</i>	<i>f/2</i>	<i>f</i>	<i>2f</i>
C14:0	4.77	6.05	6.46	6.62	5.81	6.46	6.54	6.02
C16:1	29.90	27.64	26.79	27.91	27.27	26.79	28.10	28.89
C16:0	34.44	31.24	26.56	28.21	30.21	26.56	26.66	27.20
C18:3	0.76	0.87	1.04	0.84	0.99	1.04	0.80	
C18:2	1.82	2.20	2.45	1.87	2.62	2.45	2.28	2.12
C18:1	11.06	8.41	6.34	7.39	9.61	6.34	7.04	6.48
C18:0	2.81	1.60	0.76	1.44	1.78	0.76	0.68	0.77
C20:4	3.34	4.99	4.46	5.57	5.46	4.46	4.10	4.18
C20:5	11.09	17.00	25.14	20.15	16.25	25.14	23.82	23.53
C14–C18	85.56	78.01	70.40	74.28	78.29	70.40	72.08	72.29
SFAs	42.02	38.89	33.77	36.27	37.80	33.77	33.87	34.00
MUFAs	40.96	36.05	33.13	35.30	36.88	33.13	35.15	35.37
PUFAs	17.02	25.07	33.10	28.43	25.32	33.10	30.98	30.63

the fact that the growth indices exhibit the highest values in the *f* culture medium, similar to the results of Wei et al. (2000a). Therefore, the *f* culture medium is more appropriate for the large-scale culture of the two microalgae. A low nutrient concentration restricts cell growth (Liu and Wang, 2014; Wang et al., 2006; Wei et al., 2000b), whereas a high nutrient concentration will result in an extremely high content of trace metal elements, thus inhibiting the growth of microalgae (Sun et al., 2005). Previous studies indicated that low nutrient concentration causes the accumulation of microalgae lipid but prevents the growth of microalgae. In particular, the content of PUFAs will increase with the concentration of nutrients. The opposite result was observed for MUFAs. The results of Huang et al. (2013) also verified this finding. In this study, the total lipid content and maximum lipid productivity are low under the *f/4* culture medium probably because the concentrations of N and P are less than the growth concentration. The two microalgae rapidly undergo the decline phase. Enzyme activity associated with lipid synthesis in cells decreases or is partially inactivated. Moreover, lipid accumulation is blocked, thus reducing content (Yu et al., 2011).

The results from the Nile red method show that the neutral lipid content of the two marine oil-rich microalgae accumulates in the growth plateau phase. This finding is consistent with the results obtained by Wei et al. (2000b) and Wang et al. (2010). The plateau phase is the accumulation period of neutral lipid because the nitrogen sources required by synthetic amino acid in the culture medium decrease with protein synthesis after the cells enter the plateau phase. Moreover, energy accumulation occurs in carbohydrate and lipids synthesized by the carbon from photosynthetic assimilation (Li et al., 2013; Ma et al., 2012; Xu et al., 2012; Shi and Pan, 2004). Increasing the concentration of CO<sub>2</sub> can provide rich carbon for the accumulation of carbohydrates and lipids. During the entire growth phase, the two marine microalgae initially decrease and then increase in neutral lipid because the initial microalgae are in the late stage of the logarithmic phase and the neutral lipid content of a single algal cell is high. When microalgae are cultured in the logarithmic phase, the ATP that microalgal cells split and consume in large amounts partly come from the neutral lipids (Zhu et al., 2011). Previous studies demonstrated that neutral lipid content does not increase in the logarithmic phase (Wang et al., 2014; Wei et al., 2000b), thereby causes the neutral lipid content to decrease because of its consumption in the logarithmic phase. To date, the dynamic metabolism of neutral lipids and the mechanism of the cell growth cycle remains to be clarified (Salih, 2011).

## 5 Conclusions

*Isochrysis galbana* CCMM5001 and *Nannochloropsis* sp. CCMM7001 can be potentially used as a renewable biodiesel feedstock that can reduce CO<sub>2</sub> emission. The optimum culture conditions of the two marine oil-rich microalgae are 10% CO<sub>2</sub> and *f* medium. At 10% CO<sub>2</sub>, the two microalgal strains accumulate neutral lipids of up to 490.00±4.60 (*FI* of single cell is 10<sup>-6</sup>) in single cells. Among the neutral lipids, triglycerides are the most abundant component. The maximum biomass productivity, total lipid content, lipid productivity, carbon content, and CO<sub>2</sub> fixation ability of the two microalgal strains were obtained.

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