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Reduction of CO_2 by a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor

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Abstract

The microalga incorporated photobioreactor is a highly efficient biological system for converting CO_2 into biomass. Using microalgal photobioreactor as CO_2 mitigation system is a practical approach for elimination of waste gas from the CO_2 emission. In this study, the marine microalga *Chlorella* sp. was cultured in a photobioreactor to assess biomass, lipid productivity and CO_2 reduction. We also determined the effects of cell density and CO_2 concentration on the growth of *Chlorella* sp. During an 8-day interval cultures in the semicontinuous cultivation, the specific growth rate and biomass of *Chlorella* sp. cultures in the conditions aerated 2–15% CO_2 were 0.58–0.66 d^{-1} and 0.76–0.87 g L^{-1}, respectively. At CO_2 concentrations of 2%, 5%, 10% and 15%, the rate of CO_2 reduction was 0.261, 0.316, 0.466 and 0.573 g h^{-1}, and efficiency of CO_2 removal was 58%, 27%, 20% and 16%, respectively. The efficiency of CO_2 removal was similar in the single photobioreactor and in the six-parallel photobioreactor. However, CO_2 reduction, production of biomass, and production of lipid were six times greater in the six-parallel photobioreactor than those in the single photobioreactor. In conclusion, inhibition of microalgal growth cultured in the system with high CO_2 (10–15%) aeration could be overcome via a high-density culture of microalgal inoculum that was adapted to 2% CO_2. Moreover, biological reduction of CO_2 in the established system could be parallely increased using the photobioreactor consisting of multiple units.

Keywords: Microalga; *Chlorella* sp.; Carbon dioxide; Photobioreactor; Biomass

1. Introduction

Global warming induced by increasing concentrations of greenhouse gases in the atmosphere is of great concern. Carbon dioxide (CO_2) is the principal greenhouse gas and its concentrations have increased rapidly since the onset of industrialization (Ramanathan, 1988). In 1997, 7.4 billion tons of CO_2 were released into the atmosphere from anthropogenic sources; by the year 2100, this number will increase to 26 billion tons (DOE, 1999). During the last two decades, many attempts have been made to reduce atmospheric CO_2, for example by the use of renewable energy sources or by terrestrial sequestration of carbon (IPCC, 2001).

One of the most understudied methods of CO_2 reduction is the use of microalgae that convert CO_2 from a point source into biomass. Microalgae use CO_2 efficiently because they can grow rapidly and can be readily incorporated into engineered systems, such as photobioreactors (Carvalho et al., 2006; Lee and Lee, 2003; Suh and Lee, 2003).

The CO_2 fixation rate is related directly to light utilization efficiency and to cell density of microalgae. Microalgal CO_2 fixation involves photoautotrophic growth in which anthropogenically derived CO_2 may be used as a carbon source. Therefore, biomass measurements or growth rate evaluations are critical in assessing the potential of a microalgal culture system for directly removing CO_2 (Cheng

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et al., 2006; Costa et al., 2004; Jin et al., 2006). Effects of the concentration of CO_2 in airstreams on growth of microalgae in culture have been evaluated in several studies (Chae et al., 2006; de Morais and Costa, 2007; Keffer and Kleinheinz, 2002; Kurano et al., 1995; Yoshihara et al., 1996). However, these effects remain to be largely understood. Microalgal photobioreactor can be used for CO_2 mitigation from waste gas with high concentration of CO_2 efficiently, if the effects of the CO_2 concentration in airstreams on microalgal cell growth could be well controlled.

In the present study, effects of initial cell density and CO_2 concentration in airstreams on growth of a *Chlorella* sp. culture were investigated. The efficiency of CO_2 reduction and biomass as well as lipid productivity in a semicontinuous photobioreactor system were also evaluated.

2. Methods

2.1. Microalgal cultures, medium and chemicals

A culture of *Chlorella* sp. was obtained from Taiwan Fisheries Research Institute (Tung-Kang, Taiwan). The species of *Chlorella* sp. isolated in Taiwan was unidentified. However, the partial sequence of 18S rRNA (599 bp) of the *Chlorella* sp. has been amplified and sequenced for species identification in this study. The result of sequence alignment was performed by NCBI nucleotide blast (Wu et al., 2001). The result indicates that the *Chlorella* sp. used in this study is identified as several *Chlorella* sp. strain, such as KAS001, KAS005, KAS007, KAS012, MBIC10088, MDL5-18 and SAG 211-18. The cells of *Chlorella* sp. was cultured in the modified f/2 medium in artificial sea water (per liter), including 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 metal solution (Guillard, 1975). The trace metal solution (per liter) contains 281.3 mg $NaNO_3$, 21.2 mg $NaH_2PO_4 \cdot H_2O$, 16.35 mg $Na_2 \cdot EDTA$, 11.8 mg $FeCl_3 \cdot 6H_2O$, 675 μg $MnCl_2 \cdot 4H_2O$, 37.5 μg $CoCl_2 \cdot 6H_2O$, 37.5 μg $CuSO_4 \cdot 5H_2O$, 82.5 μg $ZnSO_4 \cdot 7H_2O$, 22.5 μg Na_2MoO_4, 0.375 mg vitamin B_1, 0.188 μg vitamin B_{12} and 0.188 μg biotin.

2.2. Experimental system with photobioreactor

The microalga was incubated in a cylindrical glass reactor (30 cm length, 7 cm diameter) with 800 mL of working volume. The photobioreactor for microalgal culture and CO_2 reduction is presented schematically in Fig. 1. Cultures were placed on a bench at 26 ± 1 °C under continuous, cool white, fluorescent light. Light intensity was approximately 300 μmol m^{-2} s^{-1} at the surface of the photobioreactor. Filtered (0.22 μm) ambient air was mixed with CO_2 to give concentrations of CO_2 of 2%, 5%, 10%, and 15%. Cultures in the photobioreactor were aerated continuously with one of the mixtures at a rate of 200 mL min^{-1} (i.e., 0.25 vvm, volume gas per volume media per min).

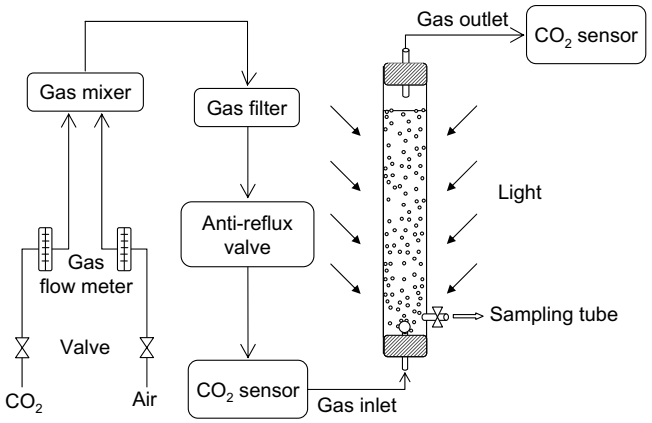

Fig. 1. Schematic diagram of the photobioreactor for the experiments on CO_2 reduction for batch and semicontinuous microalgal cultures. The airstreams with different CO_2 concentrations was adjusted by individual gas flow meter and determined via CO_2 sensor. External illumination of light intensity was provided with 300 μmol m^{-2} s^{-1} by a continuous, cool white, and fluorescent light.

2.3. Preparation of the inoculum

A stock culture of *Chlorella* sp. (approximately 1×10^5 cells mL^{-1}) was incubated in an Erlenmeyer flask containing 800 mL working volume of modified f/2 medium at 26 ± 1 °C and 300 μmol m^{-2} s^{-1}. Six days after inoculation, microalgal cells were harvested by centrifugation at 3000×*g* for 5 min, after which the pelleted cells were resuspended in 50 mL modified f/2 medium. The density of cells in the culture was then measured and the cells were separated for the further experiments.

2.4. Experimental design of batch cultivation

The photobioreactor was filled with 750 mL modified f/2 medium. The medium was aerated for 24 h and then inoculated with 50 mL of precultured *Chlorella* sp. containing either 8×10^5 cells mL^{-1} (low-density) or 8×10^6 cells mL^{-1} (high-density). The cells from a 50 mL (at the density of 3.2×10^7 cells mL^{-1}) of precultured microalga, *Chlorella* sp., were subcultured into the 800 mL culture photobioreactor as low-density and the tenfold concentrated microalgae by centrifugation were subcultured into the photobioreactor as high-density culture. Air of different CO_2 concentration was produced by mixing air and pure CO_2 at 0.25 vvm. Each air/CO_2 mixture was adjusted to desired concentration of 2%, 5%, 10%, and 15% CO_2 in airstreams. Cultures were incubated for 4–8 days. Every 8 h, each culture was sampled to determine optical density, microalgal dry weight, and culture pH.

2.5. Experimental design of semicontinuous cultivation

The semicontinuous cultivation system was set up in a single photobioreactor and a system with six-parallel photobioreactor. Each unit of photobioreactor contained

800 mL cultured microalgae. The culture was started as a batch culture. Precultured microalgae were inoculated into the photobioreactor under 2% CO_2 aeration. When cell density reached about 1×10^8 cells mL^{-1} (the value of $A_{682} > 5$), half of volume of the culture broth was replaced with fresh modified f/2 medium every 24 h. In each photobioreactor, the culture was aerated with 2%, 5%, 10% and 15% CO_2 at 0.25 vvm. Before fresh medium was added, the culture broth was sampled to estimate optical density, microalgal dry weight, lipid content, and pH. The amount of CO_2 reduced from the airstreams was estimated from the difference between the CO_2 concentrations in influent and effluent airstreams of the photobioreactors.

2.6. Microalgal cell counting and dry weight

A direct microscopic count was performed on the sample of microalgal suspension using a Brightline Hemacytometer (BOECO, Hamburg, Germany) and a Nikon Eclipse TS100 inverted metallurgical microscope (Nikon Corporation, Tokyo, Japan). Cell density (cells mL^{-1}) was measured by the absorbance at 682 nm (A_{682}) in an Ultrospec 3300 pro UV/Visible spectrophotometer (Amersham Biosciences, Cambridge, UK). Each sample was diluted to give an absorbance in the range of 0.1–1.0 if the optical density was greater than 1.0. The biomass will be underestimated when the optical density is out of the linear range. Therefore, the sample was diluted to measure getting an absorbance in the range 0.1–1.0 if the optical density was greater than 1.0. Microalgal dry weight ($g\ L^{-1}$) was measured according to the method previously reported (American Public Health Association, 1998). Culture broth of samples was removed by centrifugation and washed twice with deionized water. Finally, the microalgal pellet was collected from the deionized water by centrifugation. Dry weight was measured after drying the microalgal pellet at 105 °C for 16 h (Takagi et al., 2006).

2.7. Measurement of growth rate

The optical density of microalgal cells was converted into dry weight per liter of culture by a regression equation. Biomass was calculated from microalgal dry weight produced per liter ($g\ L^{-1}$). Specific growth rate (μ, d^{-1}) was calculated from:

$$\mu = \frac{\ln(N_2 - N_1)}{t_2 - t_1}$$

where *N1* and *N2* were the biomass (g^{L-1}) on days t_1 and t_2, respectively (Guillard, 1973). In this report, we used biomass (g^{L-1}) to quantify Chlorella sp. in culture.

2.8. pH and light measurements

Sample pH was directly determined using an ISFET pH meter KS723 (Shindengen Electric Mfg. Co. Ltd, Tokyo, Japan). The pH meter was calibrated daily using pH 4 and 7 solutions. Light intensity was measured adjacent to the bioreactor at liquid level using a Basic Quantum Meter (Spectrum Technologies, Inc., Plainfield, IL).

2.9. Lipid extraction and measurement

For the lipid extraction, the microalgal cells were obtained by centrifuging a 50-mL sample of culture at 3000×*g* for 15 min. The cells were washed with deionized water twice, lyophilized, and weighed. A sample (30 mg) was precipitated in methanol/chloroform (2/1, v/v) and sonicated for 1 h. Chloroform and 1% NaCl were added to give mixture to 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 collected. Chloroform was evaporated under vacuum in a rotary evaporator to remove organic solvent. The remaining from the evaporation was weighed as lipid (Takagi et al., 2006).

2.10. Measurement of medium nitrate content

Depletion of nutrients in microalgal culture was monitored by the determination of medium nitrate content (Tonon et al., 2002). It is a simple method for investigating the adequate content of nutrients for microalgal culture in the semicontinuous cultivation. Nitrate concentration was determined according to the method reported by Collos et al. (1999). A sample collected from photobioreactor was centrifuged at 3000×*g* for 5 min. The supernatant was collected and the absorbance was measured at 220 nm. Authentic sodium nitrate at a concentration of 0–440 μM was used as a standard.

2.11. Determinations of $CO_{2(g)}$ and $CO_{2(aq)}$

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). Free CO_2 in the aqueous solution, $CO_{2(aq)}$, was measured by a HANNA Carbon Dioxide Test Kit (KI 3818; Hanna Instruments, Woonsocket, RL).

3. Results and discussion

3.1. Evaluation of cell density and biomass

Cell density and biomass were measured more easily by optical density than by direct counting of cells or by cell dry weight (Rocha et al., 2003). Therefore, relationships between optical density and cell density and optical density and cell dry weight were established by linear regression firstly (Fig. 2). Optical density precisely predicted both cell density ($R^2 = 0.997$; $p < 0.001$) and biomass ($R^2 = 0.991$; $p < 0.001$). Therefore, the values of optical density were used to calculate the related biomass of *Chlorella* sp. in each experiment according the equations established in this study.

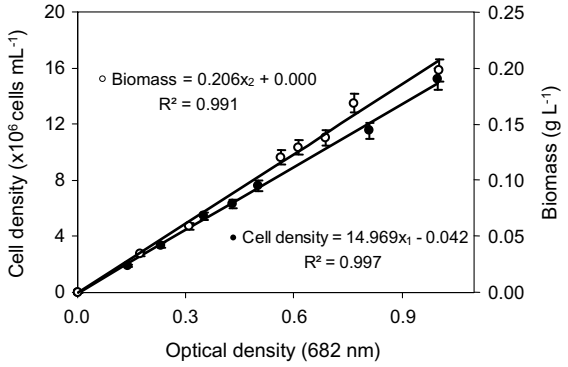

Fig. 2. Calibration curves and equations of optical density at A_{682} to the cell density (●) and the biomass (○). Left axis shows optical density vs. cell density (cells directly counted by microscope) in the *Chlorella* sp. culture. Right axis shows the optical density vs. biomass (g L^{-1}). The calibration curve, correlative equation, and R^2 value of each correlation were indicated. Each point was averaged from three independent measures.

3.2. Effect of CO_2 on microalgal culture at different cell density

To investigate the effect of CO_2 concentration on growth, *Chlorella* sp. in batch culture was incubated for 4–8 days at 26 ± 1 °C and 300 μmol m^{-2} s^{-1} and aerated with different concentrations of CO_2 at 0.25 vvm. Cultures were sampled when a stationary phase of growth was reached or a microalgal growth was significantly inhibited. Specific growth rate was calculated from the logarithmic growth phase over 1–2 days batch culture in each experiment.

As the cells grew up to plateau stage, the biomass in air, 2% and 5% CO_2 aeration with low-density cells inoculum (i.e., 8×10^5 cells mL^{-1}) were 0.537 ± 0.016 g L^{-1}, 1.211 ± 0.031 g L^{-1} and 0.062 ± 0.027 g L^{-1}, respectively. At the aeration of 2% CO_2, *Chlorella* sp. increased most rapidly at the specific growth rate of 0.492 d^{-1} and the specific growth rate markedly fell to be 0.127 d^{-1} when the cultures were aerated with 5% CO_2. The growth of *Chlorella* sp. at 10% and 15% CO_2 aeration was almost completely inhibited; therefore the specific growth rates were not available (Fig. 3a and Table 1).

In the cultures inoculated with *Chlorella* sp. at high-density (i.e., 8×10^6 cells mL^{-1}), a short lag period and steep log phase was observed when the cultures aerated with 2% and 5% CO_2 compared to those of low-density inoculum. It is worth to emphasize that the biomass and specific growth rate at 5% CO_2 aeration in high-density inoculum was 0.899 ± 0.003 g L^{-1} and 0.343 d^{-1}. The values were significantly increased as compared with those in low-density inoculum. However, the growth of *Chlorella* sp. was inhibited after 4 days of incubation under the conditions of 10% and 15% CO_2 aeration (Fig. 3b and Table 1). In the 5% CO_2 aerated cultures in high-density inoculum, the biomass production and specific growth rate were strongly enhanced. This enhancement may due to enrich-

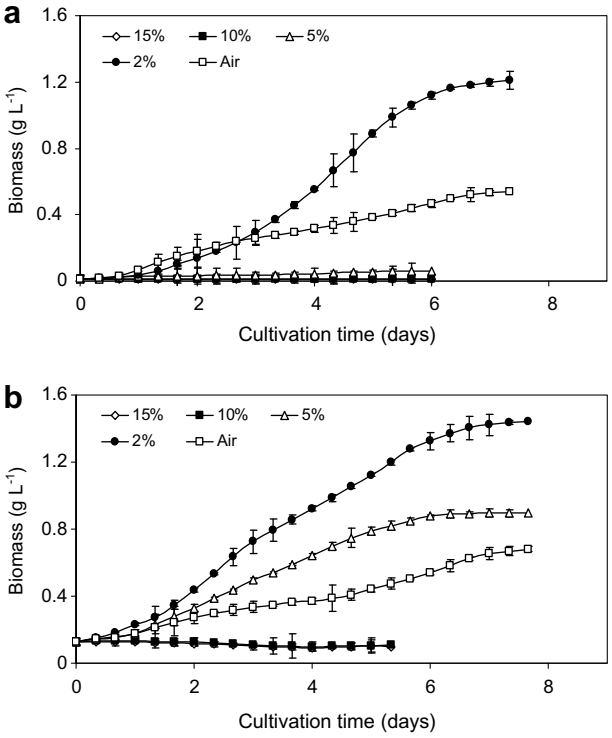

Fig. 3. Effects of different concentrations of CO_2 aeration on the growth of *Chlorella* sp. (a) shows the growth curve of *Chlorella* sp. inoculated at low-density cells (8×10^5 cells mL^{-1} in an 800-mL cultivation). (b) shows the growth curve of *Chlorella* sp. inoculated at high-density cells (8×10^6 cells mL^{-1} in an 800-mL cultivation). 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 airstreams at 26 ± 1 °C.

Table 1
The biomass production and the specific growth rate of the low- and high-density inoculums of *Chlorella* sp. growth depending on different concentrations of CO_2 aeration

CO_2 aeration	Biomass (cell dry weight, g L^{-1})[b,c]	Specific growth rate (μ, d^{-1})[d]
Low-density inoculum (8×10^6 cells mL^{-1})		
Air[a]	0.537 ± 0.016	0.230
2%	1.211 ± 0.031	0.492
5%	0.062 ± 0.027	0.127
10%	0.010 ± 0.003	–
15%	0.009 ± 0.001	–
High-density inoculum (8×10^6 cells mL^{-1})		
Air	0.682 ± 0.007	0.248
2%	1.445 ± 0.015	0.605
5%	0.899 ± 0.003	0.343
10%	0.106 ± 0.001	–
15%	0.099 ± 0.001	–

[a] CO_2 concentration in the air is around 0.03%.
[b] Biomass was measured when the cells grew up to the plateau stage in the culture.
[c] Each data indicates the mean ± SD, which were measured from three independent cultures.
[d] Specific growth rate was obtained on the exponential logarithmic growth phase by day during the cultivation. The exponential logarithmic growth phase was from 1 to 2 days batch culture with different concentrations of CO_2 aeration.

ment of available CO_2 as carbon source and the culture condition under the 5% CO_2 aeration would not be significantly changed in the culture with higher cell density inoculated.

Chlorella sp. grew rapidly in a high-density culture with CO_2 aeration. The result is confirmed by the report that the waste gas or CO_2 tolerance of microalgae was dependent on cell density (Lee et al., 2002; Yoshihara et al., 1996; Yun et al., 1997).

3.3. Effect of CO_2 on cell growth in semicontinuous cultivation

The semicontinuous culture was carried out in two stages. A batch culture had an initial cell density of 8×10^6 cells mL^{-1} (i.e., a high-density of inoculum). At 2% CO_2, cell density was allowed to increase until it reached an optical density (A_{682}) over 5 (the cell density was around 1×10^8 cells mL^{-1}), which occurred after 6–8 days of incubation. After that, half of the culture broth was replaced with fresh modified f/2 medium each day and the culture was incubated with 2%, 5%, 10%, and 15% CO_2 aeration. The growth of *Chlorella* sp. in the semicontinuous culture was constantly similar at 2%, 5%, 10%, and 15% CO_2. The average specific growth rate and biomass, respectively, were 0.58–0.66 d^{-1} and 0.76–0.87 g L^{-1} after 8 days of incubation at 2–15% CO_2 aeration. These semicontinuous cultures aerated with different CO_2 concentrations were operated for 24 days. The growth of these cultures was stable on each day. These results showed that a high concentration of CO_2 (10–15%) may directly introduce to a high-density *Chlorella* sp. culture in the semicontinuous photobioreactor system. The high CO_2 concentration did not cause harmful effects on microalgae, indicating that the CO_2 can be as carbon source for the growth of a variety of photosynthetic microalgae at high-density culture. An initial high-density of the *Chlorella* sp. cultures that was adapted to 2% CO_2 may overcome environment stress induced by higher CO_2 (10–15%) aeration.

Selection of the mutant of *Chlorella* sp. represents one approach to elevating CO_2 tolerance of microalgae (Chang and Yang, 2003). However, growth and cell density in the cultures aerated with high levels of CO_2 are still limited in the application of these mutants. Chang and Yang (2003) have isolated *Chlorella* strains NTU-H15 and NTU-H25 and found that the greatest biomass produced by each strain at 5% CO_2 was 0.28 g L^{-1} d^{-1}. The other mutant, *Chlorella* strain KR-1, showed a potential biomass of 1.1 g L^{-1} d^{-1} at 10% CO_2 (Sung et al., 1999). However, increasing the cell density in the cultures or pre-adapting cells in a low concentration of CO_2 are alternative approaches to increase CO_2 tolerance of microalgae without effects on microalgal growth (Lee et al., 2002; Yun et al., 1997). In our semicontinuous photobioreactors, *Chlorella* sp. cells that were pre-adapted to 2% CO_2 not only grew into a high-density microalgal culture but also grew fast at 10% or 15% CO_2. Our results confirmed these previous studies and provided a useful system that can be applied to conversion of CO_2 into biomass.

3.4. Effect of CO_2 on CO_2 reduction in semicontinuous culture

Semicontinuous *Chlorella* sp. culture was conducted to examine the potential of CO_2 reduction in the photobioreactor using a high-density culture. Prior to the photobioreactor being operated with microalgae present, the photobioreactor was emptied and operated for 1 day without microalgae to test for any abiotic removal of CO_2, at 2%, 5%, 10%, and 15% CO_2. During these tests, the average influent and effluent concentrations of CO_2 were similar. Thus, CO_2 was not removed via an abiotic mechanism.

The amount of CO_2 reduced from the airstreams was estimated in the semicontinuous *Chlorella* sp. cultures during an 8-day period. The difference in CO_2 concentration between the influent load and effluent load were monitored. All runs in each treatment and on each day were remarkably consistent and showed a similar pattern among the influent and effluent CO_2 measurements. The effluent CO_2 concentrations in the influent 2%, 5%, 10% and 15% CO_2 treatments was maintained at 0.8–1.0%, 3.5–3.8%, 7.9–8.4% and 12.4–12.8% CO_2 during 8-day operation, respectively. The average rate of CO_2 reduction in cultures at 2%, 5%, 10%, and 15% CO_2 in the single photobioreactor was 0.261, 0.316, 0.466 and 0.573 g h^{-1}, respectively. Thus, the overall efficiency of CO_2 reduction in the cultures was 58%, 27%, 20% and 16%, respectively (Fig. 4). Recently, de Morais and Costa (2007) reported greater efficiency of CO_2 fixation in cultures at low CO_2 concentration (6%) than in cultures at high CO_2 concentration (12%). The increasing retention of CO_2 in a microalgal photobioreactor also could significantly enhance the efficiency of CO_2

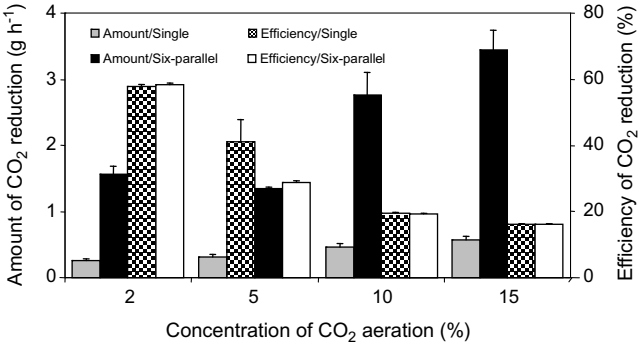

Fig. 4. Comparisons of the total amount and efficiency of CO_2 reduction in the single and the six-parallel photobioreactor of semicontinuous *Chlorella* sp. cultures under 2%, 5%, 10%, and 15% CO_2 aeration. Bars show the total amount of CO_2 reduction by single (□) and six-parallel photobioreactor (■) and; bars show the efficiency of CO_2 reduction by single (▨) and six-parallel photobioreactor (□). The total amount and efficiency of CO_2 reduction were determined by the difference of influent and effluent CO_2 loading in airstreams. Each data indicates the mean ± SD and were measured from three independent cultures.

fixation (Cheng et al., 2006). Keffer and Kleinheinz (2002) demonstrated that air dispersed in photobioreactors operated under approximately 2 s of air retention time removed up to 74% of CO_2 from an airstreams containing 0.16% CO_2. The air retention time was around 1–1.5 s in our photobioreactor; therefore, we believe that amount and efficiency of CO_2 reduction can be improved by increasing the CO_2 retention time in the photobioreactor system.

In the absence of microalgae, the medium pH was 7.5 in cultures aerated with air and pH dropped to about 6.4, 6.1, 5.8, and 5.6 at 2%, 5%, 10% and 15% CO_2, respectively. However, pH was greater in each culture of inoculated with *Chlorella* sp. Average pH was 7.6, 7.4, 7.1 and 6.8 at 2%, 5%, 10% and 15% CO_2, respectively. Free CO_2 in culture broth containing *Chlorella* sp., i.e., $CO_{2(aq)}$, was also measured. The $CO_{2(aq)}$ in the cultures was stable throughout the period of 8 days of incubation. Average $CO_{2(aq)}$ in cultures aerated with 2%, 5%, 10% and 15% CO_2 was 575, 605, 660 and 705 ppm, respectively. These values were consistent with the changes in culture pH. The $CO_{2(aq)}$ concentration was generally increased with increased influent CO_2 concentration; however, the result indicates the limit on the amount of CO_2 that can dissolve in the culture broth. Most of the influent CO_2 flowed out of the photobioreactor directly when the CO_2 concentration was more than 2%.

The efficiency of CO_2 removal or fixation in a closed culture system is dependent on the microalgal species, CO_2 concentration, and photobioreactor (Cheng et al., 2006; de Morais and Costa, 2007). Cheng et al. (2006) have demonstrated that CO_2 removal efficiency peak (55.3%) at 0.15% CO_2 and the amount of CO_2 reduction (about 80 mg L^{-1} h^{-1}) peaks at 1% CO_2 in a *Chlorella vulgaris* culture in a membrane photobioreactor. In a three serial tubular photobioreactor, 27–38% and 7–13% of CO_2, respectively, was fixed by *Spirulina* sp. and *Scenedesmus obliquus* in cultures aerated with 6% CO_2 aeration. In treatments of 12% CO_2 aeration, CO_2 fixation efficiency was only 7–17% for *Spirulina* sp. and 4–9% for *S. obliquus* (de Morais and Costa, 2007). The species dependence of efficiency of CO_2 removal or fixation may be due to physiological conditions of microalgae, such as potential of cell growth and ability of CO_2 metabolism.

3.5. Effect of CO_2 on lipid and biomass production in semicontinuous culture

Lipid and biomass productivity in the semicontinuous *Chlorella* sp. cultures were determined before the culture broth was changed each day. Table 2 summarizes the results, lipid and biomass productivity, collected from the single photobioreactor cultures under different CO_2 aeration. As a daily 50% culture broth replaced in the 800 mL semicontinuous photobioreactor aerated with 2%, 5%, 10%, and 15% CO_2, the total biomass and lipid productivity per day (400 mL of waste broth was recovered for measurement) of each photobioreactor was 0.422 g d^{-1}, 0.393 g d^{-1}, 0.366 g d^{-1} and 0.295 g d^{-1}, and 0.143 g d^{-1}, 0.130 g d^{-1}, 0.124 g d^{-1} and 0.097 g d^{-1}, respectively. In the single semicontinuous culture, both of lipid and biomass productivity decreased when the aerated CO_2 concentration was increased. However, lipid content in the cells cultured at 2%, 5%, 10%, and 15% CO_2 were very similar (approximately 32–34% of dry weight). In the semicontinuous culture, the optimum condition for biomass productivity was at 2% CO_2 aeration and lipid content was not affected even at high CO_2 aeration. Biomass productivity at 15% CO_2 aeration was 68% of that at 2% CO_2 aeration. However, our results still showed the potential growth of microalga, *Chlorella* sp., for lipid and biomass productivity in the semicontinuous system even the cells were cultivated in the condition aerated with 15% CO_2.

The lipid content of *Chlorella fusca* and *Phaeodactylum tricornutum* increased when cells were grown at increasingly higher concentrations of CO_2 (Dickson et al., 1969; Yongmanitchai and Ward, 1991). Our result was not consistent with these previous studies. Such divergent results for lipid content of microalgae cultured under CO_2 aeration may be due to differences in microalgal species, content of culture medium, and culture condition.

3.6. Performances of six-parallel photobioreactor system

The efficiency of CO_2 removal from airstreams by *Chlorella* sp. was compared between the single photobioreactor and the six-parallel photobioreactor. The effects of varying CO_2 concentration on growth of *Chlorella* sp. was similar

Table 2
Recovery of lipid and biomass productivity of the *Chlorella* sp. as waste broth in the semicontinuous photobioreactor under different concentrations of CO_2 aeration compared with single and six-parallel photobioreactor

CO_2 aeration (%)	Total lipid productivity (g d^{-1})		Total biomass productivity (cell dry weight, g d^{-1})	
	In single photobioreactor[a]	In six-parallel photobioreactors[b]	In single photobioreactor	In six-parallel photobioreactors
2	0.143 ± 0.020	0.852 ± 0.133	0.422 ± 0.061	2.560 ± 0.312
5	0.130 ± 0.011	0.773 ± 0.060	0.393 ± 0.040	2.343 ± 0.232
10	0.124 ± 0.029	0.770 ± 0.131	0.366 ± 0.089	2.154 ± 0.511
15	0.097 ± 0.007	0.601 ± 0.055	0.295 ± 0.031	1.743 ± 0.144

Each data indicates the mean ± SD, which were measured daily from day 1 to day 8.
[a] The culture volume in a photobioreactor is 800 mL. Daily waste broth was 400 mL.
[b] Sum of the production from six units of photobioreactor. The medium volume in each photobioreactor is 800 mL. Sum of daily waste broth was 2400 mL.

between the single and the six-parallel photobioreactors (data not shown). In a total volume of 4800 mL (i.e., 6×800 mL) of the six-parallel photobioreactor, the total amount of CO_2 reduced was 1.563, 2.058, 2.757 and 3.441 g h^{-1} at 2%, 5%, 10% and 15% CO_2 aeration, respectively (Fig. 4). Thus, the amount of CO_2 that reduced in the six-parallel photobioreactor was approximately six times greater than the amounts in the single photobioreactor. Therefore, the efficiency CO_2 reduction in the six-parallel photobioreactor and in the single photobioreactor was also similar (Fig. 4).

Daily recovery of lipid and biomass in the six-parallel photobioreactor were determined. In each case, the amount of lipid and biomass recovered daily in the six-parallel photobioreactor was around six times greater than the amounts recovered in the single photobioreactor (Table 2). CO_2 reduction efficiency and cell growth in both photobioreactor systems also were similar.

When microalgal cells grew in a closed photobioreactor, light decreases exponentially with the distance from light source (Suh and Lee, 2003). It will be a problem for diameter of scale-up photobioreactor with external lighting. Our results showed that our photobioreactor could be extended to parallel multiple units of photobioreactor for discharging waste gas in a large scale without decreasing biomass and lipid productivity, and efficiency of CO_2 reduction. Additionally, increasing the length of tubular photobioreactor and gas sparging into small bubbles can be considered in a scale-up system. Longer tubular photobioreactor and small bubbles could increase the retention time of gas in photobioreactor and the bubbles absorbed into cultures, and then increases the efficiency of CO_2 reduction.

4. Conclusions

Our results showed that airstreams containing a high concentration of CO_2 (2–15%) may be introduced directly into a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor. Growth, biomass productivity, lipid productivity, and the quantity of CO_2 removed from the airstreams remained constant in the photobioreactor. The maximum efficiency of CO_2 reduction reached 58% in the culture aerated with 2% CO_2. The greatest mass of CO_2 that was reduced (17.2 g L^{-1} d^{-1}) occurred at 15% CO_2. These productivity and efficiency of CO_2 reduction did not decrease when a parallel (multiple units) photobioreactor was used.

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