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A mutant strain of microalga *Chlorella* sp. for the carbon dioxide capture from biogas

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ABSTRACT

To upgrade biogas produced from the anaerobic digestion of swine wastewater, an outdoor photobioreactor was established in this study. A mutant strain of microalga *Chlorella* sp. MM-2 was firstly isolated by ethyl methane sulfonate-induced random mutagenesis. The *Chlorella* sp. MM-2 grew in the presence of gas containing $H_2S < 100$ ppm, and the growth capacity of the microalgal culture aerated with 80% CH_4 was ~70% that of the control culture (0% CH_4). In the field study, CO_2 capture efficiency of the *Chlorella* cultures, at a biomass concentration of 1.2 g L^{-1}, from the desulfurized biogas (~20% CO_2, ~70% CH_4 and $H_2S < 100$ ppm) was approximate 70% on cloudy days and 80% on sunny days. CH_4 concentration in the effluent biogas from the *Chlorella* cultures was increased to approximate 84% on cloudy days and 87% on sunny days from its original 70%. The established outdoor photobioreactor system using a gas cycle-switching operation could be used as a CO_2 capture model for biogas upgrading.

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1. Introduction

Global warming, which is induced by increasing concentrations of greenhouse gases in the atmosphere, is of great concern. There are several means of reducing the emissions of greenhouse gases by energy production from renewable sources. This issue has received increasing attention due to the exhaustion of natural sources of fossil fuels [1]. Biogas, a mixture of methane (CH_4) and carbon dioxide (CO_2) with hydrogen sulfide (H_2S) and several minor hydrocarbons, is produced from the anaerobic digestion of biological waste. It is an environment-friendly, clean and cheap fuel [2]. The main biogas resource in Taiwan is produced from the anaerobic digestion of swine wastewater. Raw biogas contains approximately 55–75% CH_4, 20–35% CO_2, 5–10% N_2 and 3000–5000 ppm H_2S. The CH_4 in biogas can be upgraded to the same standards as fossil natural gas by H_2S removal and CO_2 capture. The biogas produced from anaerobic digestion is a potential fuel for power generator [3]. However, the trace 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 used to remove H_2S from industrial and agricultural emission sources have been proposed [4–6]. After H_2S removal, however, the high CO_2 content of biogas reduces its calorific value and increases carbon monoxide and hydrocarbon emissions if desulfurized biogas is used as engine fuel [7,8]. Desulfurized biogas may require CO_2 capture to

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reduce its CO_2 concentration in order to improve engine efficiency [7]. In addition, the high CO_2 content makes the desulfurized biogas uneconomical to be compressed and transported. A biogas upgrading process can be applied in order to increase the calorific value, minimize corrosion problems, promote it to pseudo-natural gas quality and connect it to a pipeline for network distribution [1,9]. Therefore, CO_2 capture is also essential for increasing the utility of biogas.

Various research strategies on CO_2 sequestration, including physical, chemical and biological methods, have been carried out. The biological method of microalgal fixation of CO_2 by photosynthesis to convert CO_2 into a carbon source of biomass is the best potential method for CO_2 sequestration [10–13]. Microalgae have higher CO_2 fixation rates than terrestrial plants and can thus utilize CO_2 from flue gas to produce biomass [14,15]. Microalgal biomass can be used for biofuel production by pyrolysis, direct combustion or thermal chemical liquefaction [16]. The lipid fraction of microalgal biomass can be extracted and transesterified for biodiesel production [17–19].

For CO_2 capture from biogas, physical and chemical absorption methods are generally applied with fewer complications; however, these methods are needed to post-treat the waste materials for regeneration of cycling utilization. The biological methods of CO_2 capture from biogas are potentially useful and need to be evaluated. In this study, we established an outdoor photobioreactor system for CO_2 capture from desulfurized biogas produced from the anaerobic digestion of swine wastewater. For this system, the growth profiles of an isolated microalga, *Chlorella* sp. MM-2, cultivated with different concentrations of H_2S and CH_4 were evaluated. Finally, a field study of CO_2 capture from the desulfurized biogas produced from the anaerobic digestion of swine wastewater was implemented.

2. Methods

2.1. Microalga

Wild-type microalga *Chlorella* sp. obtained from the collection of Taiwan Fisheries Research Institute (Tung-Kang, Ping-Tung, Taiwan) was used to isolate the mutant that could stably grow under biogas aeration. The protocol of chemical mutagenesis and mutant isolation was followed our previous report [20]. In brief, about 5×10^7 cells of *Chlorella* sp. were treated with 25–100 mM ethyl methane sulfonate (EMS) for 1 h, and each approximate 1×10^3 cells were plated on agar plates. The plates were then cultured in a closed photobioincubator filled with biogas. The culture environment was filled with biogas; thus, the mutagen-treated microalgal cells exposed to biogas. After 5–7 days of adaptive culture, the bigger and green colonies were selected for scale up to 5 mL tube cultivation. The candidates of microalgae were sequentially seeded and grew in 100 mL flaks to verify their growth capacity under biogas aeration. In the present study, a mutant strain of *Chlorella* sp. MM-2 was obtained, the microalga was stable and able to grow under aeration with biogas.

2.2. Microalgal cultures, medium and chemicals

The *Chlorella* sp. MM-2 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} $Na_2 \cdot$ 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. The pH value of the initial growth medium was 7.4–7.6.

2.3. Measurement of microalgal cells, growth rate

Cell density (cells mL^{-1}) and biomass concentration (dry weight per liter) of cultures were measured according to the method reported previously [21]. Regression equations of the relationship between optical density and cell dry weight were established and shown as follows:

$$y_1 = 183.97x_1 - 9.1249 \quad R^2 = 0.998 \tag{1}$$

The value y_1 is cell density (10^5 cells mL^{-1}). This value was determined by a direct microscopic count performed on each sample of microalgal suspension using a Brightline Hemacytometer (BOECO, Hamburg, Germany) and a Nikon Eclipse TS100 inverted metallurgical microscope (Nikon Corporation, Tokyo, Japan). The value x_1 is optical density 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. The Eq. (2) was used to calculate the biomass concentration.

$$y_2 = 0.232x_2 + 0.054 \quad R^2 = 0.997 \tag{2}$$

The value y_2 is biomass concentration (g L^{-1}), and the value x_2 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. MM-2 in each experiment. In the present study, we used biomass concentration (g L^{-1}) for the quantification of *Chlorella* sp. MM-2 cell density in the 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 [22]. The photobioreactors were placed in an incubator at 26 ± 1 °C with a surface light intensity ~300 μmol m^{-2} s^{-1} provided by continuous, cool-white, fluorescent lights. The photobioreactor was made of glass, and the diameter of the photobioreactor was 70 mm. The gas was supplied from the bottom of the photobioreactor. The gas was premixed with air, CO_2 and H_2S for the H_2S treatment experiments. In the gas air stream, CO_2

concentration was 5% for all the cultures, and H_2S was adjusted to 50, 100, 150 or 200 ppm. The gas flow rate was adjusted by a gas flow meter (Dwyer Instruments, Inc., Michigan city, IN, USA) to give a flow rate of 0.3 vvm (volume gas per volume broth per min). The evaluation of the H_2S tolerance of the microalgal cultures aerated with 5% CO_2 and different concentrations of H_2S was began when the A_{682} value of the *Chlorella* sp. MM-2 cultures reached ~5.0 (approximate biomass concentration: 1.2 g L^{-1}). The microalgal cells of each treatment were sampled for determination of the biomass concentration every 24 h. The calculation of growth capacity was as follows:

$$\text{Growth capacity}(\%) = \frac{\text{average growth rate of experiment}}{\text{average growth rate of control}} \times 100\% \qquad (3)$$

The control culture was aerated with only 5% CO_2.

2.5. Experimental setup of microalgal cultures aerated with CH_4

The CH_4 was used in a simulated experiment to evaluate the effect of CH_4 aeration on microalgal biomass growth. The gas was prepared from pure commercial CH_4 and CO_2 cylinders and ambient air. In simulation conditions, gas containing 20, 40, 60 or 80% CH_4 were mixed and adjusted by gas flow meters. First, *Chlorella* sp. MM-2 was cultured in 2% CO_2. The evaluation of the CH_4 tolerance of the cultures aerated with 5% CO_2 and different concentrations of CH_4 was started when the A_{682} value of the *Chlorella* sp. MM-2 culture reached ~5.0 (approximate biomass concentration: 1.2 g L^{-1}). The microalgal cells of each treatment were sampled for A_{682} measurements every 24 h. The comparison of growth capacity was used to evaluate the growth of microalgal cultures aerated with different concentration of CH_4. Growth capacity was calculated with Eq. (3).

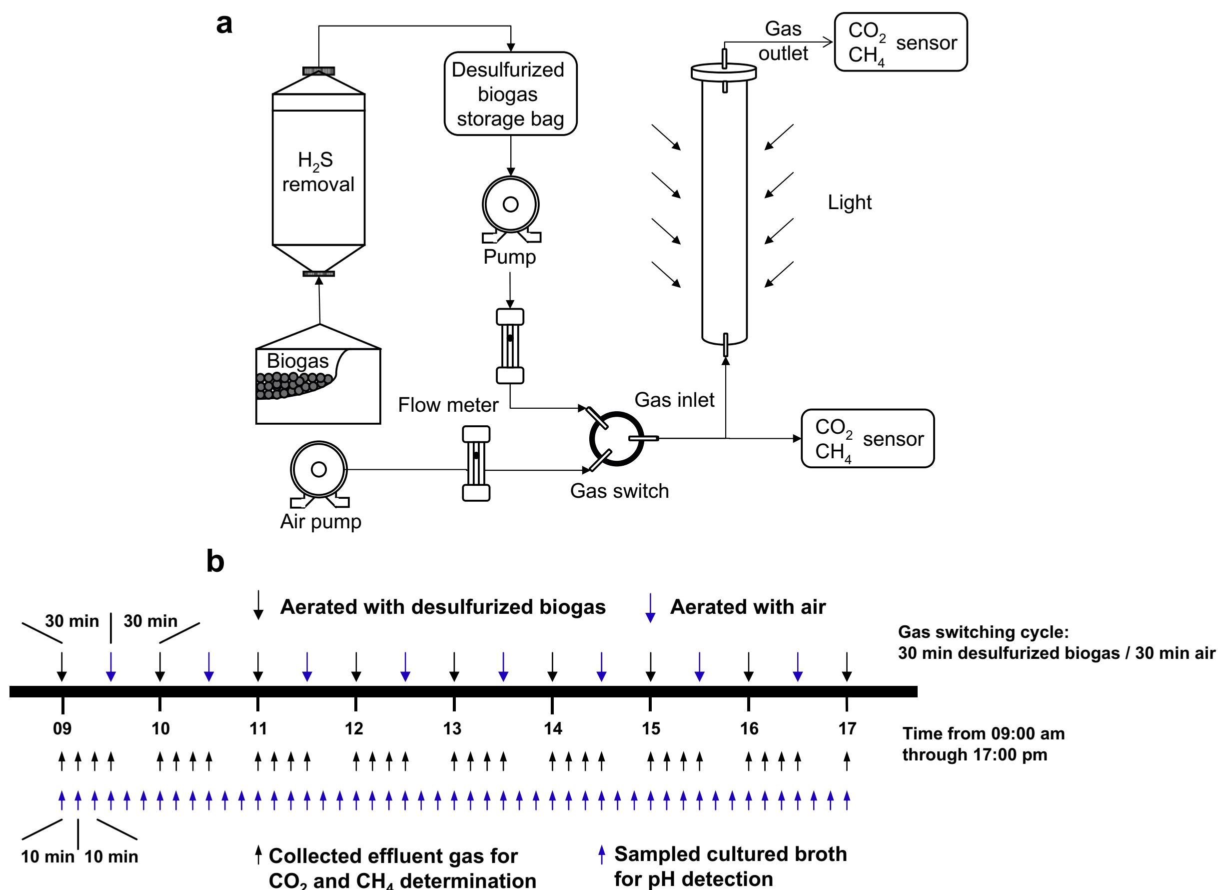

Fig. 1 – Schematic diagram of an outdoor photobioreactor system for CO_2 capture. (a) The provided biogas was desulfurized by chemical methods for H_2S removal. After biogas desulfurization, desulfurized biogas was stored in a biogas storage bag and then pumped into the photobioreactor for CO_2 capture. The desulfurized biogas was aerated into the photobioreactor by a cycle-switching operation, i.e., the desulfurized biogas was aerated for 30 min, followed by air for 30 min in a cycle controlled by the gas switch. (b) The biogas cycle-switching operation, effluent gas collection and culture sampling are shown. The upper part of the time course is the gas-switching cycle (30 min desulfurized biogas/30 min air aeration) for 8 h. The large arrows indicate the times when the gas supply was switched between desulfurized biogas and air. The small arrows indicate the times when effluent gas was collected for CO_2 and CH_4 determinations or culture broth was sampled for pH detection.

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 2.5 m in length and 20 cm in diameter. The working volume of the photobioreactor was 40 L [20]. The gas flow rate was adjustable by a gas flow meter (Dwyer Instruments, Michigan, IN, USA). The source of biogas was from the anaerobic digestion of swine wastewater on a livestock farm (Miao-Li, Taiwan). The concentrations of CH_4, CO_2 and N_2 in biogas produced from the anaerobic digestion of swine wastewater were 70 ± 5%, 20 ± 2% and 8 ± 3% (Oct. 1–Oct. 30, 2009), respectively. The biogas was desulfurized by chemical absorption in order to limit the H_2S concentration to below 100 ppm [5,6]. The microalgal cultures were performed in an outdoor photobioreactor with a total culture volume of 40 L. Culture aeration was controlled by a gas switch, and a gas-switching cycle was performed with desulfurized biogas influent load for 30 min followed by air influent load for 30 min (30 min desulfurized biogas/30 min air) for 8 h in daytime. The effluent load was sampled by a gas collection bag to determine the concentrations of CO_2 and CH_4. CO_2 capture efficiency (%) was calculated with the following formula:

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

The CO_2 elimination capacity (g m^{-3} h^{-1}) was followed as the method reported by Devinny et al. [23] and Jacob-Lopes et al. [24]. It is determined by the influent and effluent loads of CO_2 gas in streams.

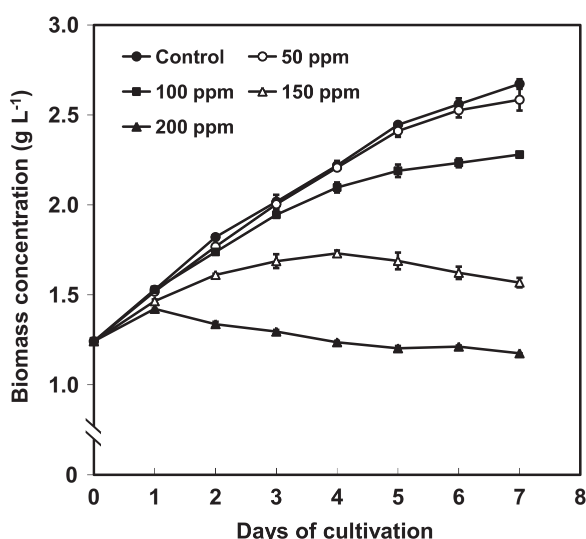

Fig. 2 – The H_2S effects on the growth of *Chlorella* sp. MM-2 cultures. The microalgal cells were cultivated at 300 μmol m^{-2} s^{-1} provided by continuous, cool-white, fluorescent lights. Gas was mixed with CO_2, H_2S and ambient air to produce airstreams containing 0, 50, 100, 150 or 200 ppm of H_2S at 0.3 vvm. The cultures were grown for 7 days, and the microalgal cells were sampled for growth determination every 24 h.

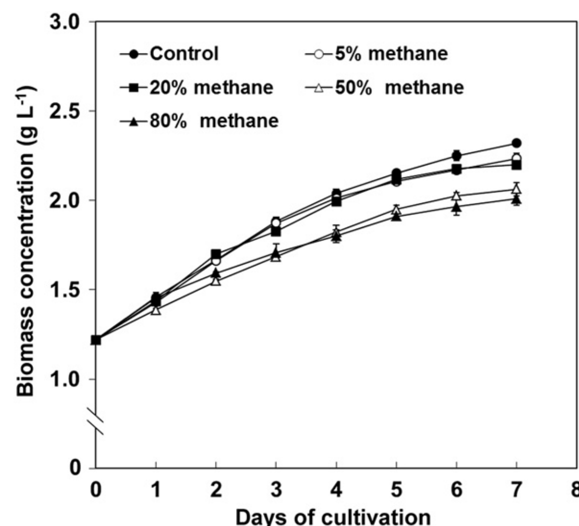

Fig. 3 – CH_4 effects on the growth of *Chlorella* sp. MM-2 cultures. The microalgal cells were cultivated in the outdoor photobioreactor system. The light intensity was at ~1500 μmol m^{-2} s^{-1} in daytime. Gas was mixed with ambient air, CO_2, and CH_4 to produce airstreams containing 0, 20, 40, 60 or 80% of CH_4 at 0.3 vvm. The cultures were grown for 7 days, and microalgal cells were sampled for growth determination every 24 h.

2.7. Chlorella sp. MM-2 cultures aerated with biogas

In the field study of biogas upgrading, an outdoor photobioreactor for CO_2 capture from the desulfurized biogas produced from the anaerobic digestion of swine wastewater was performed (Fig. 1a). The biogas initially produced from the anaerobic digestion of swine wastewater was desulfurized by a chemical absorption process to limit the H_2S concentration to below 100 ppm [5,6]. Subsequently, the desulfurized biogas (H_2S < 100 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 70 ± 5% CH_4, 20 ± 2% CO_2 and 8 ± 3% N_2 (Oct. 1–Oct. 30, 2009) was provided at 0.1 and 0.3 vvm.

The desulfurized biogas was supplied in 30-min intervals every hour from 09:00 through 17:00; i.e., a gas cycling switch was performed with desulfurized biogas influent load for 30 min and subsequently with air influent load for 30 min (30 min desulfurized biogas/30 min air) for 8 h in daytime (Fig. 1b). The initial microalgal culture density was ~1.2 g L^{-1}. The influent and effluent loads of gas were sampled for determinations of CO_2 and CH_4 concentrations every 10 min in the desulfurized biogas aeration time. Moreover, the culture broth was continuously monitored for pH changes every 10 min during the experiment.

2.8. 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).

2.9. 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).

3. Results and discussion

3.1. H_2S tolerance of the microalgal cultures

To test whether the microalgal cells could grow in conditions of aeration with biogas produced from the anaerobic digestion of swine wastewater and containing trace H_2S, the growth potential of microalgal *Chlorella* sp. MM-2 cells exposed to H_2S aeration was evaluated. The *Chlorella* sp. MM-2 in batch cultures were incubated for 7 days at 26 ± 1 °C at 300 μmol m^{-2} s^{-1} and were aerated with gas containing 0 (control), 50, 100, 150 or 200 ppm of H_2S at 0.3 vvm.

Fig. 2 shows that the growth of microalgal cells was greatly inhibited when the culture was aerated with more than 150 ppm of H_2S. The average growth rates of *Chlorella* sp. MM-2 aerated with 100, 50 and 0 ppm of H_2S were 0.214 ± 0.004, 0.241 ± 0.002 and 0.244 ± 0.003 g L^{-1} d^{-1}, respectively. The growth of microalgal cells was significantly inhibited on day 2 and day 1 of the cultures aerated with 150 ppm and 200 ppm of H_2S, respectively. The growth capacities of *Chlorella* sp. MM-2 aerated with 50 ppm and 100 ppm of H_2S were 99% and 87%, respectively, of the control growth capacity (0 ppm H_2S). These results indicate that *Chlorella* sp. MM-2 could grow under aeration with gas containing $H_2S < 100$ ppm.

The pH values of the microalgal cultures aerated with 0, 50, 100, 150 and 200 ppm of H_2S maintained at 7.9 ± 0.1, 7.8 ± 0.1, 7.5 ± 0.2, 7.1 ± 0.1 and 6.7 ± 0.2, respectively. The dissociation constant for carbonic acid was calculated using a thermodynamic model [25] that permits the prediction of the dissociation constant of inorganic carbon in sea water as a function of temperature, pH and salinity. Under the cultivation conditions (temperature ranging from 25 to 35 °C and salinity = 36) applied in the present study, the calculated pK_1 and pK_2 were 5.8–5.7 and 8.9–8.8, respectively. The calculated dissociation constant indicates that the major species of carbonic acid in the artificial sea water was $HCO_{3\,(aq)}^{-}$ at pH 6.7. Variations in pH affect the bioavailability of nutrients and the transport of substrates across cytoplasmic membrane of microalgae [26].

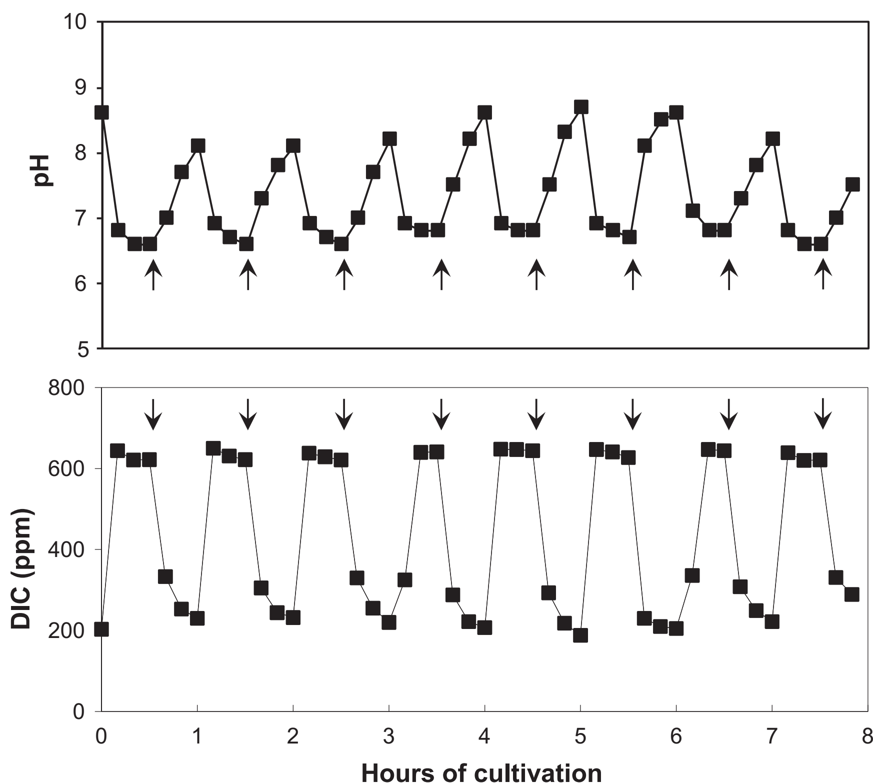

Fig. 4 – pH and dissolved inorganic carbon (DIC) variations in the microalgal culture. The *Chlorella* sp. MM-2 was cultured in the photobioreactor system intermittently aerated with desulfurized biogas. The cultures were performed at ~1500 μmol m^{-2} s^{-1} on a sunny day. Desulfurized biogas containing 70 ± 5% CH_4, 20 ± 2% CO_2, 8 ± 3% N_2 and <100 ppm H_2S was aerated at a gas flow rate of 0.1 vvm. The microalgal cells were cultivated for 8 h, and the culture broth was sampled every 10 min for the pH and DIC measurement. The arrows indicate the times when the gas supply was switched from desulfurized biogas to air.

Table 1 – Parameters of biogas upgrading by *Chlorella* sp. MM-2 cultured in the outdoor photobioreactor using the cycle-switching operation at a gas flow rate of 0.1 vvm.

Weather	Cloudy day (~800 μmol m^{-2} s^{-1})			Sunny day (~1500 μmol m^{-2} s^{-1})		
Biogas aeration time	10 min	20 min	30 min	10 min	20 min	30 min
Influent of CO_2 (%)	20.0 ± 1.0	20.1 ± 0.6	20.2 ± 0.8	20.0 ± 1.0	20.0 ± 0.5	20.0 ± 1.0
Effluent of CO_2 (%)	6.0 ± 1.0	15.1 ± 0.3	18.8 ± 0.7	4.1 ± 0.8	9.8 ± 0.8	14.2 ± 1.0
Efficiency of CO_2 removal (%)	72	25	7	80	51	29
Influent of CH_4 (%)	70.3 ± 1.0	70.5 ± 1.7	69.8 ± 1.2	71.1 ± 2.5	69.6 ± 2.9	69.0 ± 2.1
Effluent of CH_4 (%)	85.2 ± 1.5	75.3 ± 0.8	71.9 ± 0.6	87.4 ± 2.3	80.3 ± 4.0	75.3 ± 1.8

pH also affects enzyme activity and electron transport in photosynthesis and respiration; therefore, decreased pH would affect microalgal growth [27]. According to our previous study, *Chlorella* sp. can grow well at pH 6.8 [21]. Therefore, we propose that the growth of *Chlorella* sp. MM-2 was inhibited due to exposure to concentrated H_2S and not primarily because of the pH. Espie et al. [28] found that H_2S actively inhibits CO_2 transport in cyanobacteria. However, trace H_2S dissolved in water is oxidized to sulfate by photosynthetic microorganisms [29]. Therefore, at lower H_2S aeration (<100 ppm H_2S), *Chlorella* sp. MM-2 may sufficiently convert H_2S to sulfate to reduce the microalgal cell toxicity.

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

Before the treatment of biogas produced from the anaerobic digestion of swine wastewater, the microalgal CH_4 tolerance experiment was performed. *Chlorella* sp. MM-2 was cultured in an outdoor photobioreactor (40 L working volume). 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 20, 40, 60 and 80%. The microalgal cultures were sampled to evaluate growth capacity. Fig. 3 shows the growth potential of the microalgal cultures aerated with different concentrations of CH_4. The average growth rate of the control culture without CH_4 was 0.164 g L^{-1} d^{-1}. The average growth rate of the microalgal cultures aerated with CH_4 concentrations of 20, 40, 60 and 80% were 0.159, 0.155, 0.121 and 0.116 g L^{-1} d^{-1}, respectively. Converti et al. [30] reported that *A. platensis* shows potential for biogas upgrading and has a biomass productivity of 0.041 g L^{-1} d^{-1}. The biomass productivity of *Chlorella* sp. MM-2 aerated with 80% CH_4 showed high potential for biogas upgrading and CO_2 utilization. The growth capacity of *Chlorella* sp. MM-2 aerated with 20, 40, 60 and 80% of CH_4 were 97, 95, 74, and 71%, respectively, of the control capacity (without CH_4). These results indicate that the microalgal culture could be aerated with desulfurized biogas (pretreated for H_2S removal) produced from the anaerobic digestion of swine wastewater without significant growth inhibition.

3.3. pH profile of *Chlorella* sp. MM-2 cultures aerated with biogas

During the 8-h daytime desulfurized biogas/air aeration, the culture broth was sampled for pH measurements every 10 min Fig. 4 shows the pH and dissolved inorganic carbon (DIC) variations of the microalgal cultures in the photobioreactor. In the biogas/air-switching aeration cycle, increasing effluent load of CO_2 contributed to the pH decrease during the biogas aeration interval. The pH value decreased from 8.7 ± 0.2 to 6.5 ± 0.1 after 30 min of biogas aeration. After the gas was switched to air aeration, the pH value returned to 8.7 ± 0.2 after 30 min of air introduction. The fluctuations of DIC in the microalgal culture broth also followed a repetitive pattern during the gas-switching aeration cycles (Fig. 4). In addition, there was no significantly change of pH after 30 min air aeration in the blank experiment. This implied that the pH increasing (the decreased DIC) during the air aeration was due to the presence of microalgae. According to the evidence, we supposed that pH recovery in the microalgal culture during air aeration is due that the dissolved CO_2 in the broth was utilized by microalgal cells for the growth by photosynthesis. This result was confirmed by the previous report that showed a contrast change on pH and dissolved CO_2 in the microalgal cultures operated by a flue gas/air-switching aeration cycle [31]. The recovered pH values of the cultures allowed them to absorb CO_2 efficiently once again. In addition, a blank

Table 2 – Parameters of biogas upgrading by *Chlorella* sp. MM-2 cultured in the outdoor photobioreactor using the cycle-switching operation at a gas flow rate of 0.3 vvm.

Weather	Cloudy day (~800 μmol m^{-2} s^{-1})			Sunny day (~1500 μmol m^{-2} s^{-1})		
Biogas aeration time	10 min	20 min	30 min	10 min	20 min	30 min
Influent of CO_2 (%)	20.0 ± 1.0	20.1 ± 0.6	20.2 ± 0.8	20.0 ± 1.0	20.0 ± 0.5	20.0 ± 1.0
Effluent of CO_2 (%)	9.1 ± 0.2	17.8 ± 0.7	19.6 ± 0.2	5.3 ± 0.1	16.0 ± 0.7	19.5 ± 0.6
Efficiency of CO_2 removal (%)	55	11	3	74	22	3
Influent of CH_4 (%)	70.3 ± 1.0	70.5 ± 1.7	69.8 ± 1.2	71.1 ± 2.5	69.6 ± 2.9	69.0 ± 2.1
Effluent of CH_4 (%)	81.3 ± 1.5	73.3 ± 0.8	70.3 ± 0.6	85.5 ± 1.5	75.4 ± 0.8	71.8 ± 1.4

experiment (without microalgae) was performed. The blank culture (without microalgae) was aerated with air after aerating with the biogas (approximate 70% CH_4, 20% CO_2 and 8% N_2) 30 min. After biogas aeration for 30 min, the gas switch was shifted to air aeration. The pH value did not show a significantly change after air aeration for 30 min. This also implied that the pH increasing (the decreased of dissolved inorganic carbon; DIC) during the air aeration was due to the presence of microalgae. The dissolved CO_2 was the carbon source for microalgal uptake and for photosynthesis.

3.4. CO_2 capture and the growth of Chlorella sp. MM-2 cultures aerated with biogas

CO_2 capture efficiencies of the microalgal cultures operated in the outdoor photobioreactor system on a cloudy and a sunny day at gas flow rates of 0.1 and 0.3 vvm were evaluated. The CO_2 capture efficiencies of the *Chlorella* sp. MM-2 cultures at 10, 20 and 30 min after desulfurized biogas aeration were 72 ± 2, 25 ± 1 and 7 ± 1%, respectively, on the cloudy day and 80 ± 4, 51 ± 4 and 29 ± 5%, respectively, on the sunny day at a flow rate of 0.1 vvm (Table 1). These efficiencies were 55 ± 3, 11 ± 1 and 3 ± 3% on the cloudy day and 74 ± 1, 22 ± 1 and 3 ± 1% on the sunny day at a gas flow rate of 0.3 vvm (Table 2).

The average growth rates of *Chlorella* sp. MM-2 aerated with desulfurized biogas at 0.1 and 0.3 vvm were 0.276 ± 0.002 and 0.185 ± 0.001 g L^{-1} d^{-1} during intermittent aeration, respectively. Thus, the CO_2 fixation of *Chlorella* sp. MM-2 aerated with desulfurized biogas at 0.1 and 0.3 vvm were calculatedly 0.524 and 0.352 g L^{-1} d^{-1} during intermittent aeration, respectively. The results indicate that only 7.2% and 4.6% of CO_2 in the desulfurized biogas were captured and fixed into microalgal biomass. Jacob-Lopes et al. [32] reported that only a small fraction of the total CO_2 captured was effectively fixed into cyanobacterial biomass (3.10 ± 0.05%). The loss of CO_2 may due to excretion of biopolymers and release of volatile organic compounds by the cultured algal cells [24,32].

The CO_2 elimination capacity of the *Chlorella* sp. MM-2 cultures aerated with desulfurized biogas at 0.1 and 0.3 vvm was 179 and 227 g m^{-3} h^{-1} during intermittent aeration, respectively. The performance of the photobioreactors on CO_2 sequestration is mainly dependent of the microalgal species, CO_2 concentration in the inlet air stream, environmental temperature and light intensity, photobioreactor configuration, and operational mode (e.g., indoor or outdoor cultures). In this study, *Chlorella* sp. MM-2 aerated with biogas showed a potential on CO_2 elimination capacity compared with those in our previous reports using *Chlorella* sp [22,31].

The pattern of CO_2 concentration within each gas-switching cycle (30 min desulfurized biogas/30 min air) showed a similar pattern for eight cycles (Fig. 5a). These results demonstrate that 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. Our results indicate that the decrease of CO_2 capture efficiency in the microalgal cultures was caused by the continuous influent load of desulfurized biogas. In addition, higher CO_2 capture efficiencies were achieved at higher light intensities (sunny day). Higher light intensities have deeper light penetration capacities and can also cause higher photosynthetic activity in microalgal cultures. Ugwu et al. [33] reported that light conversion efficiency by the cells at high light intensity is high, i.e., the ability of the microalgal cells to absorb and process the solar light energy is high.

The CO_2 capture efficiency of the microalgal cultures in the outdoor photobioreactor aerated at a gas flow rate of 0.1 vvm

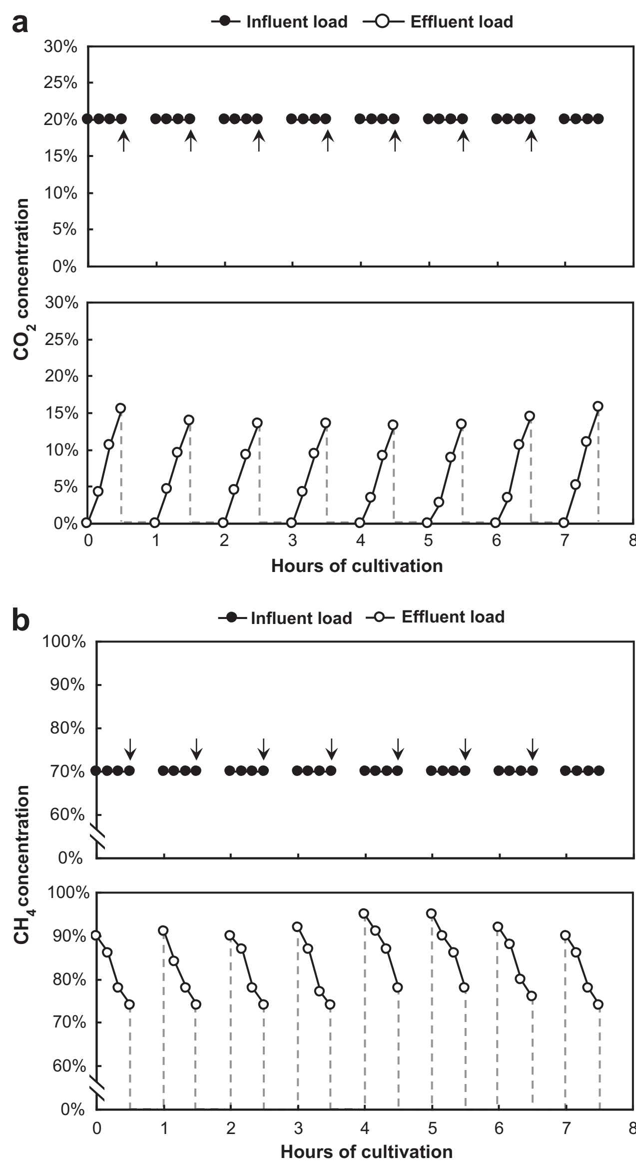

Fig. 5 – CO_2 (a) and CH_4 (b) concentrations in the influent and effluent loads of desulfurized biogas applied to microalgal cultures. The *Chlorella* sp. MM-2 cells were cultured in an outdoor photobioreactor system aerated with desulfurized biogas by the cycling-switch operation as shown in Fig. 1. The microalgal cells were cultivated at ∼1500 μmol m^{-2} s^{-1} on a sunny day. Desulfurized biogas containing 70 ± 5% CH_4, 20 ± 2% CO_2, 8 ± 3% N_2 and <100 ppm H_2S was aerated at a gas flow rate of 0.1 vvm. The cultures were grown for 8 h. The influent and effluent loads of gas were sampled every 10 min during biogas aeration. The arrows indicate the times when the gas supply was switched from desulfurized biogas to air.

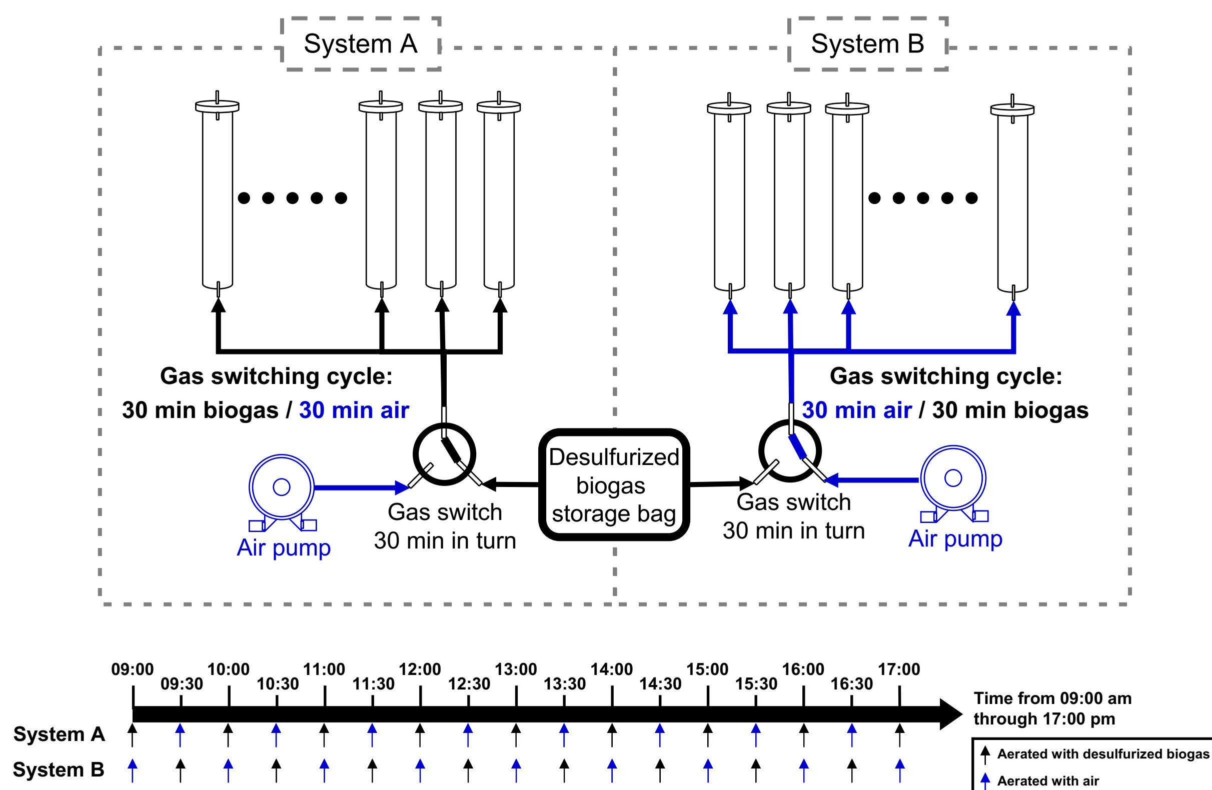

Fig. 6 – A proposed double set of photobioreactors for intermittent biogas aeration. System A and system B are used for the biogas cycle-switching operation (30 min desulfurized biogas/30 min air aeration in one system and 30 min air/30 min desulfurized biogas aeration in the other). The black arrows indicate desulfurized biogas aeration and the blue arrows indicate air aeration. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was higher than that of the culture aerated at a gas flow rate of 0.3 vvm (Tables 1 and 2). The decreasing CO_2 capture efficiency at a higher aeration rate was due to the coalescence of gas bubbles that decreased the retention time of bubbles in the culture. In addition, the decrease of surface area per unit gas volume of the bubbles can also reduce the CO_2 capture efficiency [34,35].

3.5. CH_4 enrichment capacity of Chlorella sp. MM-2 cultures aerated with biogas

Similar to the measurement of CO_2 capture efficiency, the effluent load of gas was sampled every 10 min during the desulfurized biogas aeration time within each gas-switching cycle, and the effluent load of CH_4 was measured. The patterns of CH_4 concentration in the effluent gas within each gas-switching cycle were similar and remained stable for eight cycles (Fig. 5b).

The capacities of CH_4 enrichment of the microalgal cultures operated in the outdoor photobioreactor on cloudy and sunny days at desulfurized biogas flow rates of 0.1 and 0.3 vvm were determined. The effluent loads of CH_4 from the microalgal cultures sampled at 10, 20 and 30 min after desulfurized biogas aeration were 85 ± 2, 75 ± 1 and 72 ± 1%, respectively, on the cloudy day and 87 ± 2, 80 ± 4 and 75 ± 2%, respectively, on the sunny day at a gas flow rate of 0.1 vvm (Table 1). These values were 81 ± 2, 73 ± 1 and 70 ± 1% on the cloudy day and 85 ± 2, 75 ± 1 and 72 ± 1% on the sunny day at a gas flow rate of 0.3 vvm (Table 2). The results indicate that the effluent load of CH_4 could be increased up to 80% and the CO_2 capture efficiency could reach 50% after 10 min of the desulfurized biogas aeration.

Our field study demonstrates that an outdoor photobioreactor system using a gas cycle-switching operation can capture a high percentage of CO_2 from the biogas produced from the anaerobic digestion of swine wastewater and can achieve a high level of performance in biogas upgrading. Additionally, the gas cycle-switching operation developed in the present study could be extended to a double set of photobioreactor systems. This double set of photobioreactors systems could be alternately aerated with biogases. Via gas cycle-switching operation, the biogas could be used for continuous CO_2 capture (Fig. 6).

4. Conclusion

The present study demonstrates that an outdoor microalgae-incorporated photobioreactor system is promising for biogas upgrading, i.e., decreasing CO_2 and increasing CH_4 composition of the biogas. The microalga *Chlorella* sp. MM-2 was able to utilize CO_2 for growth when aerated with desulfurized biogas (H_2S < 100 ppm) produced from the anaerobic digestion of swine wastewater. However, the demonstrated system

cannot be continuously used to upgrade biogas unless the photobioreactor system is converted to a double set of reactors for the gas cycle-switching operation.

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