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Utilization of carbon dioxide in industrial flue gases for the cultivation of microalga *Chlorella* sp.

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HIGHLIGHTS

- Three flue gases generated from a steel plant were used for *Chlorella* cultivation.
- The *Chlorella* cultures could efficiently and directly utilize CO_2, NO_X and SO_2 in flue gases.
- Operations of different flue gas aeration for microalgal cultures were optimized.
- Growth rate and lipid production of the cultures was 0.827 d^{-1} and 0.961 g L^{-1}, respectively.
- The potential application of using *Chlorella* culture to reduce CO_2 emissions from a steel plant is demonstrated.

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ABSTRACT

The biomass and lipid productivity of *Chlorella* sp. MTF-15 cultivated using aeration with flue gases from a coke oven, hot stove or power plant in a steel plant of the China Steel Corporation in Taiwan were investigated. Using the flue gas from the coke oven, hot stove or power plant for cultivation, the microalgal strain obtained a maximum specific growth rate and lipid production of (0.827 d^{-1}, 0.688 g L^{-1}), (0.762 d^{-1}, 0.961 g L^{-1}), and (0.728 d^{-1}, 0.792 g L^{-1}), respectively. This study demonstrated that *Chlorella* sp. MTF-15 could efficiently utilize the CO_2, NO_X and SO_2 present in the different flue gases. The results also showed that the growth potential, lipid production and fatty acid composition of the microalgal strain were dependent on the composition of the flue gas and on the operating strategy deployed.

1. Introduction

Global warming, which is induced by an increase in the concentration of greenhouse gases (GHG) in the atmosphere, is of great concern and has received increasing attention as the natural sources of fossil fuels are being exhausted (Favre et al., 2009). The efficient mitigation of GHG emissions has become an important international issue in scientific, and environmental fields and even in international economics and politics (Zhao and Su, 2014). Carbon dioxide (CO_2) is one of the main GHG emitted into the atmosphere. Flue gases from electric power plants and steel plants are mostly responsible for global CO_2 emissions in the world (Gielen, 2003; Kadam, 2002). Therefore, CO_2 capture and utilization of flue gases are important strategies for the sustainable operation of a power plant or steel plant.

During the recent decades, a number of post-combustion CO_2-capture methods have been developed using chemical, physical and biological methods (Kumar et al., 2011; Lee and Lee, 2003; Pires et al., 2011). Among the biological methods, the methods involving microalgal photosynthesis in particular have several merits, such as higher CO_2-fixation rates than terrestrial plants and no requirement for the further disposal of the trapped CO_2. Microalgae-based CO_2 biological fixation is regarded as a potential tactic to not only mitigate CO_2 emission but also to produce a lipid-rich microalgal biomass as a regenerative energy source (Ho et al.,

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2011; Parmar et al., 2011). The incorporation of CO_2 into energy-reserve components in a biomass, such as carbohydrates and lipids, by photosynthesis-driven microalgal fixation of CO_2 is the most promising route for CO_2 sequestration from flue gas (Brune et al., 2009; Kumar et al., 2014; Yoo et al., 2010). In light of this potential, several review articles were published (Van Den Hende et al., 2012; Wang et al., 2008a; Zhao and Su, 2014) to mainly address the effects of the operating processes on microalgal CO_2 fixation and biomass production using flue gas. However, no practical study has demonstrated the effects and efficiency of applying different types of flue gases for the cultivation of microalgae.

Microalgal biomasses can be used for biofuel production via pyrolysis, direct combustion or thermal chemical liquefaction (Mata et al., 2010). The lipid fraction of the microalgal biomass can be extracted and transesterified for biodiesel production (Chisti, 2007). Capturing CO_2 generated by industrial processes using microalgae and the subsequent utilization of the generated biomass for transportation needs would aid in achieving CO_2 sequestration and reducing our overall carbon emissions (Ho et al., 2011).

In general, the primary component of flue gas is CO_2, which is present at concentrations ranging from 3 to 25%, depending on the fuel source and the design of the plant (Packer, 2009). This CO_2 is a plentiful carbon source for microalgal cultures. The direct use of the flue gas reduces the cost of pretreatment but imposes extreme conditions on the microalgae, such as the high concentration of CO_2 and the presence of inhibitory compounds such as NO_X and SO_X (Kumar et al., 2011; Lee and Lee, 2003). Only a few microalgae species can tolerate the high levels of SO_X and NO_X that are present in industrial flue gases. Therefore, selecting microalgal strains that are suitable for bio-fixation of CO_2 form flue gases significantly affects the efficiency and cost competitiveness of the biological flue-gas CO_2 mitigation process (Jiang et al., 2013; Kumar et al., 2011; Wang et al., 2008a).

In the present study, the growth characterization, biomass production, lipid production and lipid composition of a *Chlorella* sp. mutant strain that was aerated with different flue gases were investigated. The sources of the flue gas were the coke oven, hot stove and power plant of a steel plant of the China Steel Corporation in Taiwan. Furthermore, the elimination efficiencies of CO_2, NO_X and SO_2 from the flue gases were evaluated.

2. Methods

2.1. Microalgal cultures, medium and chemicals

The microalga *Chlorella* sp. utilized, *Chlorella* sp. MTF-15, was originally obtained from the collection of the Taiwan Fisheries Research Institute (Tung-Kang, Ping-Tung, Taiwan) and was isolated by mutagenesis screening in our laboratory (Ong et al., 2010). The *Chlorella* sp. cells were grown on modified f/2 medium in artificial sea water containing 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} $NaHCO_3$ 0.3% (v/v) of a macro elemental solution and 0.3% of a trace elemental solution. The macro elemental solution contained 75 g L^{-1} $NaNO_3$ and 5 g L^{-1} $NaH_2PO_4{\cdot}H_2O$. The trace elemental solution contained 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.

2.2. Sources of flue gases

Three types of flue gases, from a coke oven, a hot stove and a power plant, were collected during August 15–November 15, 2012 from the China Steel Corporation, located in southern Taiwan (Fig. 1). Steel production processes typically dispose of large volumes of specialty gases. During the three process stages from coal to steel, three different types of flue gases are generated, namely, coke oven gas (COG), blast furnace gas (BFG) and Linz Donawitz process gas (LDG). COG is produced during coal carbonization. BFG is generated when iron ore is reduced by coke to metallic iron in blast furnaces. LDG is created from primary metal during the steel production process. Because of the large content of flammable gas in COG, BFG and LDG, they are generally combusted as fuel gas for waste utilization. The "coke oven" and "hot stove" flue gases were collected after the COG and BFG were combusted, respectively. The "power plant" flue gas was collected from the power generator, which burns BFG/LDG mixed gas.

2.3. Experimental system of indoor photobioreactors

The microalgal cells were cultured in column-type glass-fabricated photobioreactors with a working volume of 1 L per photobioreactor (ϕ 6 cm × 80 cm high). The photobioreactors were placed in an incubator at 26 ± 1 °C with a surface light intensity of approximately 300 μmol m^{-2} s^{-1} provided by continuous, cool-white fluorescent lights. The gas was supplied from the bottom of the photobioreactor with an aeration rate of 0.2 vvm. The CO_2-enriched gas was premixed with air and pure CO_2 was the control gas for the flue gas experiments. In the gas airstream, the CO_2 concentration for the cultures was adjusted to 3, 6, 12.5 or 25% in the control experiments. The flue gases were collected separately from coke oven, hot stove and power plant in the China Steel Corporation and exhausted into tanks.

Different ratios of flue gas mixed with ambient air in the tank were used for the microalgal cultures. The dilution rate (D) of the flue gas that was introduced into the photobioreactors was defined as the ratio of the volume of the specific flue gas (F) to the ambient air (A) in the mixture, i.e., $D = F/(A + F)$. For example, if 500 L of flue gas was mixed with 500 L of air, the D value was expressed as 1/2. The dilution ratios of 0 (air only), 1/8, 1/4, 1/2 and 1 (the latter being flue gas only; i.e., full flue gas) were applied in the study.

The initial biomass concentration of the *Chlorella* sp. cultures was approximately 0.3 g L^{-1}. The microalgal cells in each treatment were sampled every 24 h to determine the biomass concentration.

2.4. Experimental system of outdoor photobioreactors

To evaluate the microalgal growth performance during on-site flue-gas aeration, a *Chlorella* sp. culture system was installed adjacent to the furnace of a coke oven, hot stove and power plant of the China Steel Corporation. The diluted flue gas from the coke oven, hot stove and power plant, as mentioned earlier, was directly introduced into the microalgal culture systems using a suction pump. The flue gas was continuously supplied to the photobioreactors for 12 h during the daytime for three months (August 15–November 15, 2012). The total working volume of the microalgal cultures was 1200 L distributed in 24 photobioreactors (Fig. 1D). The culture volume of each photobioreactor was 50 L (ϕ 16 cm × 300 cm high). The flue gas was aerated at a rate of 0.2 vvm, and the inlet and outlet loads of the diluted flue gas were real-time monitored using a Landcom III portable gas analyzer (AMETEK, Inc., Paoli, PA, USA) to determine the concentrations of CO_2, NO_X and SO_2.

2.5. Determination of microalgal cell biomass and growth rate

The biomass concentration (dry weight per liter) of the microalgal cultures was determined according to the reported previously method (Kao et al., 2012b). A calibration equation considering

Fig. 1. The sources of the flue gas from the furnace of a coke oven (A), hot stove (B) or power plant (C) at the China Steel Corporation, Taiwan. The outdoor photobioreactor array for microalgal culturing is adjacent to the furnace (D). The photobioreactors are cylindrical and composed of acrylic polymer. The columns are 300 cm in length and 16 cm in diameter. The working volume of each photobioreactor is 50 L and the total volume of the photobioreactor array is 1200 L, i.e., the content of 24 photobioreactors.

the optical density and the dry weight of microalgal cells was established, as follows:

$$\text{Biomass concentration}(\text{g L}^{-1}) = 0.3038 \times A_{682} + 0.0002 \quad R^2 = 0.9993$$

Hence, the biomass concentration could be precisely calculated (R^2 = 0.9993; $p < 0.001$) using the measured optical density (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.

The optical density was used to evaluate the concentration of the *Chlorella* sp. MTF-15 biomass in each experiment. In the present study, we used the biomass concentration (g L^{-1}) to quantify the *Chlorella* sp. MTF-15 cell density in the cultures. The growth rate (g L^{-1} d^{-1}) and the specific growth rate (μ; d^{-1}) of the microalgae were calculated as follows:

$$\text{Growth rate} = \frac{W_f - W_i}{\Delta_t} \quad \text{Specific growth rate} = \frac{\ln\left(\frac{W_f}{W_i}\right)}{\Delta_t}$$

where W_f and W_i were the final and initial biomass concentration, respectively. Δ_t was the cultivation time during a specific day within the culture period of 7 days.

2.6. Lipid extraction

Lipid extraction was conducted according to the previously reported protocol (Kao et al., 2012a), with slight modifications. The microalgal cells were centrifuged and were washed twice using deionized water, and the dry biomass was obtained by lyophilization. The dried sample (200 mg) was mixed with a methanol/chloroform solution (1/2, v/v) and sonicated for 1 h. The mixture with methanol/chloroform solution was precipitated and 0.9% NaCl solution was added to yield a ratio of methanol, chloroform, and water of 1:2:1. The mixture was centrifuged and the chloroform phase was recovered. Finally, the lipids were weighed after the chloroform was removed under vacuum using a rotary evaporator.

2.7. Microalgal lipid transesterification and fatty acid profile assay

The methods used for transesterification of the microalgal lipids and the fatty acid profile assay were based on previously reported procedures (Chiu et al., 2009). A mixture of methanol (1.7 mL), sulfuric acid (0.3 mL) and chloroform (2.0 mL) was added to the

microalgal oil, and this mixture was heated at 90 °C for 40 min with thorough mixing during heating. The sample was then cooled to room temperature and mixed with 1 mL of deionized water. Finally, the organic (lower) phase containing the fatty acid methyl esters (FAMEs) was collected and the solvent was evaporated.

The fatty acid composition was determined using a FOCUS Gas Chromatograph (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a flame ionization detector (FID) and a trace GC capillary column (Thermo Fisher Scientific), which employed a cyanopropylphenyl-based phase specifically designed for the separation of FAMEs. A 30-m long column with a diameter of 0.32 mm and a 0.25-μm thick film was used. The amount of sample injected was 1 μL. The stripping gas was nitrogen, provided at a flow rate of 1.3 mL min^{-1}, and the injector and detector temperatures were 250 and 280 °C, respectively. The initial column temperature was 150 °C, at which it remained for 1 min, then the temperature was raised from 150 to 180 °C at 10 °C min^{-1}, maintained at 180 °C for 3 min, then raised from 180 to 220 °C at 1.5 °C min^{-1}, maintained at 220 °C for 1 min, and finally raised from 220 to 260 °C at 30 °C min^{-1}, and maintained at 260 °C for 5 min. The fatty acids were identified by comparison of the retention times with those of the standards using the Chrom-Card Data System software (Thermo Fisher Scientific, Waltham, MA, USA).

2.8. Gas composition analysis and measurement of the light intensity and pH

The inlet and outlet loads of the airstreams were real-time monitored using a gas analyzer. The concentration of O_2, CO_2, NO_X and SO_2 in the flue gases were measured using a Landcom III portable gas analyzer (AMETEK, Paoli, PA, USA). The pH values of the samples were determined directly using a twin compact pH meter B-213 (Horiba, Kyoto, Japan). The pH meter was calibrated daily using standard solutions of pH 4 and 7. The light intensity adjacent to the surface of the photobioreactor was measured using a LightScout Quantum Meter 3415F (Spectrum Technologies, Plainfield, IL, USA).

3. Results and discussion

3.1. Components of the different flue gases

The major components of the flue gases used in this study were analyzed, as shown in Table 1. The CO_2 content of the flue gas from the coke oven, hot stove and power plant was approximately 23–27%, 24–28% and 22–26%, respectively, indicating that the level of CO_2 in the three types of flue gases used in this work was very similar. In contrast, the levels of NO_X and SO_2 in the three types of flue gases varied significantly. It is known that NO_X and SO_2 in flue gas are the major toxic effectors for microalgal growth in cultures (Lee et al., 2000; Zhao and Su, 2014). The levels of NO_X and SO_2 were 70–80 and 80–90 ppm in the flue gas from the coke oven, 8–10 and 15–20 ppm in the flue gas from hot stove, and 25–30 and 15–20 ppm in the flue gas from power plant, respectively. The flue gas from the coke oven contained higher levels of NO_X and SO_2 because the coke oven was burning coal. According to the legislated restrictions, all flue gases should be post-combustion treated, including the use of denitrification and desulfurization processes. Therefore, the contents of NO_X and SO_2 in the flue gases were controlled at <100 ppm.

Table 1
Major components of the different flue gas were collected during August 15–November 15, 2012 from the China Steel Corporation in southern Taiwan.

	Coke oven	Hot stove	Power plant
CO_2 (%)	25 (23–27)	26 (24–28)	24 (22–26)
CO (%)	ND[b] (<0.1)	ND (<0.1)	ND (<0.1)
O_2 (%)	6.0–8.0	2.0–3.0	3.0–6.0
NO_X (ppm)[a]	70–80	8–10	25–30
SO_2 (ppm)	80–90	15–20	15–20
Temp. (°C)	160–180	120–160	150–190
Dust (mg m^{-3})	1.0–8.0	1.0–5.0	1.0–10.0
Gas flow rate (m^3 h^{-1})	180,000–280,000	260,000–400,000	250,000–400,000

[a] Nitrogen oxide (NO_X) is the mixture of nitric oxide (NO) and nitrogen dioxide (NO_2).
[b] ND indicates not detectable, i.e., the content of CO in the flue gas is <0.1%.

3.2. Growth parameters of Chlorella sp. grown with flue gas aeration

In our previous study (Chiu et al., 2008), microalgal cell growth was significantly inhibited when wild-type microalgal *Chlorella* sp. cultures were aerated using gas containing a high concentration of CO_2 (>10% CO_2). Given the high concentration of CO_2 in flue gas (>25% CO_2), the growth potential of the isolated microalga (*Chlorella* sp. MTF-15) when aerated directly with the different flue gases was first evaluated. The flue gases generated from the coke oven, hot stove and power plant of the steel plant were collected in a gas storage bag, and the gas was continuously introduced into the photobioreactor under the control of an air blower.

Fig. 2A shows the growth curves of *Chlorella* sp. MTF-15 that was aerated with air, the different flue gases or CO_2-enriched gas (25% CO_2 generated from 100% CO_2 mixed with air) for 7 days. The maximum biomass concentrations in the *Chlorella* sp. MTF-15 cultures aerated with air and 25% CO_2 were 0.938 and 1.458 g L^{-1}, respectively. When the microalgal cultures were aerated with flue gas from the coke oven, hot stove and power plant, the maximum biomass concentrations were 2.523, 1.680 and 1.872 g L^{-1}, respectively. All of the growth potentials of *Chlorella* sp. MTF-15 aerated with the three flue gases were significantly higher than those obtained using air or 25% CO_2 aeration.

The maximum growth rate of *Chlorella* sp. MTF-15 cultured with flue-gas aeration from the coke oven, hot stove and power plant was 0.515, 0.314 and 0.342 g L^{-1} d^{-1}, respectively (Table 2), which is approximately 2.0-, 1.2- and 1.3-fold higher than that obtained with 25% CO_2, respectively. These results indicated that *Chlorella* sp. MTF-15 was well adapted for cultivation using the three flue gases for CO_2 capture and utilization to produce a microalgal biomass. The higher cell concentration obtained using the flue gases may be attributed to the presence of NO_X and SO_2 in the flue gases, which served as additional nitrogen and sulfur sources to support microalgal cell growth. The details regarding the utilization of NO_X and SO_2 by the microalgal culture are discussed in Section 3.4.

The microalgal cultures were grown for 7 days, and the culture broths were sampled every 24 h for pH measurements. The pH of the broth was initially 7.0 and quickly decreased to 6.8, 6.4, 6.8 and 6.7 at 30 min because of the dissolution of CO_2, SO_2 and NO_X, and then gradually increased to 7.4, 7.1, 7.3 and 7.3 at day 7 of aeration using 25% CO_2 or the flue gas from the coke oven, hot stove or power plant, respectively (Fig. 2B). The increase in the pH of the broth with the cultivation time was observed because more dissolved CO_2 was used by the microalgae due to the daily increase in the biomass concentration in the cultures (Hulatt and Thomas, 2011; Kumar et al., 2011; Valdés et al., 2012). pH ranging 6.4–7.4 are suitable for the growth of the *Chlorella* sp. cells, according to our previous studies (Chiu et al., 2011; Kao et al., 2012b); therefore, the effect of pH changes in the *Chlorella* sp. MTF-15 cultures that were aerated with the flue gases and 25% CO_2 could be ignored.

3.3. Growth profile of Chlorella sp. cultures provided different flue-gas dilution ratios

To investigate the appropriate flue-gas dilution with air for microalgal cultivation, three flue-gas dilution ratios (i.e., 1/8, 1/4 and

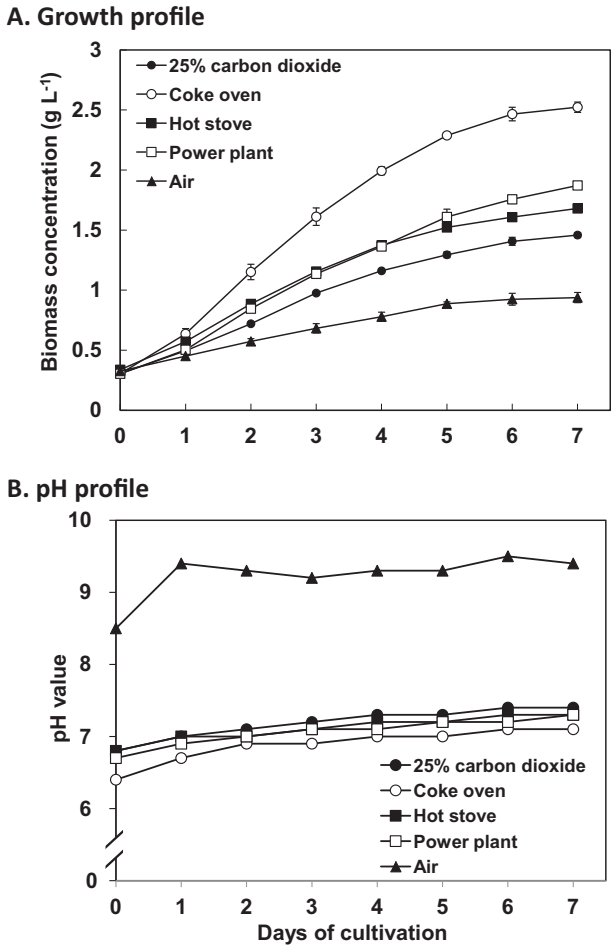

Fig. 2. Growth profile (A) and pH profile (B) of *Chlorella* sp. MTF-15 aerated with air, 25% CO_2-enriched gas or the flue gases. The initial biomass concentration was approximately 0.3 g L^{-1}. The microalgae were cultivated at a light intensity of approximately 300 μmol m^{-2} s^{-1}. The flue gas was provided at a flow rate of 0.2 vvm. The cultures were grown at 26 ± 1 °C for 7 days, and the microalgal cells and broth were sampled every 24 h for the growth and pH measurements. Each point and bar represents the mean value ± SD from three independent cultivations.

1/2) were utilized to study the effects of flue-gas dilutions on microalgal growth and lipid production. The CO_2 content in the 1/8-, 1/4- and 1/2-diluted flue gases was approximately 2–4%, 5–7% and 10–14%, respectively, in all of three flue gases used.

Fig. 3 shows the growth profile of *Chlorella* sp. MTF-15 that was aerated using different ratios of flue gases. After the 7 days of cultivation, the microalgal cell growth reached the stationary phase and the maximal biomass concentrations of the microalgal cultures were 2.855, 2.390 and 2.319 g L^{-1} in the cultures that were aerated with flue gas from the coke oven at the 1/2 dilution, from the hot stove at the 1/4 dilution and from the power plant at the 1/4 dilution ratio, respectively. Table 2 summarizes the maximum growth rate and maximum specific growth rate in the *Chlorella* sp. MTF-15 cultures aerated using different dilution ratios of the flue gas from the coke oven, hot stove or power plant. The data showed that the maximum growth rate and maximum specific growth rate were 0.528 g L^{-1} d^{-1} and 0.827 d^{-1} with a 1/2 dilution of coke-oven flue gas, 0.542 g L^{-1} d^{-1} and 0.762 d^{-1} with a 1/4 dilution of blast-furnace flue gas, and 0.449 g L^{-1} d^{-1} and 0.728 d^{-1} with a 1/4 dilution of power-plant flue gas. The difference in the best growth performances under different aeration conditions of the flue gases is most likely correlated with the difference in the compositions of the flue gases, in particular, the contents of NO_X and SO_2.

The microalgal species and the culture conditions are the most critical factors affecting growth performance when using flue gas as the carbon source. The characteristics of the microalgae used directly affect the performance of carbon fixation and biomass production (Zhao and Su, 2014). Among the microalgal candidates for flue gas utilization, *Chlorella* spp. generally exhibit better performance in terms of growth and adaptation to the culture conditions (Chiu et al., 2008; Yeh and Chang, 2012). The *Chlorella* sp. MTF-15 strain was derived from our laboratory's isolate *Chlorella* sp. MT-15. The maximum specific growth rate of the MT-15 strain reached 0.9 d^{-1} at 30 °C, and the strain could grow well in an outdoor semi-continuous cultivation system (Ong et al., 2010). However, the lipid production of the MT-15 strain was only 10–13%. In this study, the MTF-15 strain was used because it could produce approximately 35% lipid in regular indoor cultures (Table 3). In addition, the *Chlorella* sp. MTF-15 strain can endure high CO_2 concentrations (25–30%), which are similar to the CO_2 concentrations in the flue gases from the coke oven, hot stove and power plant that were used in this study. However, the growth of *Chlorella* sp. MTF-15 was slightly inhibited when the CO_2 concentration was as high as 25%. The optimal CO_2 concentrations for the growth of *Chlorella* sp. MTF-15 were in the range of 3–12% CO_2 when enriched CO_2 was supplied for growth. These results were confirmed by the results of the flue gas studies because diluting the flue gases with air was necessary to optimize the productivity of the microalgal biomass.

3.4. Flue-gas bioremediation using the microalgal culture

It was observed that the microalgal culture could effectively remove the major components of the flue gas (namely, CO_2, NO_X

Table 2
Maximum growth rate (g L^{-1} d^{-1}) and maximum specific growth rate (d^{-1}) of *Chlorella* sp. MTF-15 cultures aerated with different flue gases at different gas dilution ratios.*

Flue gas dilution ratio with air	Coke oven		Hot stove		Power plant	
	Max. growth rate[a]	Max. specific growth rate[b]	Max. growth rate	Max. specific growth rate	Max. growth rate	Max. specific growth rate
1/8	0.466 ± 0.038	0.739 ± 0.059	0.522 ± 0.042	0.744 ± 0.004	0.409 ± 0.017	0.686 ± 0.004
1/4	0.514 ± 0.072	0.754 ± 0.037	0.542 ± 0.088	0.762 ± 0.081	0.449 ± 0.016	0.728 ± 0.012
1/2	0.528 ± 0.022	0.827 ± 0.025	0.449 ± 0.007	0.667 ± 0.052	0.423 ± 0.058	0.584 ± 0.018
1[c]	0.515 ± 0.021	0.737 ± 0.071	0.314 ± 0.040	0.526 ± 0.048	0.342 ± 0.019	0.505 ± 0.002

* The max. growth rate and specific growth rate was 0.255 g L^{-1} d^{-1} and 0.488 d^{-1} in the culture aerated with 25% CO_2, 0.125 g L^{-1} d^{-1} and 0.239 d^{-1} in the culture aerated with air, respectively.

[a] Growth rate $= \frac{W_f - W_i}{\Delta_t}$ (W_f is the final biomass concentration. W_i is the initial biomass concentration. Δ_t is the cultivation time in a specific day within the culture period of 7-day).

[b] Specific growth rate $= \frac{\ln\left(\frac{W_f}{W_i}\right)}{\Delta_t}$ (W_f is the final biomass concentration. W_i is the initial biomass concentration. Δ_t is the cultivation time in a specific day within the culture period of 7-day).

[c] Full flue gas was applied.

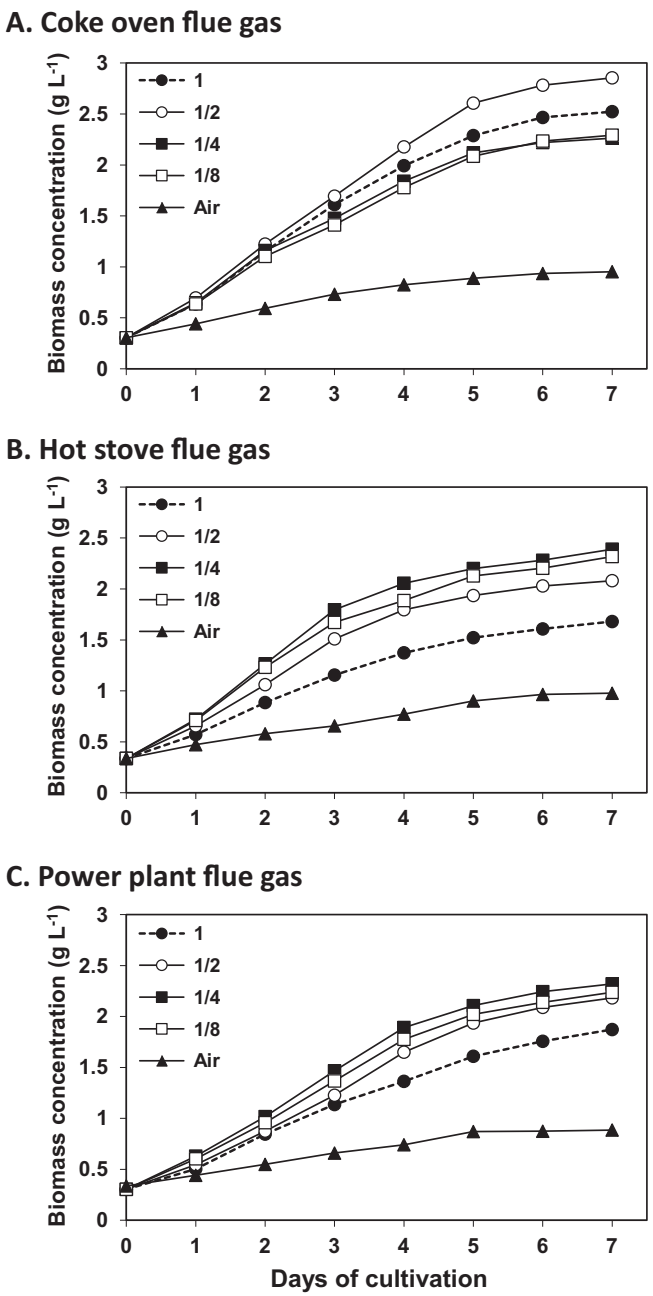

Fig. 3. Growth profiles of *Chlorella* sp. MTF-15 aerated with coke-oven flue gas (A), blast-furnace flue gas (B) or power-plant flue gas (C), provided at different gas dilution ratios (1, 1/2, 1/4, and 1/8). The initial biomass concentration was approximately 0.3 g L^{-1}, and the aeration rate was 0.2 vvm. The microalgae were cultivated at a light intensity of approximately 300 μmol m^{-2} s^{-1}. The cultures were grown at 26 ± 1 °C for 7 days, and the microalgal cells were sampled every 24 h for the growth determinations.

and SO_2) for on-site bioremediation of flue gases to achieve the dual benefits of CO_2 mitigation and air-pollution control. In this study, the removal efficiencies of CO_2, NO_X and SO_2 by microalgal cultures aerated with flue gases were determined. The CO_2 concentrations in the inlet and outlet loads of the flue gas used to aerate the microalgal cultures were monitored in real time using CO_2 gas sensors. The CO_2 removal efficiencies of *Chlorella* sp. MTF-15 under continuous aeration using different dilution ratios of flue gases were determined (Fig. 4A). As expected, the increase in the efficiency of CO_2 removal accompanied the elevated dilution factor of the flue gas applied. The data indicated that when the full flue gases were supplied to the *Chlorella* sp. MTF-15 cultures, the CO_2-removal efficiency ranged from 15 to 25% and the CO_2-fixation rate ranged from 20 to 35 g L^{-1} d^{-1}. These performances were highly dependent on the type of flue gas used. The optimal efficiency of CO_2 removal from the coke oven, hot stove and power plant flue gases was approximately 25%, 40% and 50%, respectively. It is noted that the *Chlorella* sp. MTF-15 cultures aerated with flue gas from the coke oven had relatively lower CO_2-removal and CO_2-fixation efficiencies, which might be due to the lower broth pH that arose from the coke-oven flue gas having a higher SO_2 content (Jiang et al., 2013). The pH of the culture is an important effector of the microalgal CO_2-concentrating mechanism (Kumar et al., 2011; Valdés et al., 2012). Valdés et al. (2012) indicated that the pH profile provided information about the behavior of the microalgae/photobioreactor system regarding the efficiency of CO_2 utilization. In this study, hydrolysis of SO_2 (from flue gas aeration) in the microalgal broth would result in the release of H^+, leading to a decrease in the broth's pH. This lower pH of the microalgal culture caused the lower efficiencies of CO_2 removal and CO_2 fixation.

The efficiencies of NO_X and SO_2 removal by the microalgal cultures that were aerated with flue gas were also determined (Fig. 4B and C). When *Chlorella* sp. MTF-15 was grown using the full flue gas from the coke oven, hot stove or power plant, the NO_X-removal efficiency was 65, 95 and 80%, respectively, whereas the SO_2 removal efficiency was 39, 93 and 93%, respectively. It is known that the major toxic pollutants present in the flue gases are NO_X and SO_2, which have negative impacts on the growth of microalgal species. Because the steel plant is equipped with facilities for denitrification of NO_X and desulfurization of SO_2, their contents in flue gases are controlled to less than 100 ppm. The series of *Chlorella* sp. mutants isolated in our laboratory also exhibited good tolerance of high levels of NO_X and SO_2 in flue gas (Chiu et al., 2011). Similar microalgal strains selected or adaptively cultured for using CO_2 in flue gas as an autotrophic carbon source were also able to tolerate NO_X or SO_2 concentrations as high as 100 ppm (Lee et al., 2000). It should be emphasized that the flue gases would be mixed with ambient air to dilute the CO_2 as well as NO_X and SO_2 for pilot-scale or industrial applications; therefore, the NO_X and SO_2 concentrations would be lower than those (<100 ppm) present in the flue gases that have been processed by denitrification and desulfurization. As a result, the negative effects of the NO_X and SO_2 pollutants on the selected microalgal strains could be minimized.

Table 3
Lipid content (%) and lipid production (g L^{-1}) of *Chlorella* sp. MTF-15 aerated with different flue gases at different dilution ratios.*

Flue gas dilution ratio with air	Coke oven		Hot stove		Power plant	
	Lipid content[a]	Lipid production[b]	Lipid content	Lipid production	Lipid content	Lipid production
1/8	30.0 ± 0.3	0.688 ± 0.006	36.5 ± 1.3	0.846 ± 0.031	34.5 ± 0.4	0.772 ± 0.008
1/4	22.5 ± 0.5	0.509 ± 0.010	40.2 ± 0.7	0.961 ± 0.017	32.3 ± 3.8	0.749 ± 0.087
1/2	21.5 ± 0.4	0.614 ± 0.012	41.6 ± 0.4	0.866 ± 0.009	36.3 ± 0.2	0.792 ± 0.004
1[c]	26.4 ± 0.1	0.666 ± 0.004	35.2 ± 0.1	0.591 ± 0.002	33.8 ± 0.5	0.633 ± 0.009

* The lipid content and lipid production was 35.7% and 0.521 g L^{-1} in the culture aerated with 25% CO_2, 34.0% and 0.319 g L^{-1} in the culture aerated with air, respectively.
[a] Lipid content = (lipid dry weight/biomass dry weight)/100.
[b] Lipid production = (Max. biomass production × lipid content)/100.
[c] Full flue gas was applied.

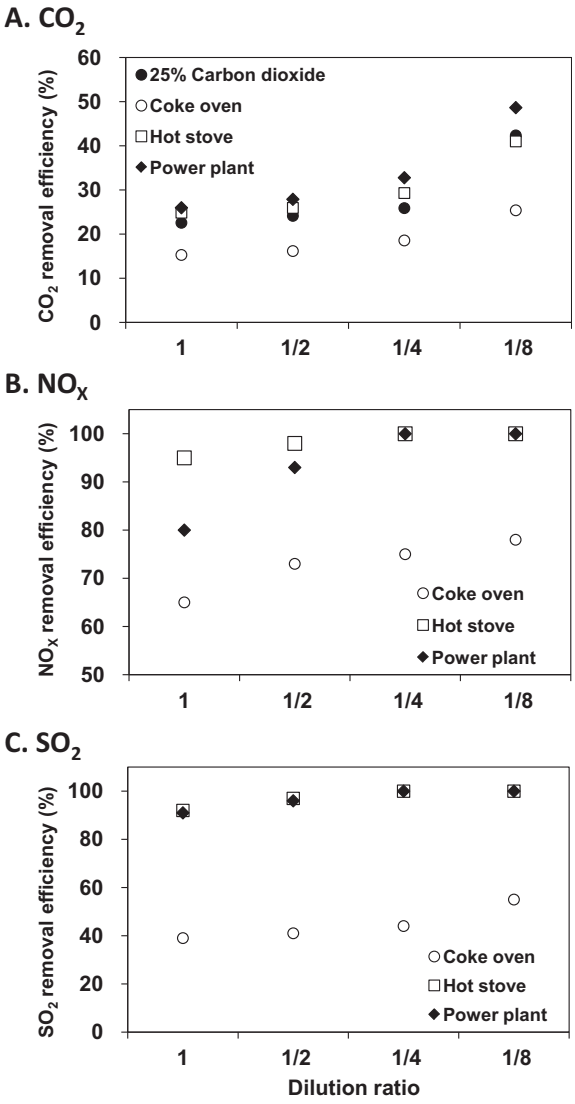

Fig. 4. Removal efficiencies of CO_2 (A), NO_X (B) and SO_2 (C) of the *Chlorella* sp. MTF-15 culture aerated with flue gases from coke oven, hot stove, power plant, and 25% CO_2-enriched gas under different dilution ratios. The biomass concentration of the microalgal culture was approximately 2 g L^{-1}, and it was cultivated during the daytime. The CO_2, NO_X and SO_2 removal efficiency was calculated from the difference in the concentrations of the inlet and outlet loads.

The inhibitory effect of SO_2 on microalgal growth can be attributed mostly to its effect on the pH value of the broth because of the release of H^+ that occurs due to the hydrolysis of SO_2 (Jiang et al., 2013). Another inhibitory effect of SO_2 on microalgal growth may be associated with the SO_4^{2-} and HSO_4^- ions that are also produced due to SO_2 hydrolysis because both types of ions have been suggested to be inhibitory factors for microalgal growth (Chiu et al., 2011). In the present study, the pH of the microalgal cultures was determined, revealing that only a slight decrease in the broth's pH was observed in the cultures that were aerated with flue gases. This pH decrease was likely attributable to the SO_2 and the CO_2 in the flue gases, and the lowest pH in the culture medium was approximately 6.4 in this study. Thus, the minor pH changes in the flue-gas aerated microalgal cultures indicated that the effect of SO_2 on microalgal growth was not significant.

Microalgae can take up nitrogen in several forms, including NH_4^+, NO_3^-, NO_2^-, NO and N_2. In general, the NO_X species emitted in incineration processes consist of 95% NO and 5% NO_2 (Wang et al., 2008b). Although extremely low amounts of NO can be dissolved in water, NO_2 has 6000-times higher solubility than NO (Dora et al., 2009). When any of the NO_X compounds dissolve in water, they form nitric acid (HNO_3) or nitrous acid (HNO_2). Both of those nitrogen acids can serve as an N-source for microalgal utilization and growth (Graham and Wilcox, 2000). We proposed that some of the NO_X in the flue gases dissolved in the microalgal culture broth and was available as an additional N-source for the microalgae; therefore, the enriched N nutrients in the microalgal cultures aerated with flue gas might be one of reasons for the increased microalgal biomass concentration and maximum growth rate.

3.5. Lipid content and production in *Chlorella* sp. cultures provided different flue gases

To investigate the effect of flue gas on lipid accumulation and production by microalgae, cultures of the *Chlorella* sp. MTF-15 strain grown for 7 days using air, 25% CO_2-enriched gas, and 1/8, 1/4, 1/2 and 1 (flue gas only) dilutions of flue gases were harvested and used for lipid extraction and quantification. The lipid contents of *Chlorella* sp. MTF-15 cultures that were aerated with different gases are shown in Table 3. The lipid content of *Chlorella* sp. MTF-15 was approximately 34.0% and 35.7% when the cultures were aerated with air or 25% CO_2. This result suggested that CO_2 enrichment of the aerated gas did not affect the lipid synthesis of the microalgal cells. The lipid content of the microalgae cells when they were grown with the full flue gases from the hot stove and power plant were also similar to that obtained using the enriched gas (35.2 and 33.8%, respectively). However, microalgal cultures cultivated with the full coke-oven flue gas had a lower lipid content (26.4%) compared with the other cultures (Table 3). Diluting the flue gas applied for culturing *Chlorella* sp. MTF-15 slightly affected the lipid content of the microalga. The maximum lipid content in the microalgal cultures was 30.0%, 41.6% and 36.3% when the coke-oven, blast-furnace and power-plant flue gas was applied in a 1/8, 1/2 and 1/2 dilution ratio, respectively.

The lipid production (calculated as (lipid content × max. biomass production)/100) of the *Chlorella* sp. MTF-15 cultures was also determined (Table 3). The data indicated that the lipid production of the microalgal cultures aerated with the different diluted flue gases ranged from 0.509 to 0.961 g L^{-1}. Lipid production in the *Chlorella* sp. MTF-15 cultures aerated with the flue gas (without dilution) from the coke oven, hot stove and power plant was quite similar. The maximum lipid production of the *Chlorella* sp. MTF-15 cultures aerated with the flue gas from the coke oven (at a 1/8 dilution), hot stove (at a 1/4 dilution) and power plant (at a 1/2 dilution) was 0.688, 0.961 and 0.792 g L^{-1}, respectively. The lipid production of *Chlorella* sp. MTF-15 cultures aerated with the flue gas from the hot stove (1/2–1/8) was higher than that obtained using flue gases from the coke oven or power plant.

Table 3 also shows that the lipid content of *Chlorella* sp. MTF-15 cultures aerated with flue gas from the coke oven was significantly lower than that of cultures aerated with the flue gases from the hot stove or power plant. The difference in the lipid-accumulation performance of the microalgal cells cultivated using different flue gases could be due to the differential NO_X content in the flue gases. The dissolved NO_X in the microalgal cultures may serve as a N nutrient that enhanced the microalgae cell growth, but microalgal cells can accumulate cellular lipids only when the nitrogen source in the culture becomes depleted (Li et al., 2008). Because the flue gas from the coke oven had a relatively higher NO_X content than did the other two types of flue gas, it might be difficult for the microalgal culture to reach N-deficiency when the flue gas with the highest NO_X content was continuously aerated into the culture. As a result, lipid accumulation cannot be efficiently induced while cultivating the microalgal strain using flue gas from the coke oven.

Table 4
Main fatty acid compositions of *Chlorella* sp. MTF-15 aerated with air, CO_2-enriched gas (25%) and different flue gases.

	Air	25% CO_2	Coke oven	Hot stove	Power plant
Lipid content (% dry weight)	34.0 ± 2.6	35.8 ± 0.7	26.4 ± 0.1	35.2 ± 0.1	33.8 ± 0.5
Fatty acid composition[a]			Relative content (%)		
C16:0	45.6 ± 1.7	42.6 ± 2.3	55.2 ± 4.5	39.7 ± 0.3	37.6 ± 0.4
C16:1	0.4 ± 0.0	1.4 ± 0.2	0.8 ± 0.0	0.8 ± 0.00	1.1 ± 0.1
C18:0	5.4 ± 0.8	4.4 ± 0.1	7.1 ± 0.8	5.4 ± 0.7	5.4 ± 0.5
C18:1	19.0 ± 0.7	22.0 ± 6.1	3.2 ± 0.1	11.7 ± 0.1	18.5 ± 0.6
C18:2	11.3 ± 0.9	11.3 ± 1.6	5.8 ± 0.2	16.3 ± 0.08	14.1 ± 0.3
C18:3	13.9 ± 0.3	14.7 ± 3.0	20.9 ± 1.3	16.4 ± 0.2	18.1 ± 0.3
Others	4.4 ± 0.4	3.8 ± 1.1	7.0 ± 0.5	9.9 ± 0.2	5.3 ± 0.3
Saturated fatty acids	51.0 ± 1.0	47.0 ± 2.4	62.3 ± 4.1	45.0 ± 0.1	43.0 ± 0.1
Unsaturated fatty acids	44.6 ± 0.5	49.3 ± 1.3	30.7 ± 0.8	45.1 ± 0.0	51.8 ± 0.1

[a] For gas chromatograph analysis of fatty acid composition, all the fatty acids were transesterified into fatty acid methyl ester.

This hypothesis may explain why *Chlorella* sp. MTF-15 aerated with coke-oven flue gas could grow rapidly but the rate of lipid accumulation was lower during the early stage of their stationary phase. Although the lipid content of *Chlorella* sp. MTF-15 aerated with full coke-oven flue gas was lower than that obtained using the other two flue gases, the lipid production of the *Chlorella* sp. MTF-15 cultures grown using the three types of flue gases was similar, due mainly to the higher growth rate obtained using coke-oven flue gas.

3.6. Fatty-acid composition of the lipids produced by Chlorella sp. cultures grown using different flue gases

Gas chromatography was used to analyze the main fatty-acid components (in terms of C16:0, C18:0, C18:1, C18:2 and C18:3) of the microalgal lipids extracted from the *Chlorella* sp. MTF-15 cultures grown using aeration with different flue gases at different dilution ratios. The lipid composition results are summarized in Table 4. There were significant variations in the fatty acid profiles when the microalgal strain was cultured using aeration with different gases, particularly in the content of C16:0 and C18:1. However, regardless of whether the flue gases, air or 25% CO_2 were used, the content of C16:0 + C18:1 (the suitable fatty acids for biodiesel synthesis) of the microalgal biomass was very similar (at approximately 60–65%). The content of fatty acid C16:0 in the microalgal biomass cultivated using coke-oven flue gas reached 55.2%, which is significantly higher than those in the cultures aerated with 25% CO_2, or the blast-furnace or power-plant flue gases. In other words, the lipids obtained from the microalgal biomass grown using coke-oven flue gas had a relatively higher content of saturated fatty acids due to its higher content of C16:0.

The length of the fatty acid chains in the lipids plays an important role in determining the characteristics of the synthesized biodiesel, such as its pour point and boiling point. C16:0, C18:0, C18:1, C18:2 and C18:3, the fatty acids commonly utilized for biodiesel synthesis (Knothe, 2008), were quantified in this study. The fatty acid profile of *Chlorella* sp. MTF-15 consists mainly of C_{16} and C_{18} fatty acids, and the major fatty acid component in the microalgal strain was C16:0. C16:0 is the major saturated fatty acid in 12 microalgal strains that represent 8 classes, including *Chlorophyceae* (Patil et al., 2007). High levels of saturated fatty acids tend to increase the stability of biodiesel because unsaturated fatty acids have poor oxidative *stability* (Demirbas, 2009). The saturated fatty acid content of *Chlorella* sp. MTF-15 aerated using coke oven flue gas was significantly higher than those of *Chlorella* sp. MTF-15 aerated using enriched-CO_2 or the flue gases of the hot stove and power plant because a relatively higher C16:0 content resulted in relatively lower C18:1 and C18:2 contents. The FAME quality of the *Chlorella* sp. MTF-15 lipids produced using aeration with the different flue gases remains to be tested. However, for the purpose of biodiesel production from a microalgal biomass, all three of the flue gases examined are suitable candidates as the CO_2 source for microalgae cultivation because the microalgal biomasses obtained had lipid compositions appropriate for biodiesel synthesis.

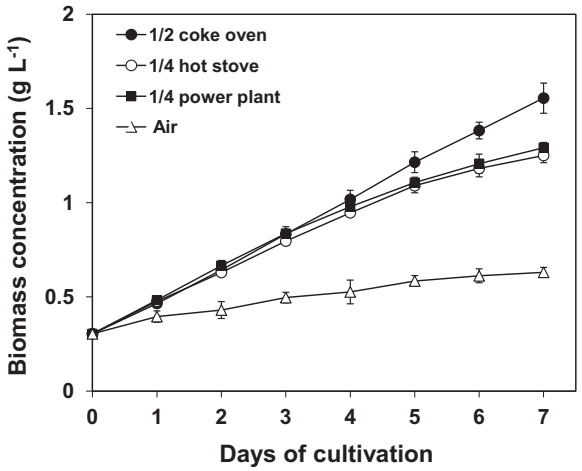

Fig. 5. Growth curves of *Chlorella* sp. MTF-15 cultures grown in an outdoor photobioreactor aerated with flue gas from the coke oven (1/2 dilution ratio with air), hot stove (1/4 dilution) or power plant (1/4 dilution) of the China Steel Corporation in southern Taiwan. The aeration rate was 0.2 vvm. The initial biomass concentration was approximately 0.3 g L^{-1}. The microalgal cells were cultivated during the daytime (1500–1800 μmol m^{-2} s^{-1}). The cultures were grown for 7 days. The microalgal cultures were sampled every 24 h to determine the growth rate and collected after 7 days to measure the lipid content. Each point and bar represents the mean value ± SD from three independent cultivations.

3.7. Outdoor cultures

To evaluate the microalgal growth performance during on-site flue-gas aeration, a *Chlorella* sp. culture system was installed adjacent to the furnace of a coke oven, hot stove and power plant of the China Steel Corporation in southern Taiwan (Fig. 1). The flue gas from the coke oven, hot stove or power plant was directly introduced into the microalgal cultures, using a suction pump at a 1/2 or 1/4 dilution continuously for 12 h during the daytime. The scale of the microalgal cultures was 1200 L, distributed in 24 photobioreactors. The culture volume of each photobioreactor was 50 L.

Fig. 5 shows the growth curves of 7-day outdoor cultures of *Chlorella* sp. MTF-15 aerated using the three flue gases. The maximum biomass concentration (g L^{-1}) and maximum growth rate (g L^{-1} d^{-1}) in the cultures aerated with the flue gas from the coke oven, hot stove or power plant was 1.555 and 0.197, 1.270 and

0.172, and 1.252 and 0.183, respectively. These values were approximately 2-fold higher than that of the cultures aerated only with air. The lipid content was 14.8, 19.6 and 19.3% in the microalgal cultures treated with the flue gas from the coke oven, hot stove or power plant, respectively. The lipid content of the microalgal biomasses obtained from the outdoor cultures was similar to that of the cultures grown using air (lipid content = 17.6%) except for the culture aerated with coke-oven flue gas.

It must be stated that environmental conditions, such as temperature and light, are difficult to control when microalgal cultivation was performed outdoor. Moreover, the outdoor cultures were treated with a light–dark cycle and the flue gas was supplied only for 12 h, during the daytime. Although there were differences between the indoor and outdoor cultures, we want to emphasize that the growth performance and biomass production in the outdoor cultures (Fig. 5) aerated with different flue gases were roughly consistent with the predictions from the results of the indoor cultures (Fig. 3). The 7-day outdoor cultures of *Chlorella* sp. MTF-15 aerated with the coke-oven flue gas had a higher maximum biomass concentration and maximum growth rate compared with those of the cultures aerated with blast-furnace or power-plant flue gas. We proposed that this result was due to the higher, but not exceeding the tolerance level of *Chlorella* sp. MTF-15, content of NO_X and SO_2 in the coke-oven flue gas. Although NO_X and SO_2 are not necessary for the growth of microalgae, adequate NO_X and SO_2 levels in the flue gas may be beneficial for microalgal growth because the NO_X and SO_2 dissolved in the culture broth can be additional nutrients. Overall, these results demonstrated that *Chlorella* sp. MTF-15 could grow well in outdoor photobioreactors that were aerated directly with flue gases from a steel-manufacturing plant.

4. Conclusions

This study showed that *Chlorella* sp. MTF-15 could grow when supplied with three types of flue gases, with efficient utilization of CO_2, NO_X and SO_2. The obtained microalgal biomass accumulated lipids with a suitable composition for biodiesel production. The performance of CO_2 fixation, NO_X/SO_2 removal, and lipid production by the microalga was greatly dependent on the composition of the flue gas and the operating strategy employed. Outdoor cultivation of the microalga by directly utilizing the flue gases from the steel plant was also successful. This study demonstrated the potential of using microalgae to mitigate CO_2 emission and produce biomass as a biodiesel feedstock.

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