Appl Biochem Biotechnol (2016) 180:452–463
DOI 10.1007/s12010-016-2109-5

Effects of Trophic Modes, Carbon Sources, and Salinity on the Cell Growth and Lipid Accumulation of Tropic Ocean Oilgae Strain *Desmodesmus* sp. WC08

Zhenyu Zhao[1,2] **· Shasha Ma**[1,2] **· Ang Li**[1,2] **·**
Pinghuai Liu[1,2] **· Meng Wang**[1,2]

Received: 23 March 2016 / Accepted: 28 April 2016 /
Published online: 5 May 2016
© Springer Science+Business Media New York 2016

Abstract The effects of trophic modes, carbon sources, and salinity on the growth and lipid accumulation of a marine oilgae *Desmodesmus* sp. WC08 in different trophic cultures were assayed by single factor experiment based on the blue-green algae medium (BG-11). The results implied that biomass and lipid accumulation culture process were optimized depending on the tophic modes, sorts, and concentration of carbon sources and salinity in the cultivation. There was no significant difference in growth or lipid accumulation with Na_2CO_3 amendment or $NaHCO_3$ amendment. However, Na_2CO_3 amendment did enhance the biomass and lipid accumulation to some extent. The highest *Desmodesmus* sp. WC08 biomass and lipid accumulation was achieved in the growth medium with photoautotrophic cultivation, 0.08 g L^{-1} Na_2CO_3 amendment and 15 g L^{-1} sea salt, respectively.

Keywords Cultivation · Microalgae · Trophic modes · Salinity · Carbon sources · Lipid accumulation · Biomass

Introduction

Microalgae are a kind of unicellular photosynthetic microorganisms which can absorb solar energy to digest water and CO_2, and then release O_2 into atmosphere. Its cell is tiny, diverse in morphology, strong in adaptability, and one of the most ancient primary producers in the world

✉ Pinghuai Liu
pinghuailiu@aliyun.com

[1] National Ministry of Education Key Laboratory of Protection and Development Utilization of Tropical Crop Germplasm Resources, Hainan University, Haikou 570228 Hainan Province, People's Republic of China

[2] Hainan Provincial Key Laboratory of Fine Chemistry, Hainan University, Haikou 570228 Hainan Province, People's Republic of China

[1]. The lipid accumulation of microalgae varies from species to cultivation conditions, ranging from 50 to 70 % of their dry weight [2]. Meanwhile, there are many nutritious elements of high levels, which proved to be significantly practical in development.

At present, the energy workers have paid more attention to the oilgae, because of the shortage of fossil fuels and the pollution. Biodiesel, also known as fatty acid alkyl esters which are made form renewable biological sources such as vegetable oils or animal fats, is a sort of environment friendly, renewable energy [3]. Although microalgae has many advantages such as high growth rate, high oil production rate, and no occupation of farmland over traditional oil crops, the cost of highly densed cultivation is still the bottleneck issue if microalgae become commercial biodiesel raw material [4]. Therefore, it is significant to research the different environments and nutritional factors on the microalgae cell growth and the influence of oil accumulation.

Lipid accumulation is typically induced by fluctuation of growth conditions or various types of stress and it can be expressed as variation in fatty acids compositions or total lipids [5]. Zhang et al. and Luo et al. indicated that fast *Desmodesmus* sp. WC08 growth under optimal conditions followed by some specific stress factors, such as nitrogen starvation, phosphors starvation, iron starvation, calcium starvation, and magnesium starvation, to obtain maximum lipid content [6, 7].

Carbon is the most important element in microalgae cells and can make up to 50 % of the dry biomass [8]. Some studies have examined the use of different organic carbon sources for algae cultivation. Malorie et al. used residual corn crop hydrolysate and silage juice as alternative carbon sources in microalgae production, which showed that the use of residual corn hydrolysate represented an interesting and efficient alternative as an organic carbon source while silage juice needed additional treatments to be implemented as a culture medium [9]. In addition, about inorganic carbon sources, Kesaano et al. showed that there was no significant difference in growth rates and lipid accumulation were observed in the algal biofilms with or without bicarbonate amendment. However, the influence of bicarbonate on photosynthetic and respiration rates was especially noticeable in biofilms. Rajdeep et al. indicated that Na_2CO_3 increased the bio-oil yield for high carbohydrate-containing algae at higher temperatures [10].

Furthermore, various studies have focused on the influence caused by salinity. In these studies, salinity stress has been reported to enhance lipid accumulation in various microalgae [11]. Microalgal growth could be affected by salinity through osmotic pressure and ionic pressure. Pancha et al. showed that *Scenedesmus* sp. CCNM 1077 grown with 400 mM salinity stress under single stage cultivation accumulated higher lipid contents but resulted in lower biomass, during two-stage cultivation, 3-day stress of 400 mM NaCl enhanced the lipid contents with negligible biomass reduction [12].

Many studies on the trophic modes and factors affecting photosynthesis of various microalgae have been made. Marudhupandi et al. revealed that the heterotrophically cultivated *Nannochloropsis salina* with various carbon yielded higher biomass and lipid content than that of the photoautotrophical cultivation [13]. Mixotrophic culture is a new method for culturing microalgae in light with organic compound as supplementary carbon source, which can enhance the lipid yield and biomass of the microalgae [8].

Desmodesmus sp. WC08 was isolated from Hainan Island in China with high biomass and high lipid content. At present, the cultivation research of *Desmodesmus* about natural conditions (temperature, illumination intensity, photoperiod, and pH) and nutritional elements (iron, calcium, magnesium) have been done. At the same times, it can be cultivated by sewage. The

study presented here evaluated the effects of adding inorganic carbon and sea salt to medium to grow algae and observe lipid accumulation in order to:

(1) Obtain the optimal inorganic carbon source and the optimal concentration,
(2) Determine an optimal salinity, and
(3) Screen an optimal trophic mode.

Materials and Methods

Microalgae Strain and Preculture Conditions

The microalgae *Desmodesmus* sp. WC08 used in this study was obtained from Wenchang (Hainan province, China) as described preciously [14]. The microalgae was cultrued at 25 ± 1 °C in 1-L flasks using BG-11 medium with an initial pH of 7.0 ± 0.1, salinity 15 g/L, and 12:12 h light/dark period under 8000~10000 lx light intensity. The flasks were manually shaken thrice a day.

Photobioreactor and Cultivation Conditions

After preculture in the the flasks, the microalgae was cultivated at 25 ± 2 °C at a light intensity of 10000 lx in a 5-L circular cylindrical laboratory scale luminescent bubble column photobioreactors (PBRs) operated at 150 rpm (there is a mini stirrer in the reactor) with a working volume of 3 L using BG-11 medium with an initial pH of 7.0 ± 0.2 and salinity 15 g L^{-1}. The medium was inoculated (10 % *v*/*v*) with an active culture of the microalgae consortium. Aeration with filtered sterile air was provided through an air purifier. Filtered air was supplied to the reactor through a 0.25-μm polytetrafluoroethylene membrane with 0.50 air volume/culture volume/minute. All the autotrophic and mixotrophic experiments were performed in batch culture with 15 days and a 12:12 light/dark cycle. The heterotrophic experiments were performed without light.

The basal BG-11 medium consisted of 1.5 g L^{-1} $NaNO_3$, 0.04 g L^{-1} K_2HPO_4, 0.075 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.036 g L^{-1} $CaCl_2 \cdot H_2O$, 0.006 g L^{-1} citric acid, 0.006 g L^{-1} ammonium ferric citrate, 0.02 g L^{-1} Na_2CO_3, 0.001 g L^{-1} $EDTA \cdot Na_2$, 2.860 g L^{-1} H_3BO_3, 0.220 g L^{-1} $ZnSO_4 \cdot 7H_2O$, 1.810 g L^{-1} $MnCl_2 \cdot 4H_2O$, 0.079 g L^{-1} $CuSO_4 \cdot 5H_2O$, 0.049 g L^{-1} $Co(NO_3)_2 \cdot 6H_2O$, and 0.390 g L^{-1} $Na_2MoO_4 \cdot 2H_2O$.

Experimental Design Method

The Influences of Trophic Modes on Biomass and Lipid Accumulation

Screening of the best organic carbon sources for biomass and lipid production was done in one variable at a time manner. Briefly, 12:12 h light/dark cycle was provided by the fluorescent lamps for the photoautotrophic and mixotrophic cultures while heterotrophic cultures were kept in the dark. Carbon (67 mmol L^{-1}) in the organic carbon sources (glucose, sodium acetate, and glycerol) was controlled to be added into the BG-11 medium (only for mixotrophy and heterotrophy). All experiments were performed in triplicate and the salinity was 15 g L^{-1}.

The Influences of Inorganic Carbon Sources on Biomass and Lipid Accumulation

Cultures were carried out in triplicate in the reactor containing BG-11 medium with various carbon sources (Na_2CO_3 and $NaHCO_3$) and the carbon molarity of Na_2CO_3 and $NaHCO_3$ were equal to the molarity of carbon source in the BG-11 medium (2.3 mmol L^{-1}). In all the parallel experiments, the salinity was determined as 15 g L^{-1}.

The Influences of Concentration of Carbon Source on Biomass and Lipid Accumulation

Cultures were carried out in triplicate in the reactor containing BG-11 medium with Na_2CO_3 concentrations of 0, 0.02, 0.04, 0.06, and 0.08 g L^{-1}. In all the parallel experiments, the salinity was 15 g L^{-1}.

The Influences of Salinity on Biomass and Lipid Accumulation

Cultures were carried out in triplicate in the reactor containing BG-11 medium with sea salt concentrations of 10, 15, 20, and 30 g L^{-1}.

Analytical Methods

Determination of Growth

Microalgal growth was monitored by direct sampling directly using a sterile disposable syringe and measuring optical density (OD) at 680 nm every 24 h intervals at a fixed time over a period of 18 days. Each parameter was tested in triplicates.

Determination of Biomass

After 15-day cultivation, microalgae was harvested by centrifugation at 8000 rpm for 6 min and cleaned by deionized water for three times. Then, the microalgae paste was dried by a vacuum freeze drier. Total biomass was expressed as gram dry weight per liter. Based on this information theoretical biomass by each litre of microalgae can be obtained from equation (1) as below:

$$P\text{biomass}(\text{g/L}) = \frac{N0(\text{g})}{V(\text{L})} \tag{1}$$

where P is biomass, N_0 is the mass of dried microalgae powder, and V is the volume of culture medium.

Determination of Total Lipid Extraction

Total lipid yield was also expressed as gram dry weight per liter while the lipid content was expressed as percentage. Lipid content was determined using a modified method of Axelsson and Gentili (2014) which was chloroform-methanol solvent base [5, 15]. In brief, 2 mL of chloroform:methanol (2:1 *v*/*v*) was added to the 0.1-g lyophilized microalgae powder (the

liquid-solid ratio was 20:1) in 15 mL solvent resistant falcon tubes and placed in an ultrasound bath extraction in the ambient temperature for 30 min then homogenized using a magnetic stirring apparatus for 3~6 h (keep sealing). The mixture was centrifuged at 4000 rpm for 5 min. After centrifugation, the supernatant was transferred to a clear tube. For rest of the microalgal dreg, the experiments above were carried out in duplicate for 5 times and pooled all the supernatant together then 0.9 % NaCl (1:0.2 $v_{supernatant}/v_{NaCl}$) was added to produce a layered solvent system. The layered solvent system was vortexed for 5 min. After a few minutes of standing, the lower layer was transferred to a clear tube and dried to constant weight using pressure blowing concentrator.

The total lipid content and total lipid yield was calculated according to Eqs. (2) and (3):

$$\text{Total lipid content } (\%) = \frac{\text{Algal lipid (g)}}{\text{Mass of Algal (g)}} \times 100 \tag{2}$$

$$\text{Total lipid yield (g/L)} = \text{Algal biomass (g/L)} \times \text{Total lipid content } (\%) \tag{3}$$

Statistical Analysis

All results were expressed as mean values ± standard deviation. The statistical differences between experimental groups were analyzed by analysis of variance (ANOVA). The analysis of least significant digit (LSD) and the graphic plotting were used by statistical product and service solutions (SPSS) and Oringin 8.0, respectively.

Results and Discussions

The Effects of Trophic Modes on Growth, Biomass, and Lipid Accumulation of *Desmodesmus* sp. WC08

Many researches have elaborated the effects of trophic modes on biomass and lipid accumulation of microalgae. But most of the researches indicated that the mixotrophic cultivation with glucose was the best trophic mode. In this research, at the first 6 days, growth period of autorophic cultivation of *Desmodesmus* sp. WC08 was short while compared to mixotrophic (glucose, sodium acetate, glycerinum) and heterotrophic (glucose, sodium acetate) clutivation. After 10 days, the growth of autotrophic cultivation exceeded all the other trophic cultivations. The result showed that glycerinum in mixotrophic cultivation and heterotrophic showed inhibitory effect on the growth of *Desmodesmus* sp. WC08. Microalgal growth was enhanced at the exponential growth phase with mixotrophic (glucose) cultivation compared to autotrophic cultivation at the control condition. Although the optical density of mixotrophic (glucose) cultivation was higher than any other trophic modes at the first 10 days, after experienced an exponential enhancement in the previous days, the subsequent growth entered into a steady phase and inclined to decline (Fig. 1).

From the Table 1, *Desmodesmus* sp. WC08 with autotrophic cultivation showed the maximum $P_{biomass}$ of 1.83 ± 0.18 g L^{-1} and there was significant difference between

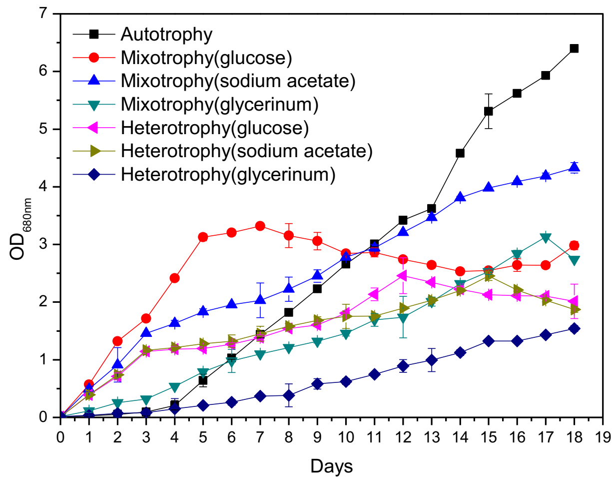

Fig. 1 Growth curves of *Desmodesmus* sp. WC08 in different trophic modes

autotrophic cultivation and other trophic cultivation ($p < 0.05$). The biomass was lowest when glycerinum was added in the mixotrophic and heterotrophic cultivation, respectively. The result attested to the inhibiting effect of glycerinum on *Desmodesmus* sp. WC08's biomass.

The highest lipid content (42.11 ± 0.61 %) and lipid yield (3.01 ± 0.34 g L^{-1}) were observed in the autotrophic cultivation and there was highly significant difference between autotrophic cultivation and other trophic cultivation ($p < 0.01$). The lipid yield of mixotrophic cultivation with glucose and sodium acetate was 1.02 ± 0.52 and 1.15 ± 0.37 g L^{-1}, respectively and had no significant difference. The lipid content and lipid yield of heterotrophic cultivation with glucose and sodium acetate had no significance either. Glyerinum in mixotrophic and heterotrophic cultivation showed considerable enhancement in lipid content, whereas it induced the lowest lipid yield in micotrophic and heterotrophic cultivation, respectively (Fig. 2).

Overall, the growth rate of mixotrophic (glucose) cultivation showed an upward trend, which was far higher than the rates of other groups at the first few days. Nevertheless, the growth rate would decline slightly subsequently. Glycerinum could increase lipid content, however, inhibiting the growth and biomass of *Desmodesmus* sp. WC08. Results of the seven trophic mode tests indicated that autotrophic cultivation were optimistic trophic mode for growth enhancement and biomass production of *Desmodesmus* sp. WC08, resulting in a significant increase ($p < 0.05$) in lipid accumulation after 18 days of culture compared to control at all tested trophic modes.

Table 1 Biomass of microalgae *Desmodesmus* sp. WC08 in different trophic modes

Trophic methods	Autortophy	Mixotrophy (glucose)	Mixotrophy (sodium acetate)	Mixotrophy (glycerinum)	Heterotrophy (glucose)	Heterotrophy (sodium acetate)	Heterotrophy (glycerinum)
Biomass (g/L)	1.83 ± 0.18[a]	0.79 ± 0.01[b]	1.01 ± 0.05[c]	0.45 ± 0.01[d]	0.58 ± 0.11[e]	0.38 ± 0.01[f]	0.13 ± 0.01[g]

Different letters indicate values are significantly different ($p < 0.05$) for each trophic modes

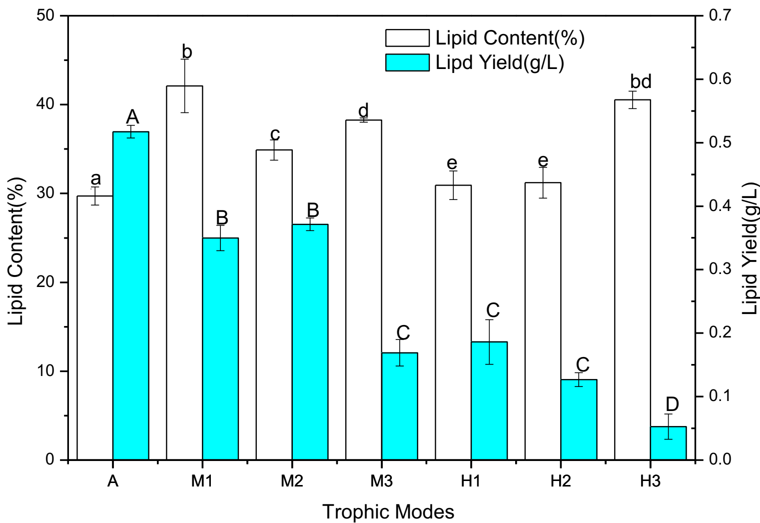

Fig. 2 The effects of various trophic methods on lipid content and lipid yield of *Desmodesmus* sp. WC08. *Different letters* indicate values are significantly different ($p < 0.05$) for each trophic modes. Where *A* is autotrophy, *M1* is mixotrophy (glucose), *M2* is mixotrophy (sodium acetate), *M3* is mixotrophy (glycerinum), *H1* is heterotrophy (glucose), *H2* is heterotrophy (sodium acetate), and *H3* is heterotrophy (glycerinum)

The Effects of Two Inorganic Carbon Sources on the Growth, Biomass, and Lipid Accumulation of Desmodesmus sp. WC08

The catalysts of carbon source supplementation on the growth of various microalgae were well documented. Supplementation of inorganic carbon, namely Na_2CO_3 [10] and $NaHCO_3$ [16], in photoautotrophic cultures were shown to be beneficial in enhancing biomass and lipid productivity. Results of the various inorganic carbon experiments indicated that Na_2CO_3 and $NaHCO_3$ were potential inorganic carbon sources for growth enhancement and lipid

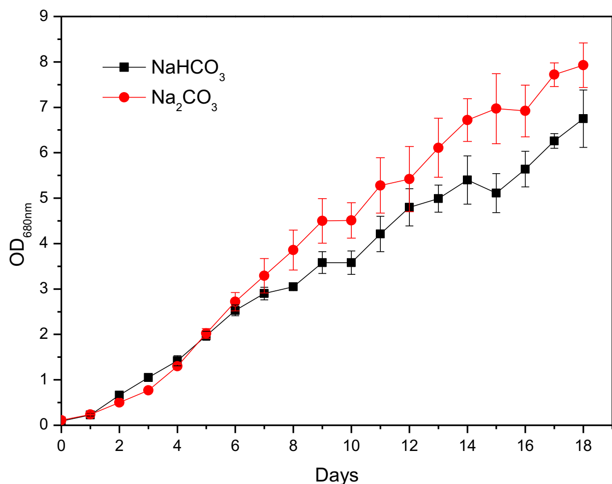

Fig. 3 Growth curves of *Desmodesmus sp. WC08* with two different inorganic carbon sources

Table 2 Biomass of microalgae *Desmodesmus sp. WC08* with two different inorganic carbon sources

Inorganic carbon sources	Na_2CO_3	$NaHCO_3$
Biomass (g/L)	2.94 ± 0.25	2.66 ± 0.37

[a] Values are significantly different ($p < 0.05$) for each carbon sources

accumulation of *Desmodesmus* sp. WC08. As shown in Fig. 3 and Table 2, *Desmodesmus* sp. WC08 grew the fastest with $NaHCO_3$ as the organic source at the first 5 days and then was exceeded by Na_2CO_3 group to get a higher growth rate and reach highest biomass of 2.94 ± 0.25 g L^{-1}, while the lowest biomass of 2.66 ± 0.37 g L^{-1} was obtained in the cultivation using $NaHCO_3$ as the carbon source. The measured lipid content and lipid yield were 33.66 ± 0.45 % and 0.90 ± 0.13 g L^{-1}, respectively, for *Desmodesmus* sp. WC08 with $NaHCO_3$ amendment. Similarly, final total lipid content and lipid yield measured from Na_2CO_3 were 32.35 ± 0.24 % and 0.95 ± 0.01 g L^{-1}, respectively. No significant difference in lipid accumulation was observed in the cultivation of *Desmodesmus* sp. WC08 with Na_2CO_3 or $NaHCO_3$ amendment (Fig. 4). The *Desmodesmus* sp. WC08 possibly did not experience carbon-limited conditions because of the large reservoir of dissolved inorganic carbon in the medium. However, a higher increase in growth rate, biomass, and lipid yield were observed in experiment amended with Na_2CO_3. The reason why CO_3^{2-} and HCO_3^- could enhance the biomass and lipid accumulation had been well demonstrated [17,18]. Briefly, the active transport of two ions (CO_3^{2-} and HCO_3^-) and the mutual transformation between two ions and CO_2 controlled by carbonic anhydrase could increase the concentration of CO_2 which surrounded ribulose 1,5-bishosphate carboxylase/oxidase. One the one hand, these combined actions could benefit the competition of ribulose 1,5-bishosphate carboxylase/oxidase's binding site to CO_2 and increase carboxylation rate; on the other hand, these combined actions could inhibit photorespiration and enhance net photosynthetic rate, however limited research on the comparison of the mechanisms between CO_3^{2-} and HCO_3^-. On the basis of Zhang et al. [19], the increasing rate of pH caused by HCO_3^- was faster than that of CO_3^{2-} supplement, and the increasing of pH could inhibit the biomass and the lipid accumulation of microalgae.

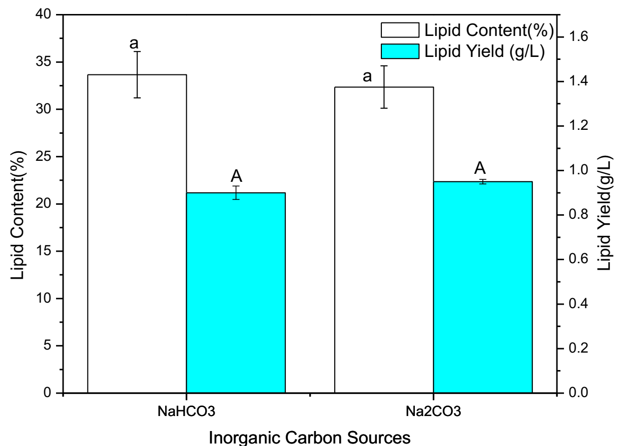

Fig. 4 The effects of two inorganic carbon sources on lipid content and lipid yield of *Desmodesmus sp. WC08. Different letters* indicate values are significantly different ($p < 0.05$) for each carbon sources

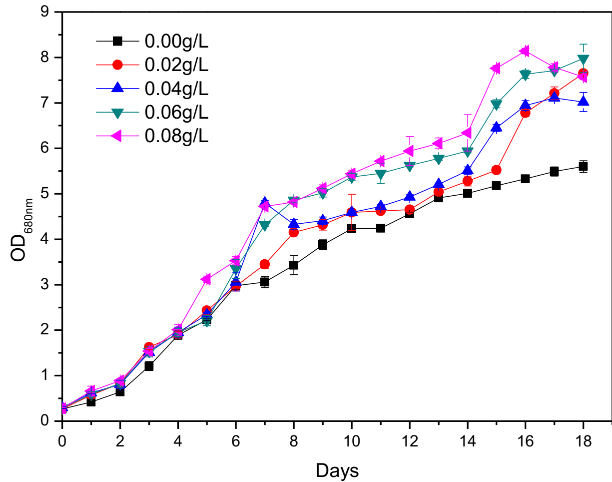

Fig. 5 Growth curves of *Desmodesmus sp. WC08* with different concentrations of Na_2CO_3

The Effects of Different Concentrations of Na_2CO_3 on the Growth, Biomass, and Lipid Accumulation of Desmodesmus sp. WC08

Desmodesmus sp. WC08 cells had a full growth cycle, as shown by the sigmoid growth models. These growth cycles comprised a more dynamic pattern in different concentrations of Na_2CO_3. Growth rate in *Desmodesmus* sp. WC08 was enhanced at the exponential growth phase with Na_2CO_3 amendment compared to the cultivation without Na_2CO_3 amendment at the control condition. Although the overall growth rates obtained were relatively similar, the highest growth, with 0.08 g L^{-1} Na_2CO_3, was achieved 17 days before. Figure 5 showed that the growth rate increased with the increasing of the concentration of Na_2CO_3.

In terms of biomass (Table 3), Na_2CO_3 (0.08 g L^{-1}) resulted in approximately 0.5-fold increase compared to control group (0.00 g L^{-1}). Supplementation of culture medium with 0.08 g L^{-1} Na_2CO_3 resulted in a final dry biomass weight of 0.99 ± 0.25 g L^{-1}, which was higher than for *Desmodesmus* sp. WC08 supplemented with 0.02, 0.04, and 0.06 g L^{-1} Na_2CO_3 groups, which produced approximately 0.89 ± 0.23, 0.83 ± 0.18, and 0.89 ± 0.14 g L^{-1}, respectively, and there was no significant difference between them ($p > 0.05$).

A slight increase in total lipid content was observed in cell growth in both 0.04 and 0.06 g L^{-1} Na_2CO_3 groups compared to control group (0.00 g L^{-1}), and there were no significant differences between them ($p > 0.05$). Meanwhile, 0.02 and 0.08 g L^{-1} Na_2CO_3 groups could increase ($p < 0.05$) lipid content significantly (Fig. 6).

Table 3 Biomass of microalgae *Desmodesmus sp. WC08* with different concentrations of Na_2CO_3

Concentration of Na_2CO_3 (g/L)	0.00	0.02	0.04	0.06	0.08
Biomass (g/L)	0.67 ± 0.190^{a}	0.89 ± 0.23^{b}	0.83 ± 0.18^{b}	0.87 ± 0.14^{b}	0.99 ± 0.25^{c}

Different letters indicate values are significantly different ($p < 0.05$) for each concentrations of Na_2CO_3

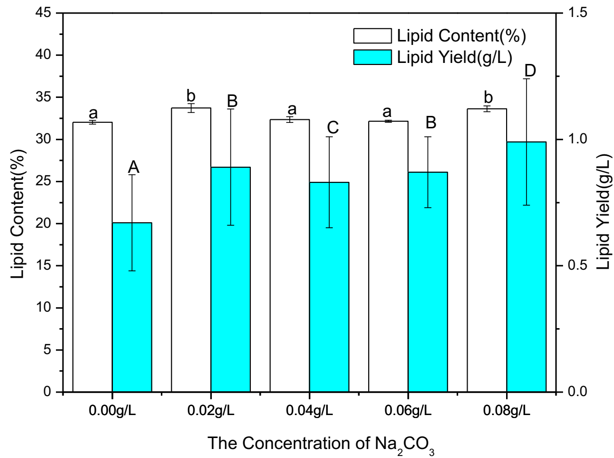

Fig. 6 The effects of different concentrations of Na_2CO_3 on lipid content and lipid yield of *Desmodesmus sp. WC08. Different letters* indicate values are significantly different ($p < 0.05$) for each concentrations of Na_2CO_3

There were some appreciable variations in maximum growth rate, biomass, and lipid accumulation observed under photoautotrophic conditions depending upon the different concentrations of Na_2CO_3, with 0.08 g L^{-1}, showing quite high growth rates, biomass, and lipid yield. Regardless of other factors, this measure would be important in choosing the optimal concentration of Na_2CO_3 to develop for a practical production system.

The Effects of Different Salinities on the Growth, Biomass, and Lipid Accumulation of Desmodesmus sp. WC08

Salinity for biomass productivity ($P_{biomass}$) with *Desmodesmus* sp. WC08 was optimized. Salinities of 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, and 40.0 g L^{-1} were established in cultures under photoautotrophic cultivation, respectively. As shown in Fig. 7, the growth of *Desmodesmus* sp. WC08 with salinities of 15.0, 20.0, and 25.0 g L^{-1} reached the apex phase

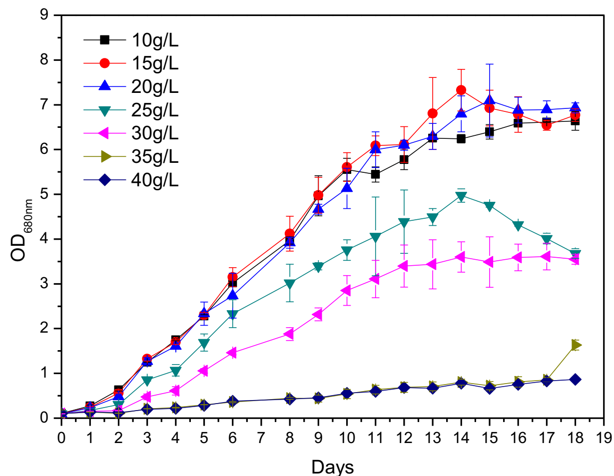

Fig. 7 Growth curves of *Desmodesmus sp. WC08* with different salinities

Table 4 Biomass of microalgae *Desmodesmus sp. WC08* with different salinities

Salinity (g/L)	10	15	20	25	30	35	40
Biomass (g/L)	4.32 ± 0.45^{a}	5.35 ± 0.30^{b}	5.05 ± 0.49^{b}	4.36 ± 0.53^{a}	3.93 ± 0.25^{a}	1.69 ± 0.27^{d}	1.69 ± 0.13^{d}

Different letters indicate values are significantly different ($p < 0.05$) for each concentrations of Na_2CO_3

at 14, 14, and 15 days, respectively. The high salinity of 35 and 40 g L^{-1} resulted in the lowest growth rate. With the increasing of salinity, the growth rate increased firstly and then decreased; the highest growth rate was under the cultivation of 15 g L^{-1} sea salt. The result revealed that the excessively high salinity could inhibit the growth of *Desmodesmus* sp. WC08.

Various sea salt stresses in the cultivation were carried out for 18 days to find optimum sea salt stress to accumulate high biomass and lipid yield as shown in Table 4 and Fig. 8. The various sea salt concentrations in the culture for biomass and lipid yield ranging from 10.0 to 40.0 g L^{-1}. The top two biomass, total cellular lipid content, and lipid yield under the salinities of 15.0 and 20.0 g L^{-1} have no significant difference ($p > 0.05$), whereas there were significant differences compared with other salinities ($p < 0.05$). The highest biomass and lipid yield of the *Desmodesmus* sp. WC08 were 5.35 ± 0.30 and 1.26 ± 0.06 g L^{-1}, respectively, under the cultivation of 15.0 g L^{-1} sea salt while the lowest biomass and lipid yield were under the cultivation of 40 g L^{-1} sea salt. These results indicated that the contribution of the culture to biomass and lipid accumulation under low-salt conditions was significant.

Conclusions

For this study, autotrophic cultivation proved to be a useable trophic mode for *Desmodesmus* sp. WC08. Two inorganic sources (Na_2CO_3 and $NaHCO_3$) could be utilized by *Desmodesmus* sp. WC08. Although there was no significant difference in growth rate, biomass, and lipid accumulation between Na_2CO_3 and $NaHCO_3$ amendment, Na_2CO_3 did enhance the biomass and lipid accumulation to some extent compared to the $NaHCO_3$ amendment. Meanwhile, the

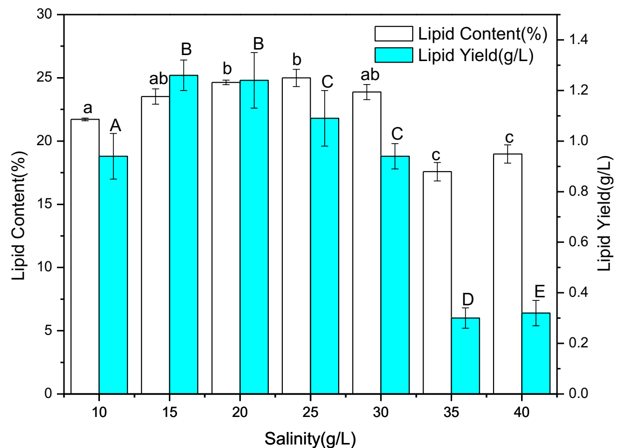

Fig. 8 The effects of different salinities on lipid content and lipid yield of *Desmodesmus sp. WC08. Different letters* indicate values are significantly different ($p < 0.05$) for each salinity

increase of the concentration of Na_2CO_3 could considerably enhance the biomass and lipid accumulation of *Desmodesmus* sp. WC08. In conditions of high salinity (35 g L^{-1} and above), the growth of *Desmodesmus* sp. WC08 was significantly inhibited while low salinity could enhance the biomass and lipid accumulation.

Acknowledgments The authors acknowledge the technical assistance of the Analytical and Testing Center of Hainan University. This work is supported by the National Science and Technology Support Program of China (2011BAD14B01), the Provincial Science and Technology Program on Modernization of Traditional Chinese Medicine of Hainan (ZY201327), and the Innovation Fund Project for Technology Based Firm (13C26244604892).

References

1. Amaro, H. M., Guedes, A. C., & Malcata, F. X. (2011). Advances and perspectives in using microalgae to produce biodiesel. *Applied Energy, 88*(10), 3402–3410.
2. Yeesang, C., & Cheirsilp, B. (2011). Effect of nitrogen, salt, and iron content in the growth medium and light intensity on lipid production by microalgae isolated from freshwater sources in Thailand. *Bioresource Technology, 102*(3), 3034–3040.
3. Li, X., Hu, H. Y., & Zhang, Y. P. (2011). Growth and lipid accumulation properties of a freshwater microalga Scenedesmus sp. under different cultivation temperature. *Bioresource Technology, 102*(3), 3098–3102.
4. Tabernero, A., Martín del Valle, E. M., & Galán, M. A. (2012). Evaluating the industrial potential of biodiesel from a microalgae heterotrophic culture: scale-up and economics. *Biochemical Engineering Journal, 63*, 104–115.
5. Mohsenpour, S. F., & Willoughby, N. (2016). Effect of CO2 aeration on cultivation of microalgae in luminescent photobioreactors. *Biomass and Bioenergy, 85*, 168–177.
6. Zhang, S., et al. (2014). Effects of concentrations of iron, calcium and magnesium on the growth and lipid accumulation of microalgal strain Desmodesmus sp. WC08. *Guangdong Editorial Society of Science and Technology Pariodicals, 4*, 126–130 [In Chinese].
7. Luo, N., Zhang, S., & Liu, P. (2016). Effects of nitrogen and phosphorus on cell growth and lipid accumulation of tropic ocean microalgae strain Desmodesmus sp. WC08. *Science and Technology of Food Industry, 37*, 223–227 [In Chinese].
8. Pancha, I., et al. (2014). Nitrogen stress triggered biochemical and morphological changes in the microalgae Scenedesmus sp. CCNM 1077. *Bioresource Technology, 156*, 146–154.
9. Gélinas, M., et al. (2015). Residual corn crop hydrolysate and silage juice as alternative carbon sources in microalgae production. *Algal Research, 12*, 33–42.
10. Shakya, R., et al. (2015). Effect of temperature and Na2CO3 catalyst on hydrothermal liquefaction of algae. *Algal Research, 12*, 80–90.
11. Caporgno, M. P., et al. (2015). Microalgae cultivation in urban wastewater: nutrient removal and biomass production for biodiesel and methane. *Algal Research, 10*, 232–239.
12. Pancha, I., et al. (2015). Salinity induced oxidative stress enhanced biofuel production potential of microalgae Scenedesmus sp. CCNM 1077. *Bioresource Technology, 189*, 341–348.
13. Marudhupandi, T., Sathishkumar, R., & Kumar, T. T. A. (2016). Heterotrophic cultivation of Nannochloropsis salina for enhancing biomass and lipid production. *Biotechnology Reports, 10*, 8–16.
14. Zhang, S., et al. (2014). Isolation and identification by 18S rDNA sequence of high lipid potential microalgal species for fuel production in Hainan Dao. *Biomass and Bioenergy, 66*, 197–203.
15. Axelsson, M., & Gentili, F. (2014). A single-step method for rapid extraction of total lipids from green microalgae. *Plos One, 9*(2), 1–6.
16. Kesaano, M., et al. (2015). Dissolved inorganic carbon enhanced growth, nutrient uptake, and lipid accumulation in wastewater grown microalgal biofilms. *Bioresource Technology, 180*, 7–15.
17. Clud, R. N., Ramsing, N. B., & Revsbech, N. P. (1992). Photosynthesis and photosynthesiscoupled respiration in natural biofilms quantified with oxygen microsensors. *Journal of Phycology, 28*, 51–60.
18. Jena, U., Das, K. C., & Kastner, J. R. (2012). Comparison of the effects of Na2CO3, Ca3(PO4)2, and NiO catalysts on the thermochemical liquefaction of microalga Spirulina platensis. *Applied Energy, 98*, 368–375.
19. Zhang, S., et al. (2014). The research progress of the factors on the biomass and lipid accumulation of microalgae. *Food and Fermestations Industry, 40*(3), 169–175 [In Chinese].