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Ability of a mutant strain of the microalga *Chlorella* sp. to capture carbon dioxide for biogas upgrading

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ABSTRACT

In this study, a culture system with outdoor microalgae-incorporating photobioreactors was utilized to upgrade biogas produced from the anaerobic digestion of swine wastewater. Using ethyl methane sulfonate (EMS) random mutagenesis, we isolated a mutant strain of microalga, *Chlorella* sp. MB-9, which was tolerant to high CH_4 and CO_2. In the field study of outdoor operation, the maximum growth rates of *Chlorella* sp. MB-9 aerated with desulfurized biogas at 0.05, 0.1, 0.2 and 0.3 vvm were 0.32, 0.311, 0.275 and 0.251 g L^{-1} d^{-1}. In addition, ~70% of the CO_2 in desulfurized biogas (~20% CO_2, ~70% CH_4, and H_2S < 50 ppm) could be captured by the *Chlorella* sp. MB-9 cultures. The CH_4 concentration in the effluent biogas from the *Chlorella* cultures increased from its original 70% up to 85–90%. The established outdoor microalgae-incorporating culture system with a gas cycle-switching operation could be efficiently used as a CO_2 capture model for biogas upgrading.

1. Introduction

Biogas is a mixture of methane (CH_4) and carbon dioxide (CO_2) with hydrogen sulfide (H_2S) and depending on the feedstock used, trace gases such as nitrogen (N_2), ammonia (NH_3), sulfur dioxide (SO_2), hydrogen sulfide (H_2S), and hydrogen (H_2) [1,2]. It is produced when certain bacteria decompose biological matter such as animal manure, organic wastes, fertilizer and biomass in an anaerobic environment. This process is referred to as anaerobic digestion. The treatment of biological waste through anaerobic digestion has many benefits. It leads biogas not only to a cleaner and healthier environment, but also produces a renewable energy source, methane [1]. The main biogas resource in Taiwan is produced via anaerobic digestion of swine wastewater. Raw biogas contains approximately 55–75% CH_4, 20–35% CO_2 and 3000–5000 ppm H_2S. Raw biogas is not high quality enough if the owner was planning on selling this gas or using it as fuel gas for machinery. The biogas produced from anaerobic digestion is a potential fuel for power generators if biogas can be upgraded to the same standards as fossil natural gas by CO_2, H_2S, and the other non-combustible component removal [2–4]. H_2S would corrode engines, pipelines and biogas storage structures if the biogas was used directly without H_2S removal. Several chemical and chemical-biological methods for the removal of H_2S from industrial and agricultural emission sources have been proposed [5–7]. Calorific values of biogas depending on the amount of methane in the gas. However, the high CO_2 content of biogas reduces its calorific value and increases carbon monoxide and hydrocarbon emissions, even if desulfurized biogas is used as engine fuel [2,8]. In addition, its high CO_2 content makes the compression and transport of desulfurized biogas uneconomical. Desulfurized biogas need to reduce its CO_2 concentration from CO_2 capture process and improve engine efficiency [2]. A biogas upgrading process can be applied to increase calorific value, minimize corrosion problems, promote it to pseudo-natural gas quality and connect it to a pipeline for network distribution [9,10]. Therefore, CO_2 capture is also essential for increasing the utility of biogas.

There are several means of reducing the emissions of CO_2 such as physical, chemical and biological methods. The biological method of microalgal fixation of CO_2 by photosynthesis, which converts CO_2 into a carbon source of biomass, is a potential method of CO_2 sequestration [11–14]. Microalgae have higher CO_2 fixation rates than terrestrial plants and can thus utilize CO_2 from flue gas to produce biomass [15–17]. Microalgal biomass can be used for biofuel production by pyrolysis, direct combustion or thermal chemical liquefaction [18]. Moreover, many microalgae are exceedingly rich in oil [19], which can be converted to many products such as renewable fuels by transesterification [19–23].

Physical and chemical absorption methods are generally cutilized for CO_2 capture from biogas with few complications; however, post-treatment of waste materials is necessary to regenerate

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cycling utilization [2]. The biological methods of CO_2 capture from biogas are potentially useful and need to be evaluated. In this study, we established an outdoor microalgae-incorporating photobioreactor system for CO_2 capture from desulfurized biogas produced from the anaerobic digestion of swine wastewater. Using this system, we evaluated the growth profiles of an isolated microalga, *Chlorella* sp. MB-9, cultivated with different concentrations of H_2S and CH_4. Finally, a field study of CO_2 capture from the desulfurized biogas produced from the anaerobic digestion of swine wastewater was implemented, and a biogas upgrading operation was demonstrated. The economic feature of proposed method was also discussed.

2. Methods

2.1. Microalga

The wild-type microalga *Chlorella* sp. was obtained from the collection of the Taiwan Fisheries Research Institute (Tung-Kang, Ping-Tung, Taiwan). The *Chlorella* sp. MB-9 was mutation-screened and isolated from our mutant strain *Chlorella* sp. MM-2 [24]. The protocol for chemical mutagenesis and mutant isolation was followed according to our previous report [25]. In brief, about 5×10^7 cells of *Chlorella* sp. were treated with 25–100 mM EMS for 1 h, and approximately 1×10^3 cells were plated on agar plates. The plates were then cultured in a closed photobioincubator filled with biogas. After 5–7 days of culture, the larger colonies were selected and verified for their growth capacity under biogas aeration. In the present study, a mutant strain of *Chlorella* sp. MB-9 was obtained that was stable and able to grow under aeration with biogas.

2.2. Microalgal cultures, medium and chemicals

The *Chlorella* sp. MB-9 cells were grown in modified f/2 medium in artificial sea water with 29.23 g L^{-1} NaCl, 1.105 g L^{-1} KCl, 11.09 g L^{-1} $MgSO_4 \cdot 7H_2O$, 1.21 g L^{-1} Tris-base, 1.83 g L^{-1} $CaCl_2 \cdot 2H_2O$ and 0.25 g L^{-1} $NaHCO_3$, with 0.3% (v/v) macro elemental solution and 0.3% trace elemental solution. The macro elemental solution was 75 g L^{-1} $NaNO_3$ and 5 g L^{-1} $NaH_2PO_4 \cdot H_2O$. The trace elemental solution was 4.36 g L^{-1} Na_2·EDTA, 3.16 g L^{-1} $FeCl_3 \cdot 6H_2O$, 180 mg L^{-1} $MnCl_2 \cdot 4H_2O$, 10 mg L^{-1} $CoCl_2 \cdot 6H_2O$, 10 mg L^{-1} $CuSO_4 \cdot 5H_2O$, 23 mg L^{-1} $ZnSO_4 \cdot 7H_2O$, 6 mg L^{-1} $Na_2MoO_4 \cdot 2H_2O$, 100 mg L^{-1} vitamin B_1, 0.5 mg L^{-1} vitamin B_{12} and 0.5 mg L^{-1} biotin.

2.3. Measurement of microalgal cells and growth rates

The value x_1 is optical density, which 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. Regression equations of the relationship between optical density and cell dry weight were established as previous study method [26] and shown as follows:

$$y_1 = 0.251x_1 + 0.148 \quad R^2 = 0.985 \tag{1}$$

The value y_1 is biomass concentration (g L^{-1}), and the value x_1 is optical density (A_{682}). Optical density precisely predicted both cell density ($R^2 = 0.998$; $p < 0.001$) and biomass concentration ($R^2 = 0.997$; $p < 0.001$). The optical density was used to evaluate the biomass concentration of *Chlorella* sp. MB-9 in each experiment. In the present study, we used biomass concentration (g L^{-1}) for the quantification of *Chlorella* sp. MB-9 cell density in culture.

2.4. Experimental setup of microalgal cultures aerated with H_2S

The microalgal cells were cultured in photobioreactors with a working volume of 800 mL; this is similar to what was done in our previous report [27]. The photobioreactors were placed in an incubator at 26 ± 1 °C with a surface light intensity of ~300 μmol m^{-2} s^{-1} provided by continuous, cool-white, fluorescent lights. The photobioreactor was made of glass and had a diameter of 70 mm. The gas was supplied from the bottom of the photobioreactor and was premixed with air, CO_2 and H_2S for the H_2S treatment experiments. In the gas airstream, the CO_2 concentration was 20% for all the cultures, and H_2S was adjusted to 50, 100, 150 and 200 ppm. The gas flow rate was adjusted to 0.3 vvm (volume gas per volume broth per min) using a gas flow meter (Dwyer Instruments, Michigan, IN, USA). The evaluation of tolerance to H_2S in microalgal cultures aerated with 20% CO_2 and different concentrations of H_2S was begun when the A_{682} value of the *Chlorella* sp. MB-9 cultures reached ~5.0 (approximate biomass concentration: 1.2 g L^{-1}). The microalgal cells in each treatment were sampled every 24 h for determination of biomass concentration. The calculation of growth capacity was as follows:

$$\text{Growth capacity } (\%) = \frac{\text{Average growth rate of experiment}}{\text{Average growth rate of control}} \times 100\% \tag{2}$$

The control culture was aerated with 20% CO_2 alone.

2.5. Experimental setup of microalgal cultures aerated with CH_4

CH_4 was used in a simulated experiment to evaluate microalgal cultures exposed to CH_4 aeration. The gas was prepared from pure commercial CH_4 and CO_2 cylinders and ambient air. In simulation conditions, gases containing 20%, 40%, 60% and 80% CH_4 were mixed and adjusted by gas flow meters. Evaluation of the CH_4 tolerance of the *Chlorella* sp. MB-9 cultures aerated with 20% CO_2 and different concentrations of CH_4 was begun when the A_{682} value of the culture reached ~5.0. The microalgal cells of each treatment were sampled for A_{682} measurements every 24 h. A comparison of growth capacity was made to evaluate the growth of microalgal cultures aerated with different concentrations of CH_4; growth capacities were calculated using Eq. (2). The calculation of biogas (CH_4) upgrading was as follows:

$$\text{Biogas upgrading } (\%) = \frac{\text{Effluent of } CH_4 - \text{Influent of } CH_4}{1 - \text{Influent of } CH_4} \times 100\% \tag{3}$$

2.6. Setup of outdoor microalgal cultures for CO_2 capture from biogas

The outdoor photobioreactor was cylindrical and made of acrylic polymer. The column was 300 cm in length and 16 cm in diameter, and the working volume of the photobioreactor was 50 L. The setup of the outdoor microalgae-incorporating photobioreactor system for CO_2 capture from the biogas produced from anaerobic digestion of swine wastewater on a livestock farm (Tai-Chung, Taiwan) is shown in Fig. 1. The concentrations of CH_4 and CO_2 in the biogas were 69 ± 1% and 20 ± 1%, respectively. The biogas was desulfurized by chemical absorption to reduce the H_2S concentration to <50 ppm [6]. The microalgal cultures were established in an outdoor photobioreactor with a total culture volume of 50 L. Culture aeration was controlled by a gas switch, and a gas-switching cycle was performed with a desulfurized biogas influent load for 30 min followed by an air influent load for 30 min (30 min desulfurized biogas/30 min air) for 8 h during the day. The effluent load was sampled using a gas collection bag to determine the concentrations of CO_2

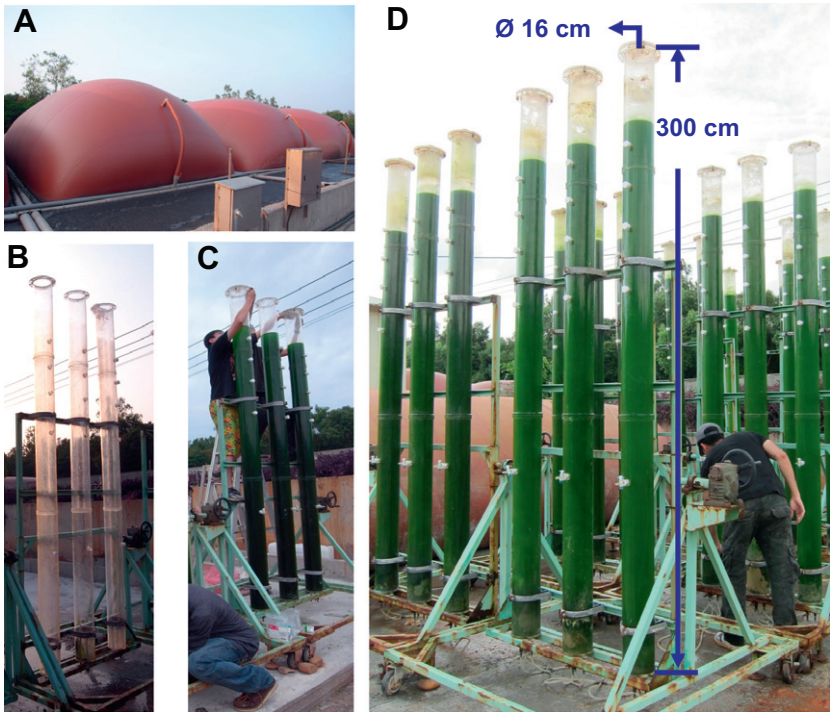

Fig. 1. The setup and operation of an outdoor microalgae-incorporating photobioreactor system for CO_2 capture from the biogas produced from anaerobic digestion of swine wastewater on a livestock farm. The pictures show biogas collection bags (A), the transparent acrylic cylindrical photobioreactor (B), setting up the photobioreactors (C) and the configuration of photobioreactor system for microalgal culture (D). The system was set up and operated on a pig farm in Tai-Chung, Taiwan.

and CH_4. The flow rate for all gases was adjusted using the gas flow meter (Dwyer Instruments). CO_2 capture efficiency (%) was calculated with the following formula:

$$CO_2 \text{ capture efficiency } (\%) = \frac{\text{Influent of } CO_2 - \text{Effluent of } CO_2}{\text{Influent of } CO_2} \times 100\% \quad (4)$$

2.7. Lipid extraction

The microalgal cells were centrifuged and washed with deionized water twice, and obtained the dry biomass by lyophilization. The dried sample (0.2 g) was mixed with methanol/chloroform solution (2/1, v/v) and sonicated for 1 h. The mixture with methanol/chloroform solution was precipitated and added chloroform and 1% NaCl solution to give a ratio of methanol, chloroform, and water of 2:2:1. The mixture was centrifuged and the chloroform phase was recovered. Finally, the lipids were weighted after chloroform was removed under vacuum by a rotary evaporator.

2.8. Microalgal lipid transesterification

Firstly, the mixture of methanol (3.4 mL), sulfuric acid (0.6 mL) and chloroform (4.0 mL) was added to the microalgal oil, and heated at 90 °C for 40 min with thoroughly mixing during heating. Second, the samples were cooled to room temperature and mixed with 2 mL dideionized water. Finally, the organic (lower) phase containing fatty acid methyl esters (FAMEs) was collected and the solvent was evaporated.

2.9. Fatty acid profile analysis

The fatty acid composition was determined FOCUS Gas Chromatograph equipped with an flame ionization detector (FID) and trace GC capillary column (Thermo Fisher Scientific), which was a cyanopropylphenyl based phase specifically designed for the separation of FAMEs. The fatty acids were identified by comparison of the retention times with those of the standards using the software Chrom-Card Data System (Thermo Fisher Scientific).

2.10. Chemical analyses

The influent and effluent loads of airstreams were sampled by a gas collection bag. CO_2 concentration was measured using a Guardian Plus Infra-Red CO_2 Monitor D-500 (Edinburgh Instruments, Livingston, UK). The detection range was from 0% to 30%. The concentration of H_2S was measured by gas detector tubes (GASTEC, Kanagawa, Japan). The concentration of CH_4 was measured by a combustible gas detector, XP-3140 (New Cosmos Electric, Osaka, Japan).

2.11. pH and light measurements

The sample pH was directly determined using an ISFET pH meter KS723 (Shindengen Electric, Tokyo, Japan). The pH meter was calibrated daily using standard solutions of pH 4 and 7. Light intensity was measured adjacent to the surface of the photobioreactor using a Basic Quantum Meter (Spectrum Technologies, Plainfield, IL, USA).

3. Results and discussion

3.1. H_2S tolerance of the microalgal cultures

In order to use the *Chlorella* sp. MB-9 for on-site cultivation by biogas, the growth potential of microalgal *Chlorella* sp. MB-9 cells exposed to H_2S aeration was evaluated. Batch cultures of *Chlorella* sp. MB-9 were incubated for 7 days at 26 ± 1 °C at 300 μmol m^{-2} s^{-1} and aerated with gas containing 0 (control), 50,

100, 150 and 200 ppm of H_2S at 0.3 vvm. All the airstreams also contained 20% CO_2.

Fig. 2 shows that the maximum growth rates and the maximum biomass concentration of microalgal cells aerated different concentrations of H_2S. The maximum growth rates of *Chlorella* sp. MB-9 aerated with 100, 50 and 0 ppm of H_2S were 0.242, 0.261 and 0.262 g L^{-1} d^{-1}, respectively. However, the maximum biomass concentration of *Chlorella* sp. MB-9 aerated with 150 and 200 ppm of H_2S was significantly lower than that aerated with 100 and 50 ppm. Afterwards, the growth of *Chlorella* sp. MB-9 aerated with 150 and 200 ppm of H_2S also decreased. These results indicate that *Chlorella* sp. MB-9 grows well under aeration with gas containing <100 ppm of H_2S.

3.2. Growth potential of the microalgal cells exposed to CH_4 aeration

Before treatment of biogas produced from the anaerobic digestion of swine wastewater, the CH_4 tolerance of the microalga *Chlorella* sp. MB-9 was evaluated. The gas was prepared with a volumetric percentage of ambient air, CO_2 and CH_4 provided by commercial pure gas cylinders. The airstreams aerating the cultures contained volumetric percentages of CH_4 of 0% (control), 20%, 40%, 60% and 80%. All of the airstreams also contained 20% CO_2. The microalgal cultures were sampled every day to evaluate growth capacity. Fig. 3A and B shows the growth of the *Chlorella* sp. MB-9 and *Chlorella* sp. (wild-type, WT) cultures aerated with different concentrations of CH_4. Fig. 3C shows the maximum growth rate of *Chlorella* sp. MB-9 and WT cultures aerated with different concentrations of CH_4. The maximum growth rates of the *Chlorella* sp. MB-9 cultures aerated with CH_4 concentrations of 20%, 40%, 60% and 80% were 0.31, 0.292, 0.276 and 0.243 g L^{-1} d^{-1}, respectively. Compared to the result of Converti et al. [28], *Arthrospira platensis* cultured by biogas aeration had a biomass productivity of 0.041 g L^{-1} d^{-1}, the biomass productivity of *Chlorella* sp. MB-9 aerated with 80% CH_4 showed a high potential for biogas upgrading and CO_2 utilization. The growth capacity of *Chlorella* sp. MB-9 aerated with 20%, 40%, 60% and 80% of CH_4 was 99%, 98%, 94% and 82% of the control growth capacity (without CH_4), respectively. These results indicate that *Chlorella* sp. MB-9 has growth potential be aerated with desulfurized biogas without significant growth inhibition.

3.3. Chlorella sp. MB-9 cultures aerated with biogas

In the outdoor field study of biogas upgrading, a system of outdoor microalgae-incorporating photobioreactors for CO_2 capture from desulfurized biogas produced from the anaerobic digestion of swine wastewater was performed (Fig. 1). The biogas initially produced from the anaerobic digestion of swine wastewater was desulfurized by a chemical absorption process to reduce the H_2S concentration to below 50 ppm [6]. Subsequently, the desulfurized biogas (H_2S < 50 ppm) was stored in a gas storage bag for CO_2 capture by the microalgal cultures in the photobioreactor controlled by a gas switch for the cycle-switching operation. The desulfurized biogas containing 69 ± 1% CH_4 and 20 ± 1% CO_2 was provided at 0.05, 0.1, 0.2 and 0.3 vvm.

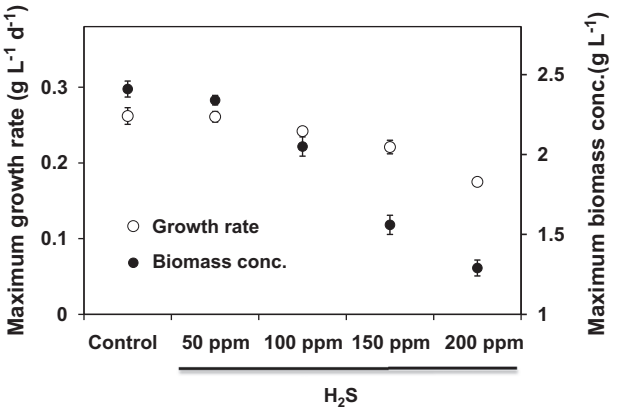

Fig. 2. Maximum growth rates and maximum biomass concentrations of *Chlorella* sp. MB-9 cultures exposed to H_2S aeration. The microalgal cells were cultivated at 300 μmol m^{-2} s^{-1} provided by continuous, cool-white, fluorescent lights. Gas was mixed with H_2S and ambient air to produce airstreams containing 0, 50, 100, 150 and 200 ppm of H_2S at 0.3 vvm.

Fig. 4A and B shows the growth of *Chlorella* sp. MB-9 cultures continuously or intermittently aerated with desulfurized biogas at 0.05, 0.1, 0.2 and 0.3 vvm. For intermittent aeration treatment, the desulfurized biogas was supplied in 30-min intervals every hour from 09:00 through 17:00; this was accomplished via a gas cycling switch with a desulfurized biogas influent load for 30 min and subsequently with an air influent load for 30 min (30 min desulfurized biogas/30 min air) for 8 h during the day. The results show that *Chlorella* sp. MB-9 grew well under desulfurized biogas aeration. Fig. 4C shows the maximum growth rates of *Chlorella* sp. MB-9 continuously or intermittently aerated with desulfurized biogas at 0.05, 0.1, 0.2 and 0.3 vvm. The maximum growth rates of *Chlorella* sp. MB-9 aerated with desulfurized biogas at 0.05, 0.1, 0.2 and 0.3 vvm were 0.322, 0.311, 0.275 and 0.251 g L^{-1} d^{-1} during continuous aeration, and 0.324, 0.321, 0.308 and 0.301 g L^{-1} d^{-1} during intermittent aeration, respectively. The maximum growth rates of *Chlorella* sp. MB-9 intermittently aerated with desulfurized biogas were higher than those of the *Chlorella* sp. MB-9 cultures continuously aerated with desulfurized biogas. This result indicates that the growth potential of *Chlorella* sp. MB-9 could be improved by intermittent aeration with desulfurized biogas. These results demonstrate that *Chlorella* sp. MB-9 can grow well in an outdoor photobioreactor aerated directly with biogas.

3.4. CO_2 capture efficiency of Chlorella sp. MB-9 cultures aerated with biogas

CO_2 capture efficiencies of the microalgal cultures in the outdoor photobioreactor system at gas flow rates of 0.05, 0.1, 0.2 and 0.3 vvm were evaluated. The average CO_2 capture efficiencies of the *Chlorella* sp. MB-9 cultures after desulfurized biogas aeration were 86.3 ± 1.9%, 80.3 ± 0.9%, 76.6 ± 1.4% and 73.7 ± 1.3% at a gas flow rate of 0.05, 0.1, 0.2 and 0.3 vvm, respectively (Table 1).

The demonstration of pattern of change in CO_2 concentration within each gas-switching cycle (30 min desulfurized biogas/30 min air) was similar over eight cycles that were operated for 8 h during the day (data not shown). In our previous study, the photobioreactor system using the cycle-switching operation could stably work for CO_2 capture from the desulfurized biogas during the 8 h interval in daytime [24]. Chiu et al. [17] recently reported that CO_2 removal from flue gas by *Chlorella* sp. MTF-7 cultures during intermittent flue gas aeration operated for 9 cycles during the day, and the patterns of the fluctuations in the values of pH and CO_2 removal efficiency were stable. These results indicate that use of the photobioreactor system with the cycle-switching operation efficiently captures CO_2 from desulfurized biogas. In the continuous biogas aeration experiments, the decrease in CO_2 capture efficiency in the microalgal cultures was caused by the continuous influent load of desulfurized biogas.

The CO_2 capture efficiency of the microalgal cultures in the outdoor photobioreactor aerated at a gas flow rate of 0.05 vvm was higher than that of the culture aerated at a gas flow rate of 0.3 vvm (Table 1). The phenomenon was showed in the previous study [17]. The coalescence of gas bubbles is the main reason for the decreasing CO_2 capture efficiency at a higher aeration rate due to the decreasing the retention time of bubbles in the

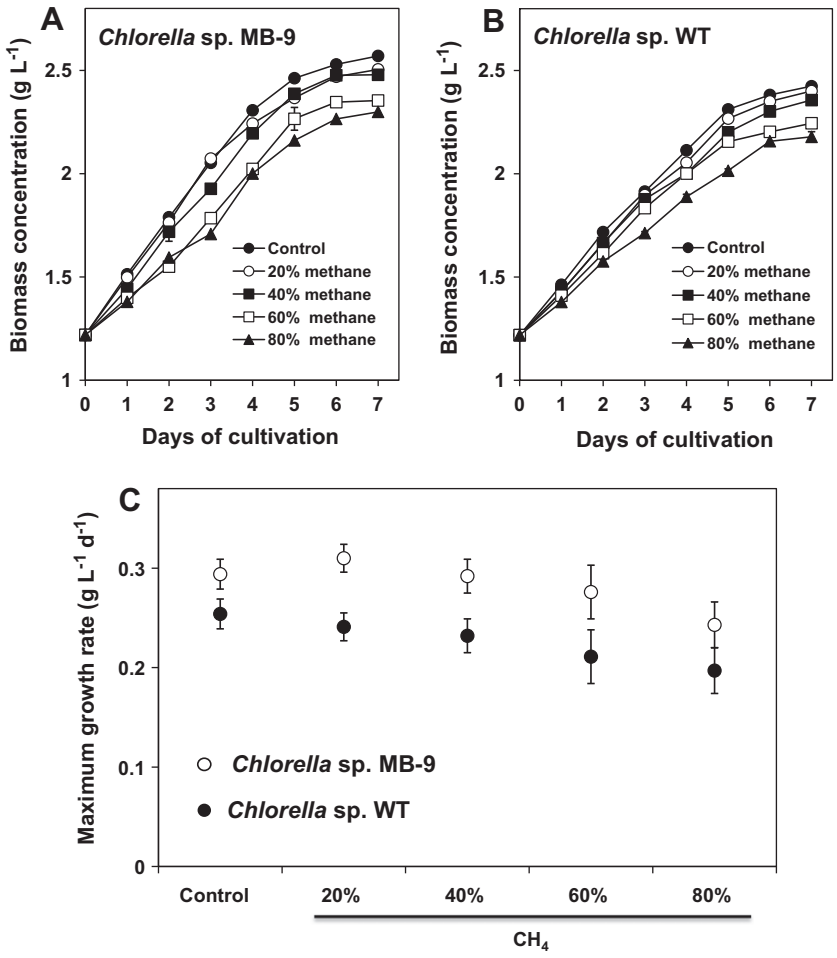

Fig. 3. Growth profiles of *Chlorella* sp. MB-9 (A) and WT (B) cultures and the maximum growth rates of *Chlorella* sp. MB-9 and WT cultures (C) aerated with different concentrations of CH_4. The microalgal cells were cultivated at 300 μmol m^{-2} s^{-1} provided by continuous, cool-white, fluorescent lights. Gas was mixed with CH_4 and ambient air to produce airstreams containing 0%, 20%, 40%, 60% and 80% CH_4 at 0.3 vvm.

photobioreactor. In addition, the decrease of bubble surface area per unit gas volume can also reduce the CO_2 capture efficiency [29,30].

3.5. Capacity of Chlorella sp. MB-9 cultures to upgrade biogas

Similar to the CO_2 capture efficiency measurements in *Chlorella* sp. MB-9 cultures, the effluent gas was sampled every 5 min during desulfurized biogas aeration within each gas-switching cycle, and the effluent load of CH_4 was measured. The CH_4 concentration in the effluent gas within each gas-switching cycle was similar and remained stable for eight cycles (data not shown).

Biogas upgrading capacity of the microalgal cultures operated in the outdoor photobioreactor at desulfurized biogas flow rates of 0.05, 0.1, 0.2 and 0.3 vvm was determined. The average effluent loads of CH_4 from the microalgal cultures after desulfurized biogas aeration were 91.1 ± 1.0%, 88.3 ± 1.5%, 86.4 ± 1.1% and 85.5 ± 1.5% at a gas flow rate of 0.05, 0.1, 0.2 and 0.3 vvm, respectively (Table 1). The results show that the ratio of the difference between influent and effluent load of CO_2 to the difference between influent and effluent load of CH_4 at a gas flow rate of 0.05, 0.1, 0.2 and 0.3 vvm were 1.27, 1.14, 1.05 and 1.07, respectively. In other words, the enrichment of CH_4 in biogas was contributed to the capture of CO_2. The photobioreactor system was used as a CO_2 bioscrubber for biogas upgrading. The higher ratio responding to the lower aeration rate may due to other substances absorption from biogas by microalgal cultures. The other substances in biogas such as, NH_3, could be absorbed in microalgal cultures [2]. The lower aeration rate contributed to higher gas retention time so that this may be the main reason that the higher ratio of the enrichment of CH_4 to the capture of CO_2 responding to the lower aeration rate.

Summarizing the results of CO_2 capture efficiency and biogas upgrading capacity of *Chlorella* sp. MB-9 cultures that were exposed to intermittent desulfurized biogas aeration, the effluent load of CH_4 could be increased up to approximately 80% from its original 70%, and the CO_2 capture efficiency could reach 50% during 30 min of biogas aeration at a gas flow rate of 0.05 vvm. Moreover, the efficiencies of CO_2 capture and biogas upgrading by the microalgal cultures could be stably maintained by the gas (biogas/air) cycle-switching operation.

3.6. Operation of a double set of photobioreactor systems

The gas cycle-switching operation developed in the present study was also extended to a double set of photobioreactor systems, and the capacity of each set was 150 L (Fig. 1). This double set of photobioreactor systems was alternately aerated with biogas at a gas flow rate of 0.05 vvm. Via the gas cycle-switching operation, the biogas could be used for continuous CO_2 capture. The results show that the ability to capture CO_2 (about 50% of CO_2 in the biogas was captured) and upgrade biogas (about a 10% increase in CH_4 concentration in the effluent load was achieved) were similar and

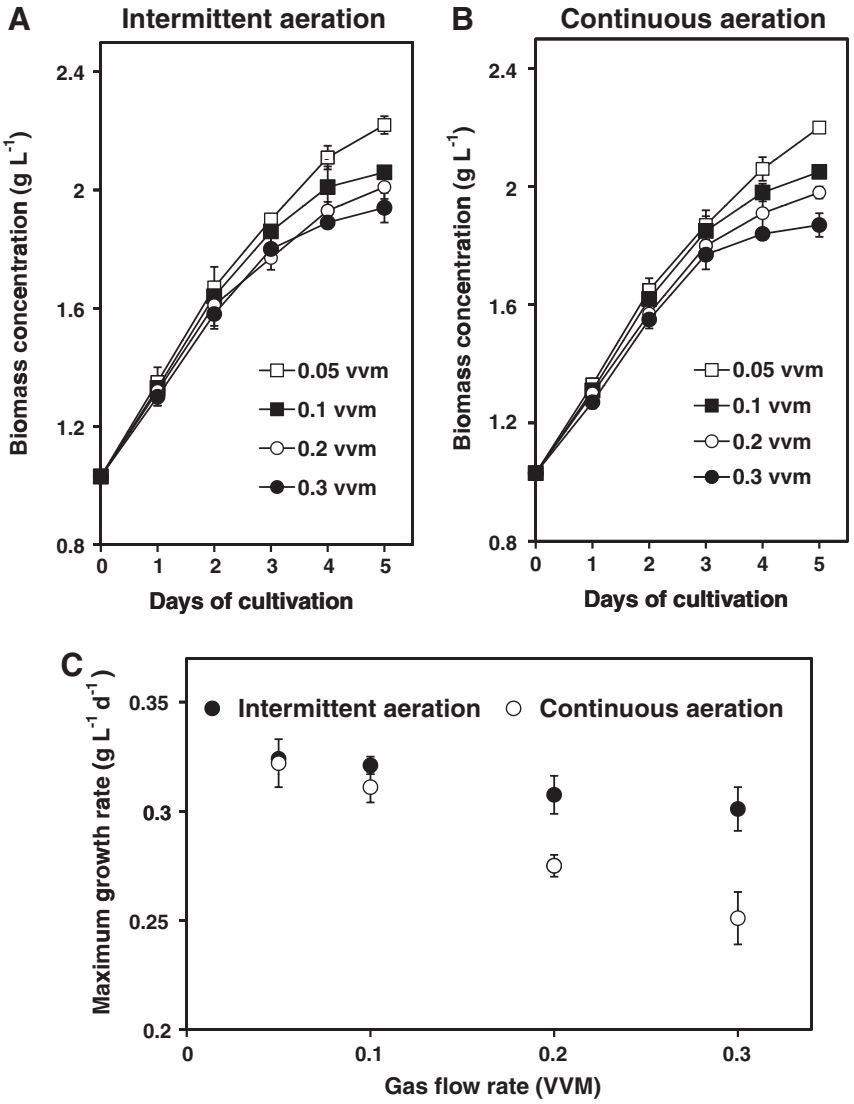

Fig. 4. The growth profiles (A and B) and the maximum growth rates (C) of *Chlorella* sp. MB-9 cultures cultivated in an outdoor photobioreactor system intermittently and continuously aerated with desulfurized biogas at gas flow rates of 0.05, 0.1, 0.2 and 0.3 vvm.

Table 1
Parameters of biogas upgrading by *Chlorella* sp. MB-9 cultured in the outdoor photobioreactor using the cycle-switching operation at different gas flow rate.

Gas flow rate	0.05 vvm	0.1 vvm	0.2 vvm	0.3 vvm
Influent of CO_2 (%)	20.3 ± 1.1	20.3 ± 1.1	20.3 ± 1.1	20.3 ± 1.1
Effluent of CO_2 (%)[a]	3.2 ± 0.6	3.8 ± 0.2	4.2 ± 0.3	5.3 ± 0.1
Efficiency of CO_2 removal (%)[b]	**86.3 ± 1.9**	**80.3 ± 0.9**	**76.6 ± 1.4**	**73.7 ± 1.3**
Influent of CH_4 (%)	69.4 ± 0.9	69.4 ± 0.9	69.4 ± 0.9	69.4 ± 0.9
Effluent of CH_4 (%)[a]	91.1 ± 1.0	88.3 ± 1.5	86.4 ± 1.1	85.5 ± 1.5
Biogas (CH_4) upgrading (%)[c]	**69.3 ± 4.7**	**63.4 ± 4.1**	**59.2 ± 3.6**	**52.1 ± 4.2**

[a] Effluent of CO_2 (%) and CH_4 (%) was measured from the effluent load of biogas.
[b] The CO_2 capture efficiency (%) was determined by the following formula: $\frac{\text{Influent of } CO_2 - \text{Effluent of } CO_2}{\text{Influent of } CO_2} \times 100\%$.
[c] The biogas (CH_4) upgrading (%) was determined by the following formula: $\frac{\text{Effluent of } CH_4 - \text{Influent of } CH_4}{1 - \text{Influent of } CH_4} \times 100\%$.

stable during biogas aeration for each set of photobioreactor systems in a continuous operation. The intermittent biogas aeration strategy via the double set of photobioreactor systems for cultivation could enhance microalgal growth, increase the utilization of the CO_2 in the biogas and also enhance the efficiency of biogas upgrading. Due to the concerns of environmental and economic aspect increasing, the environmental and economic issues should be also considered. There are many literatures discussed the environmental and economic issues [31–33]. Yang et al. [33] mentioned that the rise of biofuel development will significantly increase the prices of biofuel feedstock crops. Thus, the source of biofuel production should come from non-feedstock. Ren et al. [32] described that the way to increase the share of renewable energy in total energy supply, the biomass is thought to be the most potential renewable energy source. Microalgae are the potential candidate for biomass and biofuel production. For the economic objective satisfaction, installing the gasification equipment besides the biomass resources is the promising strategy that it will reduce the transportation cost [32]. In their study of trade-off relationship between economic and environmental characteristics, minimizing the annual energy cost contributed to the increasing the CO_2 emission [32]. In addition, Mussgnug et al. [34] concluded that the residues of microalgal biomass for anaerobic fermentation can seriously be considered as final step in future microalgae-based biorefinery concepts. Collet et al. [35] also mentioned that the biogas production from microalgal biomass by anaerobic fermentation strongly competes with others biofuel productions. This could be benefit to the economic

Table 2
Main fatty acid compositions in *Chlorella* sp. MB-9 aerated with enriched CO_2 gas and biogas[a].

	MB-9 with 20% CO_2	MB-9 with biogas (intermittent aeration)	MB-9 with biogas (continuous aeration)
Lipid content (% dry weight)	25.7 ± 1.3	22.9 ± 0.8	22.8 ± 0.6
Fatty acid composition	Relative content (%)		
16:0	52.6 ± 1.1	49.8 ± 2.6	48.8 ± 3.7
18:0	8.1 ± 0.2	7.4 ± 0.5	7.2 ± 0.5
18:1n9t	4.4 ± 0.3	8.2 ± 0.1	9.4 ± 0.8
18:1n9c	1.0 ± 0.1	3.5 ± 0.5	2.1 ± 0.1
18:2n6c	1.8 ± 0.2	1.3 ± 0.2	2.4 ± 0.3
18:3n6	11.8 ± 0.4	8.6 ± 0.6	9.4 ± 1.2
18:3n3	11.5 ± 0.4	11.8 ± 0.7	12.2 ± 0.2
Others	8.8 ± 1.1	9.4 ± 1.0	8.5 ± 0.9

Each data shown is the value presented in mean ± SE from three independent samples.
[a] For gas chromatograph analysis of fatty acid composition, all the fatty acids were transesterified into fatty acid methyl ester.

aspect of microalgal bioenergy. However, microalgal bioenergy process should be still improved to be economical. Razon and Tan [36] described the net energy analysis of biofuels from microalgae that even with optimistic assumptions regarding the performance of processing units, the results show a large energy deficit, due mainly to the energy required to culture and dry the microalgae or to disrupt the cell. Thus, until now, after the growing of microalgae and harvesting the microalgal biomass, the residues or the whole microalgal biomass for biogas production by anaerobic fermentation is now a possible strategy for being economical of microalgal biofuel production.

3.7. Lipid analysis

To investigate the effects of lipid content and lipid profiles of *Chlorella* sp. MB-9 aerated with desulfurized biogas, microalgal cells were harvested after 5 days of aeration with desulfurized biogas. A *Chlorella* sp. MB-9 culture aerated with enriched CO_2 alone (20%) was used as a control culture. The lipid contents of *Chlorella* sp. MB-9 aerated intermittently and continuously with desulfurized biogas and enriched CO_2 alone (20%) were similar (22.9 ± 0.8 and 22.8 ± 0.6 vs. 25.7 ± 1.3% dry weight). The lipid content of *Chlorella* sp. MB-9 aerated with desulfurized biogas was only slightly decreased. The lipid profile of *Chlorella* sp. MB-9 aerated with desulfurized biogas or enriched CO_2 alone (20%) was also investigated (Table 2). However, there were no significant differences between the cultures aerated with desulfurized biogas and those aerated with enriched CO_2 alone (20%). These results indicate that *Chlorella* sp. MB-9 aerated with desulfurized biogas can efficiently grow, capture CO_2 and upgrade biogas with no significant effects on lipid productivity.

4. Conclusion

The present study demonstrates that the microalga *Chlorella* sp. MB-9 was a potential strain which was able to utilize CO_2 for growth when aerated with desulfurized biogas (H_2S < 50 ppm) produced from the anaerobic digestion of swine wastewater. The demonstrated system can be continuously used to upgrade biogas by utilizing a double set of photobioreactor systems and a gas cycle-switching operation. Furthermore, our field study demonstrated that the efficiency of CO_2 capture from biogas could be maintained at 50% on average, and the CH_4 concentration in the effluent load could be maintained at 80% on average, i.e., upgrading was accomplished by increasing the CH_4 concentration in the biogas produced from the anaerobic digestion of swine wastewater by 10%.

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