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# Research Article

# The air-lift photobioreactors with flow patterning for high-density cultures of microalgae and carbon dioxide removal

A photobioreactor containing microalgae is a highly efficient system for converting carbon dioxide (CO<sub>2</sub>) into biomass. Using a microalgal photobioreactor as a CO<sub>2</sub> mitigation system is a practical approach to the problem of CO<sub>2</sub> emission from waste gas. In this study, a marine microalga, Chlorella sp. NCTU-2, was applied to assess biomass production and CO<sub>2</sub> removal. Three types of photobioreactors were designed and used: (i) without inner column (i.e. a bubble column), (ii) with a centric-tube column and (iii) with a porous centric-tube column. The specific growth rates  $(\mu)$  of the batch cultures in the bubble column, the centric-tube and the porous centric-tube photobioreactor were 0.180, 0.226 and 0.252 day<sup>-1</sup>, respectively. The porous centrictube photobioreactor, operated in semicontinuous culture mode with 10% CO<sub>2</sub> aeration, was evaluated. The results show that the maximum biomass productivity was 0.61 g/L when one fourth of the culture broth was recovered every 2 days. The CO<sub>2</sub> removal efficiency was also determined by measuring the influent and effluent loads at different aeration rates and cell densities of Chlorella sp. NCTU-2. The results show that the CO<sub>2</sub> removal efficiency was related to biomass concentration and aeration rate. The maximum CO<sub>2</sub> removal efficiency of the Chlorella sp. NCTU-2 culture was 63% when the biomass was maintained at 5.15 g/L concentration and 0.125 vvm aeration (volume gas per volume broth per min; 10% CO<sub>2</sub> in the aeration gas) in the porous centric-tube photobioreactor.

Keywords: Biomass / Carbon dioxide / Chlorella sp. / Photobioreactor

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## 1 Introduction

Global warming, which results from the increasing concentration of atmospheric carbon dioxide (CO<sub>2</sub>), has become an important environmental issue. There are many attempts at CO<sub>2</sub> recovery, including physical, chemical, and biological methods [1, 2]. Among these attempts, the biological method using microalgal photosynthesis is believed to be an effective approach to biological CO<sub>2</sub> fixation [3, 4]. By the biological approaches, CO<sub>2</sub> can be fixed in the form of microalgal biomass by photosynthesis. The photosynthetic organisms can produce proteins, fatty acids and pigments as dietary supplements for humans and animals [5]. Furthermore, lipids from microalgae are chemically similar to those in common vegetable oils and are good potential sources for biodiesel

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production [6]. The microalgae-based biodiesel, in contrast to fossil fuels, is renewable, biodegradable, and produced with low pollutant emissions [7, 8]. Thus, reducing the atmospheric CO<sub>2</sub> by microalgal photosynthesis is considered as safe and reliable for the living environment [2, 9].

Using outdoor microalgal cultures, such as open ponds, has been proposed to reduce CO<sub>2</sub> emissions [10, 11]. However, outdoor culture systems are limited to microalgal growth, are not easy to control with regard to environmental parameters, and show low productivity as a result of variable environmental temperatures, system circulation and light utilization [12]. In comparison with open culture systems, a closed photobioreactor is easy to control regarding environmental parameters [13] and can achieve high growth rates [14, 15]. A closed photobioreactor can be a bioscrubber for waste gas treatment, and the microalgal cells cultured in the photobioreactor convert the CO<sub>2</sub> from the waste gas into biomass, which is an energy-efficient and economical approach [16, 17]. Several studies have proved that using microalgal cells cultivated in photobioreactors is a useful and practical method for



CO<sub>2</sub> removal [11, 17, 18]. Several types of photobioreactors have been reported, such as tubular, flat and column photobioreactors. Vertical tubular-type photobioreactors, such as bubble and air-lift photobioreactors, have often been thought to achieve the most efficient mixing and the best volumetric gas transfer [19]. Besides, the traditional fermentation bioreactor, a bubble column equipped with a perforated draft tube, has shown significant improvements over traditional air-lift reactors in the mixing and mass transfer performance [20, 21]; however, bioreactors equipped with a perforated draft tube are rarely used in connection with a photosynthetic organism. Criteria for cultures of high density in a photobioreactor are good mixing, mass transfer and light utilization. In addition, in a high-density culture of microalgae, light utilization could be improved by good mixing, providing the flash light effect of microalgal photosynthesis [22]. As mentioned above, we tested whether a photobioreactor with a porous centric tube is potentially useful for the culture of microalgae at a high cell density.

In the present study, we designed an air-lift-type photobioreactor with a porous centric tube for culturing microalgae at high density, and its performance was compared to that of the other two designs, bubble column and centric-tube photobioreactors. The microalgal species *Chlorella* sp. NCTU-2 is native in Taiwan and was assessed as a potential candidate for growth and biomass production in this study. In order to determine the capacity of daily biomass production of the microalga, culturing in semicontinuous mode was performed. Moreover, the CO<sub>2</sub> removal efficiency was evaluated at different aeration rates and microalgal densities (*i.e.* biomass concentrations) of the culture.

## 2 Materials and methods

## 2.1 Microalgal cultures, medium and chemicals

The microalga, *Chlorella* sp. NCTU-2, was originally obtained from the collection of the Taiwan Fisheries Research Institute (Tung-Kang, Taiwan). The microalga was screened, and a potential candidate was isolated for growth and biomass production at the National Chiao Tung University, Taiwan. The microalgal cells were cultured in artificial seawater prepared as follows (per liter): 29.23 g NaCl, 1.105 g KCl, 11.09 g MgSO<sub>4</sub> · 7H<sub>2</sub>O, 1.21 g Tris-base, 1.83 g CaCl<sub>2</sub> · 2H<sub>2</sub>O, 0.25 g · NaHCO<sub>3</sub>. Micronutrients and macronutrients were prepared in the following composition in artificial seawater (per liter): 750 mg NaNO<sub>3</sub>, 44.11 mg NaH<sub>2</sub>PO<sub>4</sub> · H<sub>2</sub>O, 43.6 mg Na<sub>2</sub> · EDTA, 31.6 mg FeCl<sub>3</sub> · 6H<sub>2</sub>O, 1.8 mg MnCl<sub>2</sub> · 4H<sub>2</sub>O, 0.1 mg CoCl<sub>2</sub> · 6H<sub>2</sub>O, 0.1 mg CuSO<sub>4</sub> · 5H<sub>2</sub>O, 0.23 mg ZnSO<sub>4</sub> · 7H<sub>2</sub>O, 0.06 mg Na<sub>2</sub>MoO<sub>4</sub>, 1 mg vitamin B<sub>1</sub>, 5 μg vitamin B<sub>12</sub> and 5 μg biotin for the cultures with high cell density.

# 2.2 Measurements of microalgal density and biomass concentration

Cell density (cells/mL) was measured by spectrophotometric method using an Ultrospec 3300 pro UV/Visible spectro-

photometer (Amersham Biosciences, Cambridge, UK) at the absorbance of 682 nm ( $A_{682}$ ). 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.

Microalgal dry weight per liter (g/L) was measured according to the method previously reported [23]. Microalgal cells were collected by centrifugation and washed twice with water. The microalgal pellet was dried at 105°C for 16 h for dry weight measurement [23].

Calibration curves and equations of optical density  $(A_{682})$ to determine the cell density and the biomass concentration have been established [23]. The optical density represented the cell number of the culture depending on the concentration of the culture. A direct microscopic count was performed with a Brightline Hemocytometer (BOECO, Hamburg, Germany) and a Nikon Eclipse TS100 inverted metallurgical microscope (Nikon Corporation, Tokyo, Japan). The relationship between optical density and cell dry weight was established by linear regression: y = 0.206x. The value y is the biomass concentration (g/L) and the value x is the optical density  $(A_{682})$  [17]. Cell density and biomass concentration were measured more easily by optical density than by direct counting of cells or by cell dry weight. Therefore, the optical density can be used to precisely predict the biomass concentration ( $R^2 > 0.991$ ; p < 0.001). In this study, we used the biomass concentration to interpret the values of cell density in each experiment.

#### 2.3 Measurement of growth rate of the microalga

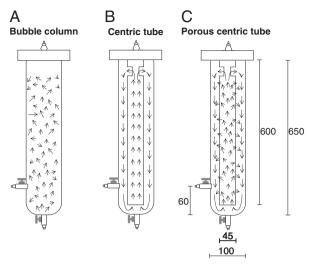
A regression equation of the cell density and dry weight per liter of culture was obtained by a spectrophotometric method [17]. The specific growth rate (, day<sup>-1</sup>) was calculated as follows:

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

where  $W_f$  and  $W_0$  are the final and initial biomass concentration, respectively. t is the cultivation time in days [23, 24]. The specific growth rate was measured and obtained from the logarithmic growth phase over 1–2 days of batch culture.

# 2.4 Photobioreactors and operation of microalgal culture

Three types of photobioreactor were designed and used in this study: (i) without inner column (*i.e.* a bubble column), (ii) with a centric-tube column and (iii) with a porous centric-tube column (Fig. 1). The working volume in the photobioreactors was 4 L. The gas supply is from the bottom of the photobioreactor. The photobioreactors were placed in an incubator at  $26\pm1^{\circ}$ C, with a light intensity of approximately  $300\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$  at the surface of the photobioreactor provided by a continuous, cool white, fluorescent light source. The light intensity was measured by counting the photons between 400 and 700 nm, using a Basic Quantum Meter (Spectrum Technologies, Plainfield, IL, USA). The outer column with a



**Figure 1.** Schematic diagram of the different types of photobioreactor and visualization of the liquid flow patterns. (A) Bubble column-type photobioreactor, (B) centric-tube photobioreactor, (C) porous centric-tube photobioreactor. The flow patterns were visualized by injecting a dye at the liquid surface. The working volume of the photobioreactors was 4 L. Gas was provided and pumped into the photobioreactors *via* a sparger at the bottom of the photobioreactor. External illumination with a light intensity of 300  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> was provided by a continuous, cool white and fluorescent light source. The arrows indicate the visualized liquid flow patterns.

diameter of 100 mm was made of glass, and the inner column with a diameter of 45 mm was made of acrylics. The heights of the outer glass column and the inner acrylic column were 650 and 600 mm, respectively. The dimensions of the three photobioreactors are also shown in Fig. 1. The flow pattern was determined through the dye technique. Batch cultures were used to inoculate the three types of photobioreactor (without inner column, with centric-tube column, and with porous centric-tube column) at an initial biomass concentration of  $1.0 \, \text{g/L}$ , and the cultures were aerated with 5%  $\text{CO}_2$  at an aeration rate of  $1.0 \, \text{L/min}$  (*i.e.*  $0.25 \, \text{vvm}$ ; volume gas per volume broth per minute).

# 2.5 Experimental design of semicontinuous cultivation

For the maintenance of biomass production, a semicontinuous culture mode was applied. The microalgal cells were pre-cultured in a batch and fed-batch culture until reaching approximately 5 g/L. After the pre-culture, the cultures were replaced with ratios of one half (1/2), one third (1/3) and one fourth (1/4) of fresh medium. Each replacement was executed when the microalgal cultures had grown back to approximately 5 g/L. The semicontinuous cultures were operated for at least two cycles of replacement (at least 15 days). The biomass productivity for each replacement was evaluated when the biomass concentration had again reached approximately 5 g/L. The obtained biomass from each replacement was also determined from the replaced broth.

## 2.6 Determination of CO<sub>2</sub> removal efficiency

The setup of the photobioreactor for  $\mathrm{CO}_2$  removal has been described previously [17]. The  $\mathrm{CO}_2$  concentration in the airstreams was sampled as influent and effluent load and measured using a Guardian Plus Infra-Red  $\mathrm{CO}_2$  Monitor D-500 (Edinburgh Instruments, Livingston, UK). The efficiency of  $\mathrm{CO}_2$  removal was determined by the difference of the  $\mathrm{CO}_2$  concentration between the influent and the effluent load of the photobioreactor with the microalgal culture. The removal efficiency (%) was determined by the following formula:

$$\frac{\text{Influent of CO}_2 - \text{Effluent of CO}_2}{\text{Influent of CO}_2} \times 100\%$$

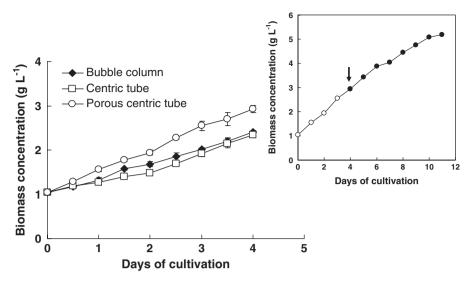
A comparison of the  $CO_2$  removal efficiencies of microalgal cultures with different aeration rates and microalgal cell densities (*i.e.* different biomass concentrations) was performed. The specific concentration of  $CO_2$  gas was provided by a commercial premixed-gas steel cylinder. The gas flow rate was adjusted with a gas flow meter (Dwyer Instruments, Michigan City, IN, USA) to give a flow rate of 0.125, 0.25, and 0.5 vvm (volume gas per volume broth per minute).

#### 3 Results and discussion

## Growth of Chlorella sp. NCTU-2 in the photobioreactors

The comparison of the growth of *Chlorella* sp. NCTU-2 cultivated in the photobioreactors without inner column, with centric-tube column and with porous centric-tube column was performed as a batch culture in an incubator at  $26\pm1^{\circ}$ C, with a light intensity of approximately  $300\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$  at the surface of the photobioreactor provided by a continuous, cool white, fluorescent light source. The cultures were provided with 5% CO<sub>2</sub> gas. The cultured samples were collected for density measurements at 12–h intervals. Fig. 2 shows the growth curves of the three cultures, and the results indicate that the microalgae cultured in the porous centric-tube photobioreactor performed at the highest growth rate.

The maximum biomass concentrations and specific growth rates of the cultures in the photobioreactors without inner column, with centric-tube column and with porous centrictube column in batch culture mode were 2.369, 2.534 and 3.461 g/L, and 0.180, 0.226 and  $0.252 \text{ day}^{-1}$ , respectively (Table 1). This result indicates that the maximum biomass concentration in the porous centric-tube photobioreactor could be enhanced by 46% and 37% compared to those in the bubble column photobioreactor and in the centric-tube photobioreactor, respectively. The culture in the porous centric-tube photobioreactor showed not only an improved maximum biomass concentration but also a better specific growth rate. Recently, Ranjbar et al. [25] reported that the maximum cell density of Haematococcus pluvialis cultured in an air-lift photobioreactor was 18% higher than that in a bubble column photobioreactor. Oncel and Sukan [26]



**Figure 2.** Growth curves of *Chlorella* sp. NCTU-2 cultured in the three different types of photobioreactor in batch and fed-batch culture mode. The cultures were aerated with 5% CO<sub>2</sub>, which was adjusted by an individual gas flow meter. The initial cell density for each culture was approximate 1 g/L. The arrow indicates the time when cultivation in the porous centric-tube photobioreactor was changed to fedbatch cultivation, in which the feed medium was supplemented with 750 mg NaNO<sub>3</sub> and 44.11 mg NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O per liter every 2 days.

Table 1. Comparison of the growth potential and CO<sub>2</sub> removal efficiency in the three types of photobioreactors.

|   | Bubble column | Centric tube | Porous centric tube |
|---|---------------|--------------|---------------------|
| Max. biomass concentration (g/L) <sup>a)</sup>          | 2.369         | 2.534        | 3.461               |
| Max. specific growth rate ( $\mu$ , day <sup>-1</sup> ) | 0.180         | 0.226        | 0.252               |
| CO <sub>2</sub> removal efficiency <sup>b)</sup>        | 24%           | 23%          | 35%                 |

- a) The cultures were performed in batch culture at an initial biomass concentration of 1 g/L.
- b) The comparison of the CO<sub>2</sub> removal efficiencies among these three types of photobioreactors was made when the cultures had reached a biomass concentration of approximately 2 g/L; the cultures were aerated with 5% CO<sub>2</sub> at an aeration rate of 1.0 L/min (*i.e.* 0.25 vvm).

demonstrated that an air-lift photobioreactor culture yielded a maximum biomass concentration value of 2.21 g/L whereas a bubble column photobioreactor culture yielded only a maximum biomass concentration value of 1.87 g/L. The air rising randomly through the photobioreactor was the only driving force for culture mixing in the bubble column photobioreactor [25, 26]. The air-lift-type photobioreactor with the centric tube could provide a regular circulation of the culture in that the air rising from the inner column made the circulating liquid flow out of the inner column whereupon it was gravitationally forced downward (as shown in Fig. 1) [27, 28]. The regular circulation of the culture resulted in a more effective mixing for growth [26]. Ranjbar et al. [25] also reported that the light regime inside a photobioreactor could be improved and a high-density growth was attainable by using an air-lifttype photobioreactor. In our designed photobioreactor, there are perforations of 5 mm diameter regularly distributed along the porous centric tube. A similar concept has also been reported in which the liquid flowing through the rising zone could horizontally flow through the perforations [29]. The result indicated that the growth of Chlorella sp. NCTU-2 cultured in a photobioreactor with a porous centric tube was even 37% higher than when cultured in a photobioreactor with a centric tube. The perforations along the centric tube in the porous centric-tube photobioreactor could provide a shorter mixing time; therefore, this photoreactor possesses a better mixing efficiency [30]. In a high-density culture, the main limitation is the light penetration, which will decrease due to the self-shading effect of the microalgal cells. However, a photobioreactor providing a light/dark zone could minimize the self-shading effect [31]. The perforations along the centric tube could increase the frequency with which the microalgal cells are exposed to light/dark cycles. The more frequent light/dark cycles affect the productivity and the yield of biomass and have been reported to lead to higher growth and photosynthesis rates [32, 33].

# 3.2 CO<sub>2</sub> removal of *Chlorella* sp. NCTU-2 in the photobioreactors

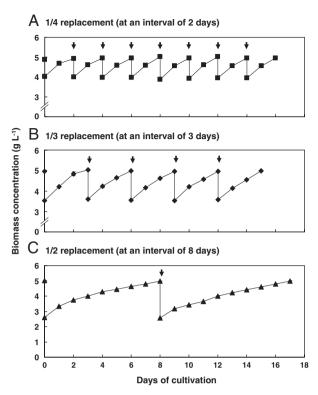
The CO<sub>2</sub> removal efficiency of *Chlorella* sp. NCTU-2 cultivated in the three types of photobioreactor was evaluated and compared. The microalgal cells were cultured in batch cultures. When the biomass concentration reached 3 g/L, the microalgal cells were centrifuged and resuspended in fresh medium. Then, the

microalgal cells were divided into equal parts and cultured in the photobioreactors at a biomass concentration of approximately 2 g/L and with 5% CO2 aeration at 0.25 vvm. The CO2 removal efficiency was determined by measuring the influent load and the effluent load. Table 1 shows a comparison of the CO2 removal efficiencies of Chlorella sp. NCTU-2 cultivated in photobioreactors without inner column, with centric-tube and with porous centrictube column. The results show that the CO2 removal efficiency in the porous centric-tube photobioreactor is 45 and 52% higher than those in the bubble column and centric-tube photobioreactors, respectively. The CO<sub>2</sub> removal efficiency in the porous centric-tube photobioreactor showed the highest efficiency of CO2 removal, which is probably due to the higher mixing efficiency and the higher photosynthetic rate. A similar result was also reported by Xu et al. [29] that the quality of mixing is critical for the performance of a bioreactor, and a shorter mixing time was obtained with an air-lift reactor with a net draft tube, which also provided a horizontal flow, in comparison with the mixing time in a bubble column reactor and an air-lift reactor without a net draft tube. The result has also been confirmed by Grobbelaar [34] who indicated that higher mixing resulting in more frequent light/dark cycles would enhance the photosynthetic efficiency.

# 3.3 Biomass productivity of *Chlorella* sp. NCTU-2 in semicontinuous cultivation

Before the operation of semicontinuous cultivation, the microalgal cells were cultivated in a porous centric-tube photobioreactor. When the cell density reached approximately 3 g/L (beginning of the early stationary phase), the mode was changed to fed-batch cultivation in that the feed medium was only supplied with 750 mg NaNO<sub>3</sub> and 44.11 mg NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O per liter every 2 days (as shown in Fig. 2). After 6 days of cultivation, the biomass concentration reached approximately 5 g/L. This almost corresponds to the maximum biomass concentration of a Chlorella sp. NCTU-2 culture grown in the porous centric-tube photobioreactor at a high growth rate. The growth potential would be decreased due to the decreasing light utilization at the higher biomass concentration of the culture. For the maintenance of the highdensity culture and of biomass production, the semicontinuous culture mode was then applied. For the semicontinuous culture mode, the growth profiles of Chlorella sp. NCTU-2 cultured in the centric-tube photobioreactor aerated with 5% CO2 and operated with 1/4 (i.e. one fourth of the volume of the culture broth was replaced by fresh medium at intervals of 2 days), 1/3 (one third of the broth replaced at 3-day intervals) or 1/2 (one half of the broth replaced at 8-day intervals) replacement are shown in Fig. 3. A steady-state growth profile was seen with each broth replacement during semicontinuous culture. The steady-state growth profile indicated that continuous growth of the microalgae cultivated in the porous centric-tube photobioreactor could be sustained in a high-density culture.

Table 2 shows the performance of the broth replacement strategies in the semicontinuous culture mode. At a high culture density (biomass concentration from 2.47 to 4.94 g/L), the specific growth rates and biomass productivities in the 1/4, 1/3 and 1/2 replacement were 0.106, 0.118 and 0.132 day<sup>-1</sup>, and 0.61, 0.53 and 0.51 g L<sup>-1</sup> d<sup>-1</sup>, respectively. Compared with



**Figure 3.** Growth profiles of *Chlorella* sp. NCTU-2 cultured in semicontinuous culture mode with 5% CO<sub>2</sub> aeration and operated by 1/4, 1/3 and 1/2 replacements in the porous centric-tube photobioreactor. The microalgal cells were pre-cultured in a fedbatch culture until they reached approximately 5 g/L. After the pre-culture, the culture medium was replaced with volume ratios of one half (1/2 replacement; the cultured broth was replaced at intervals of 2 days), one third (1/3; 3 days), and one fourth (1/4 replacement; 8 days) of fresh medium. The cultures were continuously operated for about 18 days. The arrows indicate the times when cultured broth was removed and fresh medium was added.

our previous study [17], the biomass productivity in the maintained semicontinuous culture mode was  $0.458\,\mathrm{g}\,L^{-1}\,\mathrm{d}^{-1}$ . The results indicate that, for the porous centric-tube photobioreactor in semicontinuous culture mode, the maximum biomass productivity was  $0.61\,\mathrm{g/L}$  when one fourth of the culture broth was recovered from the culture every 2 days.

# 3.4 CO<sub>2</sub> removal efficiency at a variety of culture densities at different aeration rates

For the study of  $\rm CO_2$  removal, microalgal cells were collected and cultured in a biomass concentration range from 1.03 to 5.15 g/L. Different culture densities were obtained by condensing the microalgal cells by centrifugation. The microalgal cells were resuspended in fresh medium for further experiments. All the cultures at a variety of microalgal densities were provided with premixed 10%  $\rm CO_2$  gas at different aeration rates. The  $\rm CO_2$  removal efficiency was evaluated by measuring the influent load and effluent load airstreams at different aeration rates and cell

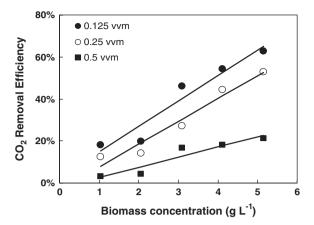
**Table 2.** Performance of the different broth replacement strategies in semicontinuous cultivation.<sup>a)</sup>

| Replacement<br>ratio<br>each time <sup>b)</sup> | Time interval<br>between<br>replacements<br>(days) | Recovered<br>volume<br>with each<br>replacement<br>(L) | Specific growth rate (μ, day <sup>-1</sup> ) | Biomass productivity $(gL^{-1} day^{-1})^{c)}$ |
|---|--|--|--|--|
| 1/4   | 2  | 1  | 0.106  | 0.61   |
| 1/3   | 3  | 4/3  | 0.118  | 0.53   |
| 1/2   | 8  | 2  | 0.132  | 0.51   |

- a) All the cultures were performed in the porous centric-tube photobioreactor.
- b) In this semicontinuous cultivation strategy, the initial cultures were grown as fed-batch cultures until the cell density reached 5 g/L. After that, the cultured broth was replaced with 1/4, 1/3 or 1/2 the volume with fresh medium and cultivation was continued until the cell density again reached 5 g/L. The semicontinuous cultures were performed at least for two cycles of replacements (for at least 15 days) (as shown in Fig. 3).
- Biomass productivity was defined as total recovered biomass divided by reactor working volume and by day.

densities of the microalgae. Fig. 4 shows the correlation between  $CO_2$  removal efficiency, culture aeration rate and biomass concentration. The regression lines and the equations were as follows:  $y_1 = 0.1204x + 0.0295$  for 0.125 vvm,  $y_2 = 0.108x - 0.0308$  for 0.25 vvm, and  $y_3 = 0.0483x - 0.0223$  for 0.5 vvm. Here, the value of  $y_i$  is the  $CO_2$  removal efficiency (%) and the value x is the biomass concentration (g/L).

The result indicates that an increasing CO<sub>2</sub> removal efficiency could be achieved by a lower aeration rate. Also, the CO2 removal efficiency could be increased by cultivation at a high density. Mandeno et al. [35] have revealed that the CO<sub>2</sub> removal efficiency decreased with increasing gas flow. This may have resulted from bubble coalescence. The amount of coalescence would have increased with the increased gas flow rate. As the bubbles coalesce, the bubble surface area per unit gas volume would decrease and the larger bubbles would rise faster than the smaller ones. Moreover, CO2 absorption from the bubbling gas would also decrease with decreasing surface area per unit gas volume of the bubbles. In order to obtain a higher CO2 removal efficiency, high-density cultivation was performed because, under these conditions, more CO2 was consumed by the microalga Chlorella sp. NCTU-2. Furthermore, an increase in CO2 removal efficiency during high-density cultivation may also result from the high-density culture broth causing a higher viscosity, which would in turn increase the gas retention time for CO<sub>2</sub> absorption. The optimal conditions for CO<sub>2</sub> removal in this study were culturing Chlorella sp. NCTU-2 at a high biomass concentration of 5.15 g/L and aeration of the culture at 0.125 vvm. The maximum efficiency of CO<sub>2</sub> removal was 63% (with 10% CO<sub>2</sub> in the aeration gas). Keffer and Kleinheinz [16] have demonstrated that the maximum CO2 reduction was 74% with Chlorella vulgaris cultured in a bubble column photobioreactor, but the CO<sub>2</sub> concentration for aeration was 100-fold lower than that in this study. In this study, the CO2 removal efficiency could still reach 63% when using the porous centric-tube photobioreactor with a high-density culture, although the CO2 concentration for aeration was 100-fold higher than that reported



**Figure 4.**  $CO_2$  removal efficiency in *Chlorella* sp. NCTU-2 cultures operated at different aeration rates and biomass concentrations. The microalgal cultures were pre-cultured in a fed-batch culture. Then, different densities of cultured microalgal cells were obtained by centrifugation. The microalgal cells were resuspended in fresh medium to achieve biomass concentrations of 1.03, 2.06, 3.09, 4.12, and 5.15 g/L. The cultures were operated in the porous centric-tube photobioreactor and aerated with 10%  $CO_2$  at different gas flow rates of 0.125, 0.25, and 0.5 vvm. The  $CO_2$  removal efficiency was determined by measuring the  $CO_2$  concentration difference between the influent load and the effluent load, which were monitored by  $CO_2$  sensors.

by Keffer and Kleinheinz [16]. Compared with our previous study [17], due to the achievement of a high-density culture in the porous centric-tube photobioreactor, the CO<sub>2</sub> removal efficiency was increased from 20 to 53% at the same aeration rate and CO<sub>2</sub> concentration. By using the porous centric-tube photobioreactor with semicontinuous cultivation strategy, a high culture density could be maintained, up to approximately 5 g/L; besides, a high efficiency of CO<sub>2</sub> removal could be achieved.

## 4 Conclusion

A porous centric-tube photobioreactor was designed for the culture of microalgae. The photobioreactor can be used for maintaining a high-density culture of the microalga *Chlorella* sp. NCTU-2 in semicontinuous culture mode. In the semicontinuous cultures, the maximum biomass productivity could reach approximately  $0.6 \, \mathrm{g \, L^{-1} \, d^{-1}}$  with 1/4 volume of broth replacement every 2 days, and >60% of  $\mathrm{CO}_2$  could be removed from the aeration gas containing 10%  $\mathrm{CO}_2$ .

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# Conflict of interest

The authors have declared no conflict of interest.

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