Bioresource Technology 100 (2009) 833–838

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Lipid accumulation and CO_2 utilization of *Nannochloropsis oculata* in response to CO_2 aeration

Sheng-Yi Chiu [a], Chien-Ya Kao [a], Ming-Ta Tsai [a], Seow-Chin Ong [a], Chiun-Hsun Chen [b], Chih-Sheng Lin [a,*]

[a] Department of Biological Science and Technology, National Chiao Tung University, No. 75 Po-Ai Street, Hsinchu 30068, Taiwan
[b] Department of Mechanical Engineering, National Chiao Tung University, Hsinchu 30068, Taiwan

ARTICLE INFO

Article history:
Received 29 March 2008
Received in revised form 26 June 2008
Accepted 27 June 2008
Available online 22 August 2008

Keywords:
Nannochloropsis oculata
Lipid
Biomass
Carbon dioxide

ABSTRACT

In order to produce microalgal lipids that can be transformed to biodiesel fuel, effects of concentration of CO_2 aeration on the biomass production and lipid accumulation of *Nannochloropsis oculata* in a semicontinuous culture were investigated in this study. Lipid content of *N. oculata* cells at different growth phases was also explored. The results showed that the lipid accumulation from logarithmic phase to stationary phase of *N. oculata* NCTU-3 was significantly increased from 30.8% to 50.4%. In the microalgal cultures aerated with 2%, 5%, 10% and 15% CO_2, the maximal biomass and lipid productivity in the semicontinuous system were 0.480 and 0.142 g L^{-1} d^{-1} with 2% CO_2 aeration, respectively. Even the *N. oculata* NCTU-3 cultured in the semicontinuous system aerated with 15% CO_2, the biomass and lipid productivity could reach to 0.372 and 0.084 g L^{-1} d^{-1}, respectively. In the comparison of productive efficiencies, the semicontinuous system was operated with two culture approaches over 12 d. The biomass and lipid productivity of *N. oculata* NCTU-3 were 0.497 and 0.151 g L^{-1} d^{-1} in one-day replacement (half broth was replaced each day), and were 0.296 and 0.121 g L^{-1} d^{-1} in three-day replacement (three fifth broth was replaced every 3 d), respectively. To optimize the condition for long-term biomass and lipid yield from *N. oculata* NCTU-3, this microalga was suggested to grow in the semicontinuous system aerated with 2% CO_2 and operated by one-day replacement.

1. Introduction

Greenhouse gases are accumulating dramatically in Earth's atmosphere as a result of human activities and industrialization. In addition, the increasing concentration of greenhouse gases causes serious global warming increasing the temperatures of the surface air and subsurface ocean. Carbon dioxide (CO_2) is the main greenhouse gas. Many attempts including physical and chemical treatments have been used to recover CO_2 from atmosphere. In biological approach, microalgae appear more photosynthetically efficient than terrestrial plants and are the candidates as efficient CO_2 fixers (Brown and Zeiler, 1993).

In recent years, the bioregenerative methods using photosynthesis by microalgal cells have been made to reduce the atmospheric CO_2 to ensure a safe and reliable living environment. As the result of mild conditions for CO_2 fixation, there is no requirement for further disposal of recovered CO_2 (Lee and Lee, 2003; Cheng et al., 2006; Jin et al., 2006). Marine microalgae are expected as a proper candidate due to their high capability for photosynthesis and easily cultured in sea water which solubilizes high amount of CO_2. The CO_2 fixation by microalgal photosynthesis and biomass conversion into liquid fuel is considered a simple and appropriate process for CO_2 circulation on Earth (Takagi et al., 2000).

Lipids from microalgae are chemically similar to common vegetable oils and have been suggested being a high potential source of biodiesel (Dunahay et al., 1996; Chisti, 2007). Microalgal oil most accumulated as triglycerides can be transformed to biodiesel (Lee et al., 1998; Zhang et al., 2003). The biodiesel compared with fossil-driven diesel, that is renewable, biodegradable, and low pollutant produced (Vicente et al., 2004). The advantages of biodiesel from microalgae are that microalgae are easy to culture and less area occupation for cultivation (Chisti, 2007). In addition, microalgal-based biodiesel is a potential renewable resource for displacement liquid transport fuels derived from petroleum (Chisti, 2008).

Nannochloropsis oculata is an interesting microorganism in the field of marine biotechnology because of its high lipid content. Many microalgae can accumulate lipids due to excess photosynthate and some species can accumulate amount of lipids under heterotroph or environment stress, such as nutrient deficiency (Takagi et al., 2000) or salt stress (Takagi et al., 2006). In this study, we investigated the effects of CO_2 concentration in airstreams on the biomass production and lipid accumulation of *N. oculata* NCTU-3 cultures. We also evaluated the efficiency of lipid productivity in

* Corresponding author. Tel.: +886 3 5131338; fax: +886 3 5729288.
E-mail address: lincs@mail.nctu.edu.tw (C.-S. Lin).

0960-8524/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2008.06.061

a semicontinuous system for *N. oculata* NCTU-3 cultures. For a long-term operation, the total biomass and lipid productivity in the semicontinuous system operated by one-day and three-day replacement were evaluated.

2. Methods

2.1. Microalgal cultures, medium, and chemicals

The microalga *N. oculata* NCTU-3 was originally obtained from the collection of Taiwan Fisheries Research Institute (Tung-Kang, Taiwan) and screened for its potential ability of growth and biomass production at National Chiao Tung University, Taiwan (data not shown). The *N. oculata* NCTU-3 cells were grown in the modified f/2 medium in artificial sea water which has the following composition (per liter): 29.23 g NaCl, 1.105 g KCl, 11.09 g $MgSO_4{\cdot}7H_2O$, 1.21 g tris-base, 1.83 g $CaCl_2{\cdot}2H_2O$, 0.25 g $NaHCO_3$, and 3.0 mL of trace elemental solution. The trace elemental solution (per liter) includes 75 g $NaNO_3$, 5 g $NaH_2PO_4{\cdot}H_2O$, 4.36 g $Na_2{\cdot}EDTA$, 3.16 g $FeCl_3{\cdot}6H_2O$, 180 mg $MnCl_2{\cdot}4H_2O$, 10 mg $CoCl_2{\cdot}6H_2O$, 10 mg $CuSO_4{\cdot}5H_2O$, 23 mg $ZnSO_4{\cdot}7H_2O$, 6 mg Na_2MoO_4, 100 mg vitamin B_1, 0.5 mg vitamin B_{12} and 0.5 mg biotin.

2.2. Preparation of microalgal inoculum

A stock culture of *N. oculata* NCTU-3 (approximately 1×10^5 cells mL^{-1}) was cultured in an Erlenmeyer flask with 800 mL working volume of modified f/2 medium under 26 ± 1 °C and 300 μmol m^{-2} s^{-1}. After 6 d culture, the microalgal cells pelleted by centrifugation at 3000 × *g* for 5 min were resuspended with 50 mL fresh medium and separated for further experiments. Light intensity was measured from the light-attached surface of the photobioreactor using a Basic Quantum Meter (Spectrum Technologies, Inc., Plainfield, IL, USA).

2.3. Experimental system with photobioreactor

The *N. oculata* NCTU-3 was cultured in a cylindrical glass photobioreactor (30 cm length, 7 cm diameter) with 800 mL of working volume placed at 26 ± 1 °C under continuous, cool white, fluorescent lights. The setup of photobioreactor for microalgal culture system was described in the previous research (Chiu et al., 2008). Light intensity was approximately 300 μmol m^{-2} s^{-1} at the surface of photobioreactor. Gas provided as different concentrations of CO_2 mixed with ambient air was prepared with a volumetric percentage of CO_2 and filtered (0.22 μm) to give CO_2 concentrations of 2%, 5%, 10%, or 15%. The microalgal cultures were aerated continuously with gas provided via bubbling from the bottom of reactor with an aeration rate of 200 mL min^{-1} (i.e., 0.25 vvm, volume gas per volume broth per min). A pre-cultured *N. oculata* NCTU-3 was inoculated in cylindrical glass photobioreactor in 800 mL culture volume at an initial biomass concentration (calculated dried weight of microalgal cells per liter, g L^{-1}) of 0.01 g L^{-1} (approximate 7×10^5 cells mL^{-1}) as a batch culture. Different concentrations of CO_2 aeration were mixed with air and pure CO_2, and adjusted by gas flow meter (Dwyer Instruments, Inc., Michigan, IN, USA) to give a flow rate of 0.25 vvm.

2.4. Microalgal cell counting and dry weight

A direct microscopic count was performed with Brightline Hemocytometer (BOECO, Hamburg, Germany) and a Nikon Eclipse TS100 inverted metallurgical microscope (Nikon Corporation, Tokyo, Japan). Cell density (cells mL^{-1}) was measured by an Ultrospec 3300 pro UV/Visible spectrophotometer (Amersham Biosciences, Cambridge, UK) at the absorbance of 682 nm (A_{682}). Each sample was diluted to give an absorbance in the range 0.1–1.0 if optical density was greater than 1.0.

Microalgal dry weight per liter (g L^{-1}) was measured according to the method previously reported (American Public Health Association, 1998). Microalgal cells were collected by centrifugation and washed twice with deionized water. Microalgal pellet was dried at 105 °C for 16 h for dry weight measurement (Takagi et al., 2006).

2.5. Measurement of growth rate

A regression equation of the cell density and dry weight per liter of culture was obtained by a spectrophotometric method (Chiu et al., 2008). Specific growth rate (μ d^{-1}) was calculated as follows:

$$\mu = \frac{\ln(W_f/W_o)}{\Delta t}$$

where W_f and W_0 were the final and initial biomass concentration, respectively. $\triangle t$ was the cultivation time in day (Ono and Cuello, 2007).

2.6. Chemical analyses

Sample pH was directly determined with an ISFET pH meter KS723 (Shindengen Electric Mfg Co. Ltd., Tokyo, Japan).

Determination of nitrate content in broth was followed by the spectrometric method reported by Collos et al. (1999). Broth from microalgal cultures was collected and centrifuged at 3000 × *g* for 5 min. The absorbance of supernatant was measured at 220 nm. A standard curve was determined from authentic sodium nitrate at concentrations from 0 to 440 μM.

The CO_2 concentration in airstreams, $CO_{2(g)}$, was measured using a Guardian Plus Infra-Red CO_2 Monitor D-500 (Edinburgh Instruments Ltd, Livingston, UK).

Lipid extraction was according to the modified method reported by Takagi et al. (2006). The microalgal cells were obtained by centrifugation at 3000 × *g* for 15 min. Cells were washed with deionized water twice, and the dried biomass was obtained by lyophilization. A sample (30 mg) was mixed with methanol/chloroform solution (2:1, v/v) and sonicated for 1 h. After precipitation of mixture with methanol/chloroform solution, chloroform and 1% NaCl solution were then added to give a ratio of methanol, chloroform, and water of 2:2:1. The mixture was centrifuged at 1000 × *g* for 10 min and the chloroform phase was recovered. Finally, chloroform was removed under vacuum in a rotary evaporator and the remainder was weighed as lipid.

2.7. Measurement of lipid content by fluorescent spectrometry

For fast determination of lipid content, a fluorescent spectrometric method was applied. In the method, the microalgal cells were stained with Nile Red (Sigma, St. Louis, MO, USA) followed the protocol reported by de la Jara et al. (2003). In brief, 1 mL of 1 × 10^6 cells suspension was added 50 μL of Nile Red in acetone working solution as a concentration of 0.1 mg mL^{-1} for lipid staining. The mixture was gently inverted for mixing and incubated at 37 °C in darkness for 10 min. In the detection, the fluorometer with a 480 nm excitation filter and a 580 nm emission filter were used. Non-stained cells were used as an auto-florescence control. The relative florescence intensity of Nile Red was calculated as florescence intensity of Nile Red stained subtracted auto-florescence intensity signal (Lee et al., 1998; Liu et al., 2008). The following equation of the correlation curve indicated fluorescent intensity of Nile Red staining *vs.* lipid content measured by gravimetric method.

$y = 1.680x + 5.827$ $R^2 = 0.994$ ($p < 0.001$)

The value y is total lipid content determined by gravimetric method. The value x is the relative arbitrary unit obtained Nile Red fluorescent spectrometric method.

2.8. Setup of semicontinuous culture system

Before the *N. oculata* NCTU-3 cultures applied to the semicontinuous system aerated with various CO_2 concentrations, the microalga was grown in a batch culture and aerated with air. When the cell density in the batch culture reached to about 1×10^7 cells mL^{-1}, the culture was changed into the aeration of 2% CO_2. After 4–6 d cultivation aerated with 2% CO_2, cell density of the culture reached up to about 1×10^8 cells mL^{-1}. The culture was then replaced half of broth with fresh medium each day and performed for 3 d. After that, the culture was also replaced half of broth with fresh medium at the forth day and aerated with 2, 5, 10, and 15% CO_2. After 4 d culture, the sampling time was at 6, 12, and 24 h everyday and the culture was replaced half of broth with fresh medium daily.

2.9. Statistics

All values are expressed as mean ± standard deviation (SD). A Student's t test was used to evaluate differences between groups of discrete variables. A value of $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Growth of N. oculata NCTU-3 aerated with different CO_2 concentration

Effect of CO_2 concentration in airstream on the growth of *N. oculata* NCTU-3 was investigated in a batch culture incubated at 26 ± 1 °C and 300 μmol m^{-2} s^{-1}. The initial biomass inoculum was 0.01 g L^{-1} (about 7×10^5 cells mL^{-1}) and the cultures were aerated with air (CO_2 concentration is approximate 0.03%), 2%, 5%, 10%, and 15% CO_2. The cultures were sampled at an 8-h interval. The specific growth rate was calculated from the cultures in each experiment. Fig. 1 shows the microalgal growth aerated with different CO_2 concentrations. After 6–8 d, the growth of air and 2% CO_2 aerated cultures were reached a plateau stage and the biomass concentration of *N. oculata* NCTU-3 were 0.268 ± 0.022 and 1.277 ± 0.043 g L^{-1}, respectively. Whereas, the growth of microalga aerated with 5%, 10%, and 15% CO_2 were completely inhibited.

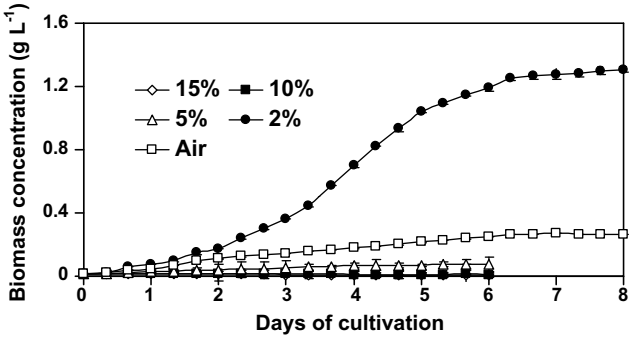

Fig. 1. Effect of the concentrations of CO_2 aeration on the growth of *N. oculata* NCTU-3. In the cultures, approximate 0.01 g L^{-1} of microalgal cells was inoculated and cultivated under air, 2%, 5%, 10%, and 15% CO_2 aeration. All experiments were carried out in triplicate. The cultures were illuminated at 300 μmol m^{-2} s^{-1} and bubbled with a flow rate of 0.25 vvm airstream at 26 ± 1 °C.

The specific growth rate in the air and 2% CO_2 aerated cultures were 0.194 d^{-1} and 0.571 d^{-1}, respectively. The culture aerated with 2% CO_2 showed an optimal growth potential. When the *N. oculata* NCTU-3 culture aerated with 2% CO_2, not only the biomass was greatly produced but also the specific growth rate was enhanced compared with those in the culture aerated with air. This result was confirmed by Hu and Gao (2003). They indicated that microalga, *Nannochloropsis* sp., grew best in an enriched CO_2 aeration compared with air aeration. It may due to enough carbon sources for microalgal growth without carbon source limitation. The significant inhibition of high CO_2 aeration, 5–15%, was also confirmed by the reports that the concentration of CO_2 aeration above 5% could be harmful to microalgal cells and inhibit the microalgal growth (Silva and Pirt, 1984; Sung et al., 1999; Chang and Yang, 2003; de Morais and Costa, 2007a,b).

3.2. Lipid content of microalga at different growth phases

The microalgal cells from logarithmic, early stationary phase and stationary phase were collected to measure lipid content and supernatant from the collected samples was also obtained for determining the nitrate content in broth. The result showed that the lipid accumulation in microalgal cells was associated with growth phases. The lipid content of *N. oculata* NCTU-3 cells at logarithmic, early stationary phase and stationary phase were 30.8, 39.7, and 50.4%, respectively. This result indicated that lipid accumulation increases as *N. oculata* NCTU-3 approaches into stationary phase. The decreased nitrate content in the broth of *N. oculata* NCTU-3 culture from logarithmic phase to stationary phase was found (data not shown). It is hinted that the *N. oculata* NCTU-3 culture from logarithmic phase to stationary phase would accompany with the nitrate depletion. Roessler (1988) reported that the nutrient deficiency induced an increase in the rate of lipid synthesis in a diatom, *Cyclotella cryptica*, and resulted in lipid accumulation in the cells. It is also indicated that lipid accumulation is related to nitrogen depletion as a nutrient deficiency (Roessler et al., 1994; Takagi et al., 2000). The result is confirmed by these previous reports that the microalga, *N. oculata* NCTU-3, shows the metabolic effect of nitrogen depletion related to the increasing lipid accumulation.

3.3. Effect of CO_2 concentration on cell growth in semicontinuous cultures

For the study of lipid accumulation in response to higher CO_2 aeration, the microalgal cells pre-adapted to CO_2 were applied. In the experiment, *N. oculata* NCTU-3 cells were pre-adapted to 2% CO_2 before the microalga was inoculated into the semicontinuous cultures. Moreover, a high density (approximate 0.4 g L^{-1}) of inoculum was applied in the cultures. The semicontinuous system was operated for 8 d and the growth was stable by each day replacement and was maintained at logarithmic growth potential. The results showed that the growth profiles of *N. oculata* NCTU-3 aerated with 2%, 5%, 10%, and 15% CO_2 in the semicontinuous system were similar (Fig. 2). The average specific growth rate and maximum cell density (i.e., biomass concentration) were from 0.683 to 0.733 d^{-1} and from 0.745 to 0.928 g L^{-1} at different concentrations of CO_2 aerated cultures, respectively (Fig. 2). High CO_2 aeration (5–15%) may be a harmful effect on the microalgal cells growth as shown in Fig. 1. But increasing the inoculated cell density and pre-adapting to 2% CO_2 culture could promote the growth capacity of microalga in the cultures aerated with higher CO_2 concentrations. The results indicated that increasing cell density and pre-adapting microalgal cells in an adequate CO_2 concentration is an alternative approach for the application of high CO_2 aeration without drastic harmful effects on microalgal cell growth.

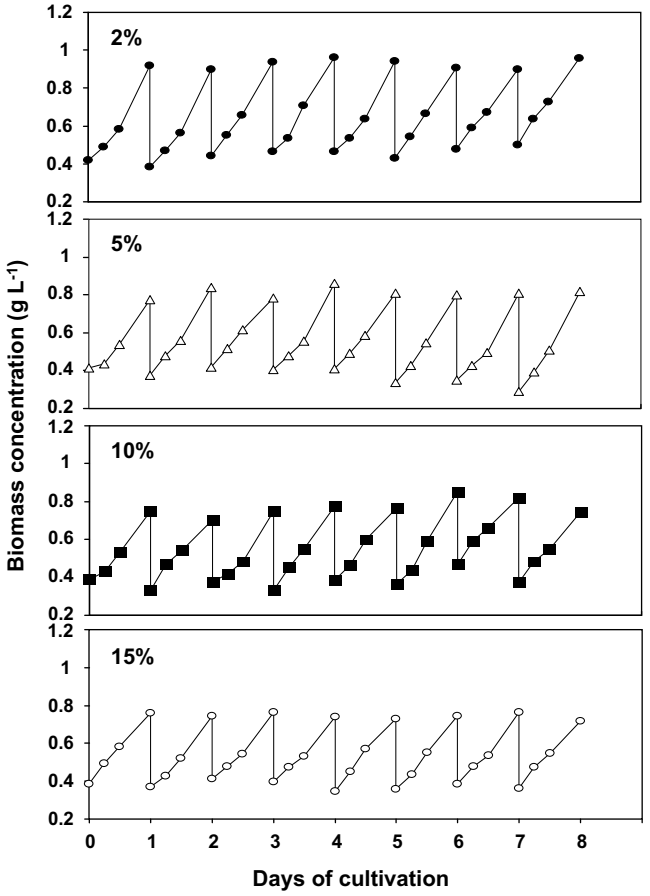

Fig. 2. Growth profiles of *N. oculata* NCTU-3 cultured in the semicontinuous system aerated with 2%, 5%, 10%, and 15% CO_2. In the cultures, approximate 0.4 g L^{-1} of microalgal cells was inoculated and cultivated under an illumination at 300 μmol m^{-2} s^{-1} and bubbled with a flow rate of 0.25 vvm airstreams at 26 ± 1 °C for 8 d. Amount of 50% of cultured broth was replaced with the fresh modified f/2 medium at interval of 24 h.

3.4. Biomass and lipid productivity in semicontinuous culture

In the semicontinuous culture system, the *N. oculata* NCTU-3 cells were collected at the time before culture replaced each day for determination of biomass and lipid productivity. Table 1 summarizes the biomass and lipid productivity of *N. oculata* NCTU-3 cultures aerated with various CO_2 concentrations. As increasing CO_2 concentration of aeration from 2 to 15%, both biomass and lipid productivity were generally decreasing (Table 1). It is reported that the lipid content was increasing associated with the increasing CO_2 concentration of aeration in *Chlorella fusca* and *Phaeodactylum tricornutum* cultures (Dickson et al., 1969; Yongmanitchai and Ward, 1991). The data in this study showed an inverted result may due to different microalgal species, growth condition, and medium content (Hu and Gao, 2006). Our results showed that the pH of cultures with 2%, 5%, 10%, and 15% CO_2 aeration was maintained at pH 7.8, 7.7, 7.3, and 7.0, respectively. Yung and Mudd (1966) reported that the carbon assimilation of lipid synthesis was decreased with decrease of pH. This may be possibly because the higher pH having higher available bicarbonate for carboxylation of lipid synthesis. This inference supports the result that lipid accumulated in *N. oculata* NCTU-3 may be mainly affected by pH and lipid content of the microalgal cultures was decreased with decrease of broth pH.

Table 1
Daily recovery of biomass and lipid of *N. oculata* NCTU-3 cultured in the semicontinuous system aerated with different CO_2 concentrations

CO_2 aeration	Total biomass productivity (cell dry weight, g L^{-1} d^{-1})	Total lipid productivity (g $L^{-1}d^{-1}$)	Percentage of lipid content (%)
2%	0.480 ± 0.029	0.142 ± 0.049	29.7 ± 2.0
5%	0.441 ± 0.044	0.113 ± 0.035	26.2 ± 1.9
10%	0.398 ± 0.069	0.097 ± 0.026	24.6 ± 1.7
15%	0.372 ± 0.022	0.084 ± 0.021	22.7 ± 1.9

The semicontinuous cultures were performed for 8 d and a half of broth was replaced each day. The culture volume in photobioreactor is 800 mL. Daily waste broth was 400 mL. Each data indicates the mean ± SD, which were measured daily from d-1 to d-8.

3.5. Optimal CO_2 concentration applied in semicontinuous cultures

In the semicontinuous system, *N. oculata* NCTU-3 could grow well under high CO_2 (up to 15% CO_2) aeration, shows the potential of the microalgal culture for CO_2 removal. Therefore, the CO_2 removal efficiency in the semicontinuous system cultured with *N. oculata* NCTU-3 was determined by the measurement of influent and effluent of CO_2 airstream. The method and operation was established and described in our previous study (Chiu et al., 2008).

The amount of CO_2 between influent and effluent, and CO_2 removal efficiency were recorded. The CO_2 concentrations in the effluent of 2, 5, 10 and 15% CO_2 aerated cultures were maintained at 0.9–1.1, 3.8–4.1, 8.3–8.7, and 12.9–13.2% CO_2 over 8 d cultivation, respectively. The CO_2 removal efficiency in the cultures aerated with 2%, 5%, 10%, and 15% CO_2 were 47%, 20%, 15%, and 11%, and the amount of CO_2 removal in the cultures were 0.211, 0.234, 0.350, and 0.393 g h^{-1}, respectively. The efficiency of CO_2 removal in the culture aerated with low CO_2 concentration was higher than those aerated with high CO_2 concentration (de Morais and Costa, 2007a,b; Chiu et al., 2008). The CO_2 removal efficiency in a closed photobioreactor system is dependent on microalgal species, photobioreactor, and concentration of CO_2 aeration (Cheng et al., 2006; de Morais and Costa, 2007a,b). This assumption was confirmed by the study in *Chlorella* sp., the study showed more CO_2 removal capacity but lower biomass productivity in a microalgal culture treated with low CO_2 aeration (Chiu et al., 2008). Cheng et al. (2006) demonstrated a *Chlorella vulgaris* cultured membrane-photobioreactor obtained a maximum rate of microalgal CO_2 fixation at 2% of CO_2 aeration. Different photobioreactors could also bring different gaseous transfer efficiency, light harvesting efficiency, and mix efficiency (Carvalho et al., 2006). In the present study, amount of CO_2 removal was 0.211, 0.234, 0.350, and 0.393 g h^{-1}. However, total biomass productivity was 0.480, 0.441, 0.398, and 0.372 g L^{-1} d^{-1} in the cultures with 2%, 5%, 10%, and 15% CO_2 aeration, respectively. The microalgal cultures aerated with higher CO_2 showed lower biomass productivity. This result may due to that when the microalgal cells aerated with higher CO_2, most of the CO_2 is consumed for metabolic activity and less of CO_2 is fixed to become cellular components, i.e., biomass. The higher metabolic activity may contribute to the microalgal cells to subsist on higher CO_2 stress. The results showed that the maximal CO_2 utilization efficiency was from the cultures aerated with 2% CO_2 airstreams. It is also indicated that the optimal concentration of CO_2 aeration in the system based on the efficiency of biomass and lipid productivity was 2% CO_2.

3.6. Comparison of productive efficiencies in semicontinuous system with different culture approaches

The comparison of productive efficiencies in the semicontinuous systems in which the culture broth were replaced at an interval of 24 h (one-day replacement) or 72 h (three-day replacement)

was performed. In the systems, approximate 0.4 g L^{-1} of *N. oculata* NCTU-3 cells was inoculated and the microalgal cultures were replaced half (for one-day replacement) or three fifth (for three-day replacement) of broth with fresh medium in the semicontinuous system after the cultures was aerated with 2% CO_2. Fig. 3 shows the stable growth profiles of *N. oculata* NCTU-3 cultured with one-day and three-day replacement. In the cultures, the broth was replaced at logarithmic phase in one-day replacement and replaced before the cells reached to early stationary phase in three-day replacement. The growth profiles of both one-day and three-day replacement cultures were stable over 12 d cultivation. Table 2 shows the biomass and lipid productivity of *N. oculata* NCTU-3 cells in the semicontinuous culture system with one-day and three-day replacement. The total volume of replaced broth was 4800 mL in one-day replacement and only 1,920 mL in three-day replacement over 12 d. The lipid content of microalga in the three-day replacement was significantly higher than that in the one-day replacement culture (41.2% *vs.* 30.7%). However, the total biomass and total lipid yield in the three-day replacement culture were only 24% and 32% compared with those in the one-day replacement culture, respectively. It means that the culture broth being daily replacement could be more efficient not only for biomass production but also for lipid yield. In conclusion, the total biomass and lipid yield in the semicontinuous culture operated by one-day replacement were more efficient compared with those in three-day replacement, although the *N. oculata* NCTU-3 cells in the three-day replacement could increase lipid accumulation because of nutrition-deficient effect.

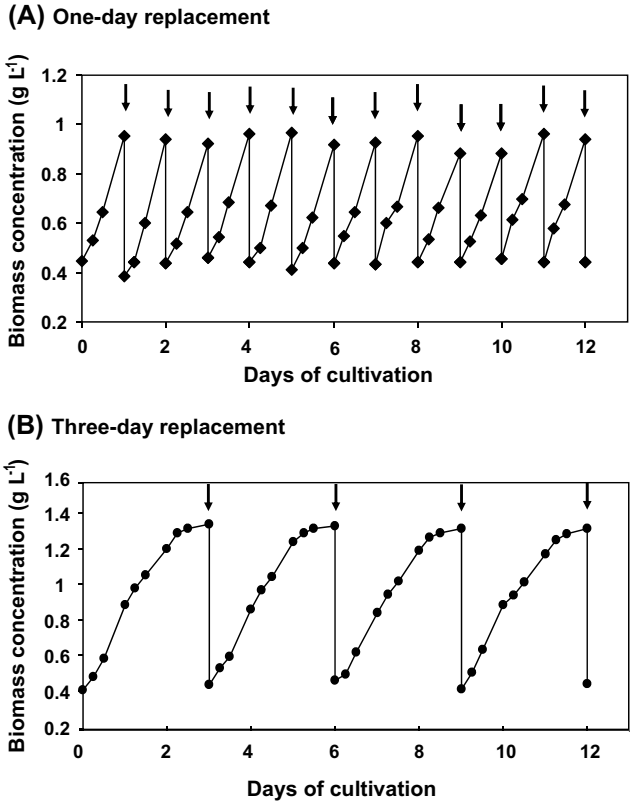

Fig. 3. Growth profiles of *N. oculata* NCTU-3 cultured in the semicontinuous system with 2% CO_2 aeration and operated by one-day and three-day replacements. In the cultures, approximate 0.4 g L^{-1} of microalgal cells was inoculated and cultivated under an illumination at 300 μmol m^{-2} s^{-1} and bubbled with a flow rate of 0.25 vvm airstreams at 26 ± 1 °C. The cultivations were continuously operated for 12 d. Amount of half and three fifth of cultured broth was replaced with the fresh modified f/2 medium at interval of 24 h (one-day replacement; half broth was replaced each day) and 72 h (three-day replacement; three fifth broth was replaced every 3 d), respectively. The arrows indicate the time when the cultured broth was removed and fresh medium was added.

Table 2
Biomass and lipid productivity of *N. oculata* NCTU-3 cultured in the semicontinuous system aerated with 2% CO_2 under the treatments of one-day and three-day replacement

Culture	Total biomass productivity (cell dry weight, g L^{-1} d^{-1})	Total lipid productivity (g L^{-1} d^{-1})	Percentage of lipid content (%)
One-day replacement	0.497 ± 0.032	0.151 ± 0.021	30.7 ± 2.4
Three-day replacement	0.296 ± 0.009	0.121 ± 0.035	41.2 ± 1.9

The semicontinuous cultures were performed for 12 d. The cultural broth was replaced by half (for one-day replacement) or three fifth (for three-day replacement) with fresh medium at interval of 24 and 72 h, respectively. The total biomass and lipid productivity were measured from the total replaced broth divided by day. The total replaced broth volume was 4800 mL in one-day replacement and only 1920 mL in three-day replacement over 12 d. Each data indicates the mean ± SD.

4. Conclusions

The results showed the lipid accumulation of *N. oculata* NCTU-3 could be increased from logarithmic growth phase to stationary growth phase. The *N. oculata* NCTU-3 pre-adapting to 2% CO_2 cultured in a semicontinuous system with a high cell density of inoculum could grow well in the system aerated with higher CO_2 concentration (5–15% CO_2); however, increasing biomass production and lipid accumulation would not be followed as the cultures aerated with higher CO_2. Achieving the optimal condition for a long-term biomass and lipid yield in the semicontinuous system, the microalga could be cultured with 2% CO_2 aeration in one-day replacement operation.

Acknowledgements

The work was financially supported by the Research Grants of NSC 94-2218-E-009-032, NSC 95-2218-E-009-018 and NSC 96-2218-E-009-002 from National Science Council, Taiwan. This work was also partially supported by the Ministry of Education, under Grant of MoE ATU Program, Taiwan.

References

American Public Health Association, 1998. Methods for biomass production. In: Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Baltimore, MD, USA.
Brown, L.M., Zeiler, K.G., 1993. Aquatic biomass and carbon dioxide trapping. Energy Conv. Manag. 34, 1005–1013.
Carvalho, A.P., Meireles, L.A., Malcata, F.X., 2006. Microalgae reactors: a review of enclosed system designs and performances. Biotechnol. Prog. 22, 1490–1506.
Chang, E.H., Yang, S.S., 2003. Some characteristics of microalgae isolated in Taiwan for biofixation of carbon dioxide. Bot. Bul. Acad. Sin. 44, 43–52.
Cheng, L., Zhang, L., Chen, H., Gao, C., 2006. Carbon dioxide removal from air by microalgae cultured in a membrane-photobioreactor. Sep. Purif. Technol. 50, 324–329.
Chisti, Y., 2007. Biodiesel from microalgae. Biotechnol. Adv. 25, 294–306.
Chisti, Y., 2008. Biodiesel from microalgae beats bioethanol. Trends Biotechnol. 26, 126–131.
Chiu, S.Y., Kao, C.Y., Chen, C.H., Kuan, T.C., Ong, S.C., Lin, C.S., 2008. Reduction of CO_2 by a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor. Bioresour. Technol. 99, 3389–3396.
Collos, Y., Mornet, F., Sciandra, A., Waser, N., Larson, A., Harrison, P.J., 1999. An optical method for the rapid measurement of micromolar levels of nitrate in marine phytoplankton cultures. J. Appl. Phycol. 11, 179–184.
de la Jara, A., Mendoza, H., Martel, A., Molina, C., Nordström, L., de la Rosa, V., Díaz, R., 2003. Flow cytometric determination of lipid content in a marine dinoflagellate, *Crypthecodinium cohnii*. J. Appl. Phycol. 15, 433–438.
de Morais, M.G., Costa, J.A.V., 2007a. Biofixation of carbon dioxide by *Spirulina* sp and *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. J. Biotechnol. 129, 439–445.

de Morais, M.G., Costa, J.A.V., 2007b. Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. Energy Conv. Manag. 48, 2169–2173.

Dickson, L.G., Galloway, R.A., Patterson, G.W., 1969. Environmentally-induced changes in the fatty acids of *Chlorella*. Plant Physiol. 44, 1413–1416.

Dunahay, T.G., Jarvis, E.E., Dais, S.S., Roessler, P.G., 1996. Manipulation of microalgae lipid production using genetic engineering. Appl. Biochem. Biotechnol., 223–231.

Hu, H., Gao, K., 2003. Optimization of growth and fatty acid composition of a unicellular marine picoplankton, *Nannochloropsis* sp., with enriched carbon sources. Biotechnol. Lett. 25, 421–425.

Hu, H., Gao, K., 2006. Response of growth and fatty acid compositions of *Nannochloropsis* sp. to environmental factors under elevated CO_2 concentration. Biotechnol. Lett. 28, 987–992.

Jin, H.F., Lim, B.R., Lee, K., 2006. Influence of nitrate feeding on carbon dioxide fixation by microalgae. J. Environ. Sci. Health Part A-Toxic/Hazard. Subst. Environ. Eng. 41, 2813–2824.

Lee, J.S., Lee, J.P., 2003. Review of advances in biological CO_2 mitigation technology. Biotechnol. Bioprocess Eng. 8, 354–359.

Lee, S.J., Yoon, B.D., Oh, H.M., 1998. Rapid method for the determination of lipid from the green alga *Botryococcus braunii*. Biotechnol. Tech. 12, 553–556.

Liu, Z.Y., Wang, G.C., Zhou, B.C., 2008. Effect of iron on growth and lipid accumulation in *Chlorella vulgaris*. Bioresour. Technol. 99, 4717–4722.

Ono, E., Cuello, J.L., 2007. Carbon dioxide mitigation using thermophilic cyanobacteria. Biosyst. Eng. 96, 129–134.

Roessler, P.G., 1988. Changes in the activities of various lipid and carbohydrate biosynthetic enzymes in the diatom *Cyclotella cryptica* in response to silicon deficiency. Arch. Biochem. Biophys. 267, 521–528.

Roessler, P.G., Bleibaum, J.L., Thompson, G.A., Ohlrogge, J.B., 1994. Characteristics of the gene that encodes acetyl-CoA carboxylase in the diatom *Cyclotella cryptica*. Ann. N. Y. Acad. Sci. 721, 250–256.

Silva, H.J., Pirt, S.J., 1984. Carbon dioxide inhibition of photosynthetic growth of *Chlorella*. J. Gen. Microbiol. 130, 2833–2838.

Sung, K.D., Lee, J.S., Shin, C.S., Park, S.C., Choi, M.J., 1999. CO_2 fixation by *Chlorella* sp. KR-1 and its cultural characteristics. Bioresour. Technol. 68, 269–273.

Takagi, M., Watanabe, K., Yamaberi, K., Yoshida, T., 2000. Limited feeding of potassium nitrate for intracellular lipid and triglyceride accumulation of *Nannochloris* sp UTEX LB1999. Appl. Microbiol. Biotechnol. 54, 112–117.

Takagi, M., Karseno, Yoshida, T., 2006. Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride in marine microalgae *Dunaliella* cells. J. Biosci. Bioeng. 101, 223–226.

Vicente, G., Martínez, M., Aracil, J., 2004. Integrated biodiesel production: a comparison of different homogeneous catalysts systems. Bioresour. Technol 92, 297–305.

Yongmanitchai, W., Ward, O.P., 1991. Growth of and omega-3 fatty acid production by *Phaeodactylum tricornutum* under different culture conditions. Appl. Environ. Microbiol. 57, 419–425.

Yung, K.H., Mudd, J.B., 1966. Lipid synthesis in the presence of nitrogenous compounds in *Chlorella pyrenoidosa*. Plant Physiol. 41, 506–509.

Zhang, Y., Dubé, M.A., McLean, D.D., Kates, M., 2003. Biodiesel production from waste cooking oil. 1. Process design and technological assessment. Bioresour. Technol. 89, 1–16.